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FULL PAPER

Circulating microRNA-16 and microRNA- 106a as novel biomarkers in inflammatory bowel disease

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Inflammatory Bowel Disease (IBD) includes Crohn's Disease (CD), and Ulcerative Colitis (UC), the two main types of IBD. The IBD incidence is rising globally and among the young age. Endoscopy has a principal role in rating IBD mucosal lesions. Though, it is expensive and invasive. MicroRNAs could be included in regulation of particular genes and included in inflammation. Disorders in microRNAs levels might cause disease development. The objective of our study is to investigate the role of microRNA-16 and microRNA- 106a levels as indicators for diagnosis and assessing the IBD activity. The values of miR 16 and miR 106a were measured in plasma from 20 healthy volunteers (group I) and 40 UC patients (group II) and 40 CD patients (group III) by quantitative real time-PCR (qRT-PCR). Endoscopic severity index was calculated based on laboratory and endoscopic findings. Significant upregulation of miR 16 and miR 106a levels in group II and group III than group I. A positive correlation was demonstrated between both miR 16 and miR 106a with endoscopic severity index. ROC curves of miR 16 for detecting UC showed sensitivity and specificity of 92.5 % and 90 % particularly. ROC curves of miR 106a for differentiating active from inactive CD showed sensitivity and specificity of 90 % and 75 % particularrly. both miR 16 and miR 106a could act as promising biomarkers of IBD diagnosis and assessing its activity aiding in its proper management.

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KEYWORDS

miR 16; miR 106a; endoscopic severity index; IBD.

Introduction

Inflammatory Bowel Disease (IBD) includes Crohn's Disease (CD), and Ulcerative Colitis (UC), the two principal types of IBD, which share pathological and clinical criteria [1]. The occurrence of IBD is rising globally and among the young age [2]. The IBD etiology is not precisely obvious. Some papers have demonstrated that atypical immunity reaction towards the gut flora in genetically vulnerable subjects is in charge of the disease [1]. Research has showed that there is a correlation between IBD development and the adaptive immunological reaction. It has been lately informed that the innate immunological response has a role in IBD pathogenesis as in GIT inflammatory reaction [3]. The IBD diagnosis is depending on clinical, laboratory, and endoscopic findings. Mostly, the diagnosis

of CD or UC is obvious. Endoscopy has a principal task in rating lesions of mucosa. Besides, it is expensive and invasive with complications [4,5]. Likewise, if the disease is just colonic, discriminating between the 2 diseases would be inadequate in up to 15% of patients owed to unclear observations on tissue biopsy or colonoscopy [6]. Up to now, no optimal indicator to detect and assess IBD. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are utilized in monitoring. However, they are nonspecific, because their levels rise in various inflammatory diseases or stay at normal levels in active IBD cases [7]. Fecal calprotectin is non-invasive indicator for assessing disease revert. Nevertheless, it is yet nonspecific because it is increased in extra intestinal inflammatory diseases, as celiac disease [8]. Hence, there is enhancing need for more specific diagnostic biomarkers for IBD and can discriminate between various types [9].

MicroRNAs are non-coding RNAs consisting of 17-22 nucleotides and significantly existing in the cells which are included in different physiological and pathological activities [10]. MicroRNAs are included in the control of variable biological procedures, for example intestinal barrier function, immunological reaction regulation, and GIT microflora interactions, to produce chronic inflammation of the mucosa [11]. MicroRNAs could be included in regulation of particular genes and included in cell discrimination and organogenesis. Disorders in microRNAs levels might cause disease development [3]. Abnormal microRNA expression in the tissues as well as peripheral blood was found in IBD cases compared to healthy group. But, the findings are contradictory in some studies regarding the correlations between microRNA values and disease activity [12]. The purpose of study was to investigate the role of the levels of microRNA-106a and microRNA-16 in Crohn's Disease (CD), and Ulcerative Colitis (UC) in IBD diagnosis and assessing its activity.

Aim of the work

This works aims to investigate the role of microRNA-16 and microRNA- 106a levels as indicators for diagnosis and assessing the IBD activity and correlate them with endoscopic severity index.

Experimental

Research subjects

Between August 2023 and February 2024, this case-control study was performed at Zagazig Faculty of Medicine Medical Biochemistry and Clinical Pathology Departments, Egypt. 80 IBD patients were collected for the study at Tropical Medicine and Internal Medicine Departments, 40 patients in UC (group II) and 40 patients in CD group (group III). The diagnosis of IBD was established on the clinical, laboratory, radiological, endoscopic, and histological findings. Colonoscopy was performed to all patients to assess the endoscopic severity index. Crohn's Disease Endoscopic Index of Severity (CDEIS) scores for CD and UCEIS scores for UC were assessed after carrying out colonoscopy to evaluate the endoscopic activity CDEIS >3 for CD and UCEIS >1 for UC are the cut off levels of disease flaring in both diseases [13,14]. Each of the UC group and CD groups were subdivided into 2 subgroups: patients with active disease and patients with inactive disease, each is twenty in number. Twenty healthy candidates of the same age and sex contributed as a control group (group I). Thorough history taking, serum samples for CBC, ESR were obtained. A written permission was collected from all candidates beforehand the study. Individuals with celiac disease, familial adenomatous polyposis, and infectious colitis were excluded from the study. The research was executed in agreement with Declaration of Helsinki .The research was approved by the IRB Committee with approval number (ZU-IRB # 10946 /25-7-2023).

Research method

Acquisition of venous specimen

Five ml of venous blood samples were obtained on EDTA for real-time PCR assessment of plasma miR-16 and miR-106a values. MiRNA extraction was conducted utilizing miRNeasy kits from Qiagen, Germany. All phases were executed consistent with the directions of the kit.

Production of cDNA

Then miRNA reverse transcription was carried out by miScript IIRT kit Qiagen .The cDNA were transferred to a freezer.

Augmentation of miRNA expression values

The amplification was performed in a twenty µL mix involving 5µL of the cDNA, one hundred pmol/mL of every primer miRNA-16, miRNA 106a or RNU6, 10 µL 2x Master Mix, and four µL distiled H_2O. The amplification was applied by Real time Cycler as stated by the subsequent process: initial start step 95 ºC for fifteen min, and then 40 rounds of 95 ºC for fifteen second, 55 ºC for 30 second, and lastly 70 ºC for thirty seconds. The amplitude of change of the miRNA value observed in patients in comparison to control was asseseed by the 2-ΔΔCt way.

Results

TABLE 1 Basic data of the studied groups

Variable	Control group (n=20)	ulcerative colitis patients		Crohn's disease patients			
		inactive ulcerative colitis (n=20)	active ulcerative colitis (n=20)	inactive Crohn's Disease (n=20)	active Crohn's Disease (n=20)	*P*-value	*Post hoc*
Age (years)						0.990	P1=0.850
							P2=0.875
Mean±SD							P3=0.729
Range							P4=0.925
	30.2±4.87	29.9±5	30.45±5.37	29.65±4.56	30.05±5.21		P5=0.729
	(25-42)	(24-41)	(25-43)	(23-40)	(24-43)		P6=0.801
Hemoglobin						<0.001*	P1<0.001*
							P2<0.001*
Mean±SD							P3<0.001*
Range							P4<0.001*
	14.25±1.09	10.7±0.41	8.6±0.5	10.73±0.44	8.48±0.47		P5<0.001*
	(12.5-16)	(10-11.5)	(8-9.5)	(10-11.5)	(8-9.5)		P6<0.001*
ESR						<0.001*	P1<0.001*
Mean±SD							P2<0.001*
Range							P3<0.001*
							P4<0.001*
	3.55±1.5	18.4±2.54	39.95±3.39	18.4±2.54	40.5±3.17		P5<0.001*
	(1-6)	(15-24)	(33-45)	(15-24)	(33-45)		P6<0.001*
Variable						P value	
Female	**N**	9	10	9	10	11	0.967 ---------
Sex	**%**	45.0%	50.0%	45.0%	50.0%	55.0%	
Male	**N**	11	10	11	10	9	
	%	55.0%	50.0%	55.0%	50.0%	45.0%	

(f)= ANOVA test
P1=control group vs inactive ulcerative colitis
P2= control group vs active ulcerative colitis
P3= control group vs inactive Crohn's Disease
P4= control group vs active Crohn's Disease
P5= inactive ulcerative colitis vs active ulcerative colitis
P6= inactive Crohn's Disease vs active Crohn's Disease

Table 1 presents that, there was non-significant variance between the groups under the study concerning age and sex. In contrast, there was significant variance between the groups under investigation concerning hemoglobin, where cases with active Crohn's disease group showed the least mean Hb values followed by active ulcerative colitis group, then inactive ulcerative colitis group and inactive Crohn's disease cases.

Likewise, there has been significant difference between the considered groups regarding ESR, where cases with active Crohn's disease group presented the highest mean ESR values followed by active ulcerative colitis group, then inactive Crohn's disease group and inactive ulcerative colitis cases.

TABLE 2 Comparison of endoscopic severity index of the diseased studied groups

Variable	ulcerative colitis patients		Crohn's disease patients		tests		
	inactive ulcerative colitis (n=20)	active ulcerative colitis (n=20)	inactive Crohn's Disease (n=20)	active Crohn's Disease (n=20)	*f*	*P*-value	*Post hoc*
Endoscopic Severity Index					98.419	<0.001*	P1<0.001* P2<0.001*
Mean±SD	0.9±0.31	3.35±1.14	2±0.73	5±0.79			
Range	(0-1)	(2-5)	(1-3)	(4-6)			

(f)= ANOVA test

P1= inactive ulcerative colitis vs active ulcerative colitis

P2= inactive Crohn's Disease vs active Crohn's Disease

Table 2 reveals a significant variance between the studied groups concerning endoscopic Severity Index where cases with active Crohn's disease group showed the higher mean values than inactive Crohn's disease. As well, active ulcerative colitis group showed higher mean levels than inactive ulcerative colitis group.

TABLE 3 Comparison of level of miRNA 16 and the level of miRNA 106-a between control group and ulcerative colitis groups

Variable	Control group (n=20)	ulcerative colitis patients (n=40)	tests	
			t	*P*-value
miRNA 16 Mean±SD	1±0.02	1.65±0.49	-5.878	<0.001*
Range	(0.97-1.03)	(0.97-3.4)		
miRNA 106-a			-7.934	<0.001*
Mean±SD	0.99±0.02	2.2±0.68		
Range	(0.96-1.04)	(0.96-3.4)		

(t)= Independent Samples Test

Table 3 presents a significant variance among the studied groups concerning level of miRNA 16 expression and the level of miRNA 106-a, where cases with ulcerative colitis group showed higher mean levels than control group.

TABLE 4 Comparison of level of miRNA 16 and the level of miRNA 106-a between control group and Crohn's disease groups

Variable	Control group (n=20)	Crohn's disease patients (n=40)	tests	
			t	*P*-value
miRNA 16 Mean±SD	1±0.02	2.45±1.02	-6.327	<0.001*
miRNA 106-a Mean±SD	0.99±0.02	3.37±1.04	-10.222	<0.001*

Table 4 illustrates that, a considerable variance between the studied groups concerning level of miRNA 16 expression and the level of miRNA 106-a, where cases with Crohn's disease group showed higher mean levels than control group.

As shown in, Figure 1, there was significant positive correlation between miRNA 16 level and endoscopic severity index in UC group. Likewise, there was significant positive relation between miRNA 106a level and endoscopic severity index within ulcerative colitis group.

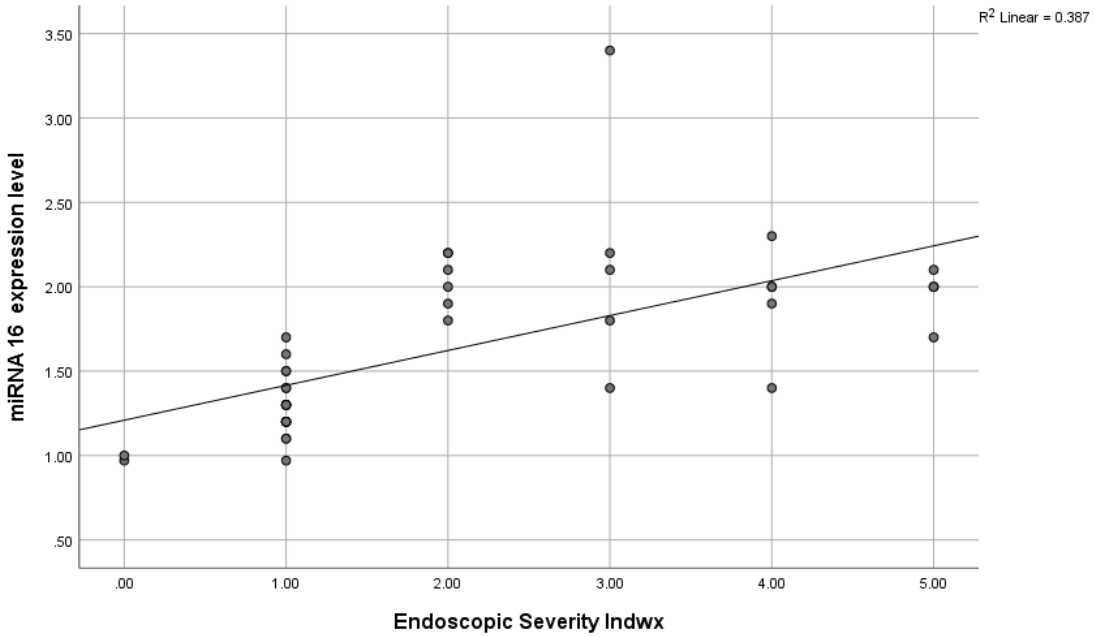

FIGURE 1 Scatter diagram illustrating positive correlation between miRNA 16 and endoscopic severity index in ulcerative colitis

As shown in Figure 2, there was statistically significant positive correlation between miRNA 106a level and endoscopic severity index in CD group. Moreover, there was significant positive correlation between miRNA 16 level and endoscopic severity index within Crohn's disease group.

Table 5 demonstrates a significant difference between the studied ulcerative colitis groups concerning miRNA 16 expression level and the of miRNA 106-a expression level, where cases with active ulcerative colitis showed higher mean levels when compared to inactive ulcerative colitis group .

Table 5 also presents a significant difference between the studied Crohn's disease groups concerning miRNA 16 expression level and the of miRNA 106-a expression level, where cases with active Crohn's disease presented higher mean levels when compared to inactive Crohn's disease group.

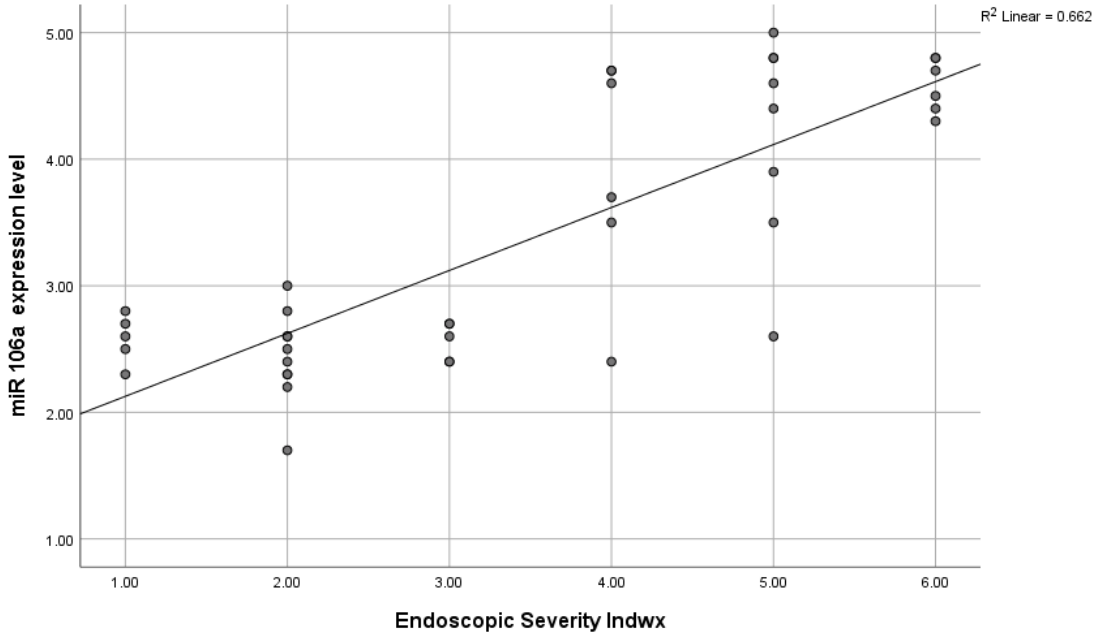

FIGURE 2 Scatter diagram illustrating positive correlation between miRNA 106-a and endoscopic severity index in Crohn's disease

TABLE 5 Comparison of expression levels of miRNA 16 and miRNA 106-a between active and inactive ulcerative colitis cases and between active and inactive Crohn's disease cases

Variable	ulcerative colitis patients		tests		Crohn's disease patients		tests	
	inactive ulcerative colitis (n=20)	active ulcerative colitis (n=20)	*t*	*P* value	inactive Crohn's Disease (n=20)	active Crohn's Disease (n=20)	*t*	*P*-value
miRNA 16 expression level Mean±SD	1.27±0.20	3.35±1.14	-7.462	<0.001*	1.63±0.33	3.27±0.79	-8.530	<0.001*
miRNA 106-a expression level Mean ± SD	1.69±0.39	2.72±0.48	-7.423	<0.001*	2.51±0.28	4.24±0.74	-9.778	<0.001*

(t) Independent Samples Test

TABLE 6 Predictive values of the miRNA 16 to diagnose ulcerative colitis

Variables	AUC	95%CI	Cutoff	Sensitivity	Specificity	PVP	PVN	Accuracy
miRNA 16 expression level	0.943	0.877-1	1.015	92.5%	90%	94.9%	85.7%	91.7%
miRNA 106a expression level	0.942	0.876-1	1.035	92.5%	95%	97.3%	86.4%	93.3%

AUC=Area under curve, PVP=Predictive value for positive, PVN= Predictive value for Negative, and CI= Confidence Interval. Analysis by the ROC curve was performed to test the predictive power of miRNA 16 expression as relevant factors for ulcerative colitis at cut off= 1.015, it achieved an area under curve (AUC) of 0.943 and had a sensitivity of 92.5% and a specificity of 90% as shown in Figure 3 and Table 6.

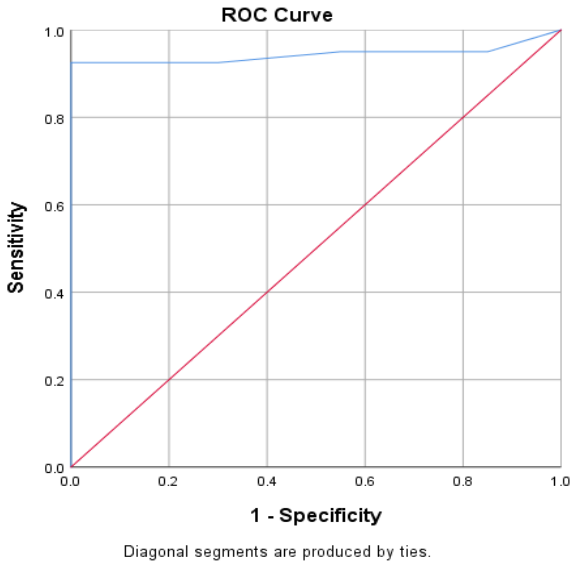

FIGURE 3 ROC curve illustrating predictive values of the miRNA 16 to diagnose ulcerative colitis Regarding the predictive power of miRNA 106a expression level as relevant factors for ulcerative colitis at cut off= 1.035, it achieved (AUC) of 0.942 and had a sensitivity of 92.5% and a specificity of 95% as demonstrated in Table 6 .

TABLE 7 Predictive values of the miRNA 16 to diagnose Crohn's disease patients

Variables	AUC	95%CI	Cutoff	Sensitivity	Specificity	PVP	PVN	Accuracy
miRNA 16 expression level	.934	.862-1	1.015	92.5%	90%	94.9%	85.7%	91.7%
miRNA 106a expression level	1.000	1.0	1.95	97.5%	100%	100%	95.2%	98.3%

Analysis by the ROC curve was performed to test the predictive power of miRNA 16 expression as relevant factors for Crohn's disease at cut off= 1.015, it achieved (AUC) of 0.934 and had a sensitivity of 92.5% and a specificity of 90%, as shown in Table 7. Regarding the predictive power of miRNA 106a expression level as relevant factors for Crohn's disease at cut off= 1.95, it achieved (AUC) of 1.0 and had a sensitivity of 97.5% and a specificity of 100%, as demonstrated in Table 7 and Figure 4.

Regarding the predictive power of miRNA 106a expression level as relevant factors at cut off= 2.05 for differentiation between active and inactive ulcerative colitis, it achieved (AUC) of 0.948 and had a sensitivity of 90% and a specificity of 85%, as depicted in Figure 5.

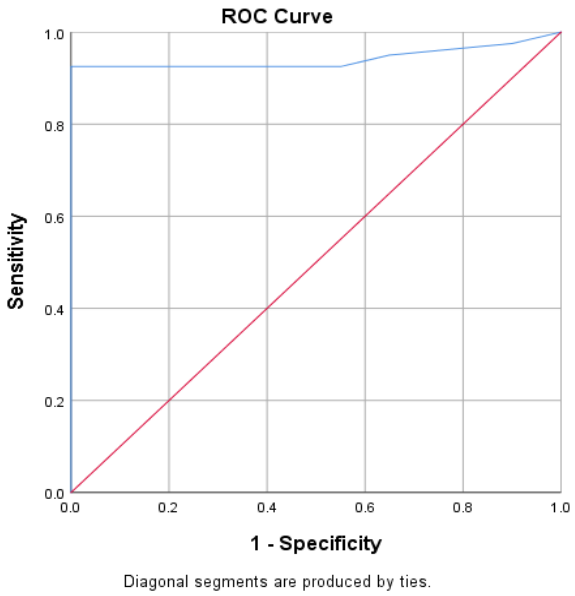

FIGURE 4 ROC curve illustrating predictive values of the miRNA 106-a to diagnose Crohn's disease patients

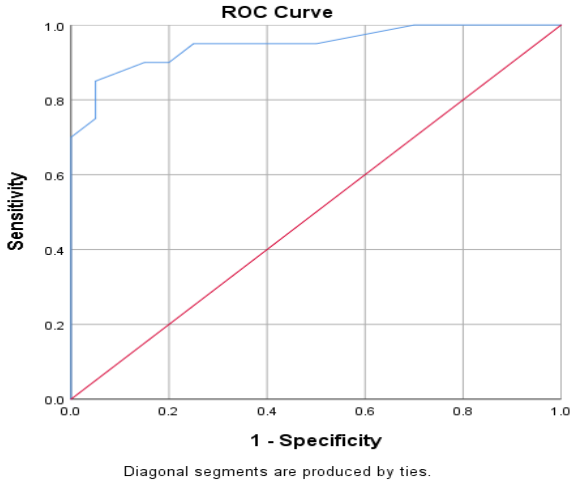

FIGURE 5 ROC curve illustrating predictive values of the miRNA 106-a to differentiate active from inactive ulcerative colitis patients

Analysis by the ROC curve was performed to test the predictive power of miRNA 16 expression as relevant factors for differentiate active from and inactive ulcerative colitis patients as it achieved (AUC) of 0.974 and had a sensitivity of 90% and a specificity of 80%, as demonstrated in Figure 6.

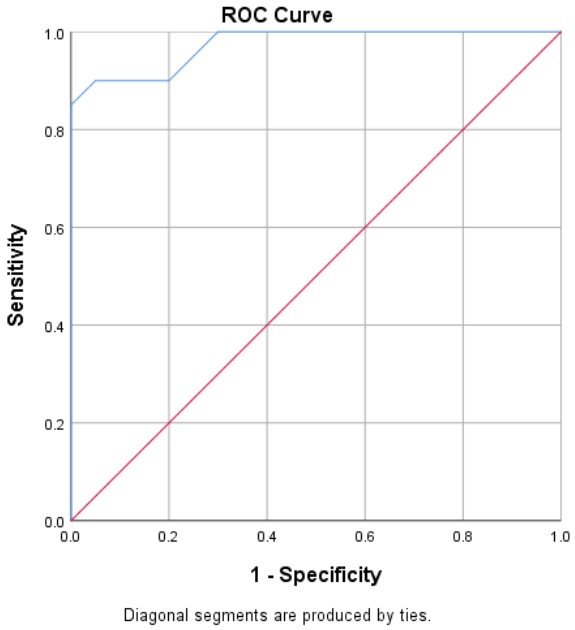

FIGURE 6 ROC curve illustrating predictive values of the miRNA 16 to differentiate active from and inactive ulcerative colitis patients

Regarding the predictive power of miRNA 106a expression level as relevant factors at cut off= 2.9 for differentiation between active and inactive Crohn's disease, it achieved an area under the (AUC) of 0.946 and had a sensitivity of 90% and a specificity of 95%, as illustrated in Figure 7.

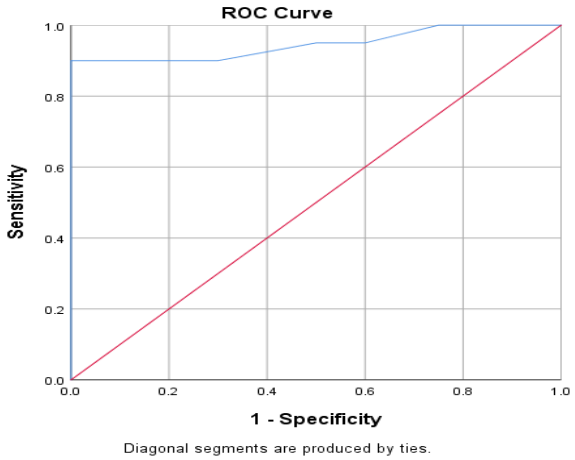

FIGURE 7 ROC curve illustrating predictive values of the miRNA 106-a to differentiate active from inactive Crohn's disease patients

Regarding the predictive power of miRNA 16 expression level as relevant factors at cut off= 1.85 for differentiation between active and inactive Crohn's disease, it achieved (AUC) of 0.949 and had a sensitivity of 90% and a specificity of 75%, as displayed in Figure 8.

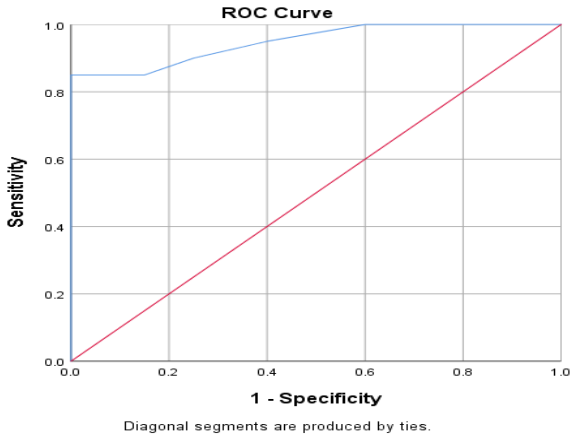

FIGURE 8 ROC curve illustrating predictive values of the miRNA 16 to differentiate active from inactive Crohn's disease patients

Discussion

Inflammatory bowel disease (IBD) is mostly related to Crohn's disease (CD) and ulcerative colitis (UC). In 15% of cases, conflicting endoscopy or histological data make it difficult to discriminate between the two conditions. Combination of clinical symptoms, endoscopic assessment, and histological investigation help in IBD diagnosis and treatment. Nevertheless, there is an increasing need for non-invasive biomarkers that might aid in prognosis, early identification of IBD, and monitoring of disease activity [15].

Small non-coding RNA molecules called microRNAs have a major role in post-transcriptional gene control. They possess an impact on several biological processes, such as immune system response and inflammation. Several miRNAs have been approved to be dysregulated in the setting of IBD in recent study, which suggests that these miRNAs may aid as possible indicators for the diagnosis and monitoring of the disease [16].

The ability of two dysregulated miRNAs linked to IBD to regulate inflammatory processes and their detection in blood have drawn attention to them. miRNA-16 and miRNA-106a are the miRNAs in question. Since their expression is different in blood samples from IBD patients than from healthy control, these miRNAs can be used as non-invasive biomarkers for IBD [17].

The aim of this study was to assess the role of microRNA-16 and microRNA- 106a as indicators in IBD diagnosis and assessing its activity.

The current study demonstrated that concerning the expression of miRNA 16 and miRNA 106-a, there was a significant variance between the groups under investigation; Crohn's disease and ulcerative colitis groups had greater mean levels than the control group. In agreement with our findings, Wu *et al.* [18] found that patients with either UC or CD have greater expression levels of miR-16 compared to healthy volunteers. Similar findings were obtained by Paraskevi *et al.* [19] who also stated that on comparing CD patients with UC patients, the expression of miR-16 is markedly greater in CD patients. Schönauen *et al*. [20] discovered that IBD patients had greatly higher blood and fecal levels of miR-16 than healthy control, and that the expression levels directly related with disease activity. Atanassova *et al.* [21] also showed that in cases with CD, there was a correlation between the disease activity and augmented serum miR-16 expression. In contrast, miR-16 expression declines to values observed in healthy controls in UC patients with severe activity. Tian *et al. [*22] revealed that when UC patients were compared to IBD patients and healthy group, there was upregulation in miR-16 expression. This displays that miR-16

inhibits the production of adenosine A2a receptor protein (A2aAR) at a post-transcriptomic level and signifies that the changed expression of miR-16 may be linked with IBD. These findings suggest that miR-16 and A2aAR might probably be useful therapeutic targets for IBD patients in terms of controlling inflammation and adjusting therapy.

In addition, Habib *et al.* [23] investigated the levels of miRNA-106 expression in the blood of several IBD patients in relation to the levels in a healthy group and the variable expression levels in the variable activity stage groups. IBD (UC and CD) patients have considerably greater levels of miRNA-106 expression than the control group; these levels increase with the activity of the illness. Furthermore, a marked upregulation in serum miRNA 106 was seen in CD rather than UC. Fasseu *et al.* [24] indicated that miR106 levels were considerably greater in IBD patients than in control groups, according to research on blood and sigmoid colon samples from these cases.

The reason for the higher microRNA-16 and microRNA- 106a levels in IBD patients is not fully understood, but it is thought to be related to both ulcerative colitis and Crohn's disease are characterized by a persistent inflammatory response in the GIT. It is possible that changes in miRNA expression result from the dysregulated immune reaction under these conditions. The inflammatory response may be adjusted by miRNA-16 and miRNA-106a, and the overexpression of these genes may be a result of the inflammation in affected persons. In IBD, the gastrointestinal mucosa is inflammed and then goes through a healing process. It is probable that miRNA-106a and miRNA-16 regulate cell division, death, and tissue remodeling. The increased expression levels of these miRNAs in the patient groups may signify a greater demand for tissue regeneration and repair in ulcerative colitis and Crohn's disease. Both CD and UC are influenced by complex genetics. Certain genetic variations may add to the dysregulation of miRNA expression in a number of diseases. Genetic variation related to miRNA processing, stability, or target binding can affect the expression levels of miRNA-16 and miRNA-106a. The onset and development of inflammatory bowel disease (IBD) have been linked to environmental variables and gut flora [10]. These variables could have an impact on the gut's miRNA-106a and miRNA-16 expression. In Crohn's disease and ulcerative colitis, dysbiosis (an unbalanced gut microbiota) and exposure to specific environmental triggers may be factors in the variant expression of these miRNAs [24].

The present study showed that regarding the levels of miRNA 16 and miRNA 106-a, there was a significant variance between the ulcerative colitis groups under study. Mean levels of these expressions were greater in conditions of active ulcerative colitis than in the group with inactive ulcerative colitis. These results were in accordance with Habib *et al*. [23] who revealed that increased UC activity was correlated with a significant rise in serum miRNA 106 expression levels. When comparing the mean levels of miRNA 106-a and miRNA 16 expression between the ulcerative colitis groups under study, there was a statistically significant difference. The patients with active ulcerative colitis showed greater mean levels than the inactive ulcerative colitis group. Zahm *et al*. [25] stated peripheral blood miRNAs levels have been shown to discriminate between active IBD cases and healthy controls. Duttagupta *et al.* [26] demonstrated that patients with active UC had greater blood levels of miR-106a expression.

Current findings clearly showed that concerning the expression levels of miRNA 106-a and miRNA 16, there was a significant variance between the Crohn's disease groups under study. Specifically, patients with active Crohn's disease exhibited higher mean levels than the inactive Crohn's disease group. This

was in accordance with Habib *et al.* [23] who showed that increased CD activity was correlated with a significant rise in serum miRNA 106 expression levels. Regarding the levels of miRNA 106-a and miRNA 16 in the investigated Crohn's disease groups, there was a significant variation; patients with active Crohn's disease exhibited greater mean levels than the inactive Crohn's disease group. Paraskevi *et al.* [19] showed that as compared to healthy controls, active CD patients' peripheral blood had considerably higher levels of miR-106a. Zahm *et al*. [25] mentioned when compared to healthy controls, the peripheral blood of pediatric CD patients who are actively ill has shown a considerable increase in miR-106a expression. Duttagupta *et al.* [26] demonstrated that those with active Crohn's disease had higher blood levels of miR-106a expression. Wang *et al.* [27] showed higher levels of peripheral blood miRNA in patients with Crohn's disease and ulcerative colitis, and these levels are more connected with activity variables than ESR and CRP, which is in agreement with our findings. Omidbakhsh *et al.* [28] discovered that miR106a and miR362-3p help to differentiate between individuals with active CD and those with inactive disease, as well as between control participants. The highest increase in expression of miR-106a was seen in patients with active CD. Thus, these miRNAs might be employed to distinguish between these two illnesses. Unlikely, Taganov *et al.* [29] showed that the patients with active CD, Crohn's ileitis, and Crohn's colitis subgroups did not differ in their miRNA expression. This indicates that individuals with Crohn's colitis, ileitis, and active UC do not have common expression of miRNAs between tissue biopsies and blood samples.

This finding may be clarified by the fact that active Crohn's disease is characterized by persistent inflammation in the digestive system, whereas inactive Crohn's disease indicates a period of reduced or quiet inflammation in the gastrointestinal tract. MiRNA-106a and miRNA-16 are known to control the immune system and inflammation. These miRNAs' elevated expression levels in active Crohn's disease propose that they may play a role in starting and sustaining the inflammatory cascade.

Concerning the expression level of miRNA 106a was determined to have predictive value as important determinants for ulcerative colitis. At cutoff = 1.035, it obtained (AUC) of 0.942, predictive values for positive (PVP) = 97.3% and negative (PVN) = 86.4%, accuracy of 93.3 percent, and sensitivity and specificity of 92.5 percent and 92.5 percent, respectively. Regarding the predictive power of miRNA 106a expression level as relevant factors for Crohn's disease at cut off=1.005, the results showed (AUC) of 0.987, sensitivity of 92.5%, specificity of 85%. Habib *et al.* [23] illustrated that the prediction capacity of miRNA 106a expression level as significant determinants for UC and CD was shown to be 73.2% accurate, with (AUC) of 0.70, sensitivity of 72.8%, specificity of 68.7%, and accuracy of 73.2%.

Conclusion

Since the augmented expression of microRNA-106a and microRNA-16 has been related to augmented disease activity in the peripheral blood of IBD cases, these biomarkers may be significant in both diagnosis IBD and definition of the degree of disease activity. MiRNA-106a and miRNA-16 show altered expression in IBD, and their detection in the bloodstream makes them promising biomarkers for the diagnosis and prognosis of IBD. To confirm their therapeutic significance and provide uniform evaluation techniques, more research is required. By approving early diagnosis and adapted treatment strategies, the discovery of non-invasive biomarkers like miRNA-16 and miRNA-106a has the potential to improve the care and outcomes of IBD patients.

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Authors' Contributions

Heba Fawzy shared as corresponding author , typing, editing, statistical analysis and methodology. Sherif Swilam , Mahmoud Amer and Abd El-Fattah Mohammed aided in typing, selecting subjects and clinical methodology. Huda E.M. Said and Doaa M.Hendawy shared in lab investigations and typing.

Conflict of Interest

No relevant conflicts of interest.

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References

[1] M.Z. Cader, A. Kaser, Recent advances in inflammatory bowel disease: mucosal immune cells in intestinal inflammation, *Gut*, **2013**, *62*, 1653-1664. [Crossref], [Google Scholar], [Publisher]

[2] A.N. Ananthakrishnan, Epidemiology and risk factors for IBD, *Nature Reviews Gastroenterology & Hepatology*, **2015**, *12*, 205-217. [Crossref], [Google Scholar], [Publisher]

[3] A. Geremia, P. Biancheri, P. Allan, G.R.Corazza, A. Di Sabatino, Innate and adaptive immunity in inflammatory bowel disease, *Autoimmunity Reviews*, **2014**, *13*, 3-10. [Crossref], [Google Scholar], [Publisher]

[4] V. Annese, M. Daperno, M.D. Rutter, A. Amiot, P. Bossuyt, J. East, M. Ferrante, M. Götz, K.H. Katsanos, R. Kießlich, I. Ordás, European evidence based consensus for endoscopy in inflammatory bowel disease, *Journal of Crohn's and Colitis*, **2013**, *7*, 982-1018. [Crossref], [Google Scholar], [Publisher]

[5] G.A. Calin, M. Ferracin, A. Cimmino, G. Di Leva, M. Shimizu, S.E. Wojcik, M.V. Iorio, R. Visone, N.I. Sever, M. Fabbri, R. Iuliano, A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia, *New England Journal of Medicine*, **2005**, *353*, 1793-1801. [Crossref], [Google Scholar], [Publisher]

[6] W.J. Tremaine, Is indeterminate colitis determinable?, *Current Gastroenterology Reports*, **2012**, *14*, 162-165. [Crossref], [Google Scholar], [Publisher]

[7] J.D. Lewis, The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease, *Gastroenterology*, **2011**, *140*, 1817-1826. [Crossref], [Google Scholar], [Publisher]

[8] Y.M. Shastri, D. Bergis, N. Povse, V. Schäfer, S. Shastri, M. Weindel, H. Ackermann, J. Stein, Prospective multicenter study evaluating fecal calprotectin in adult acute bacterial diarrhea, *The American Journal of Medicine*, **2008**, *121*, 1099-1106. [Crossref], [Google Scholar], [Publisher]

[9] A.A. Soubières, A. Poullis, Emerging role of novel biomarkers in the diagnosis of inflammatory bowel disease, *World Journal of Gastrointestinal Pharmacology and Therapeutics*, **2016**, *7*, 41. [Crossref], [Google Scholar], [Publisher]

[10] J.A. Weber, D.H. Baxter, S. Zhang, D.Y. Huang, K. How Huang, M. Jen Lee, D.J. Galas, K. Wang, The microRNA spectrum in 12 body fluids, *Clinical chemistry*, **2010**, *56*, 1733-1741. [Crossref], [Google Scholar], [Publisher]

[11] D.G. Johnston, M.A. Williams, C.A. Thaiss, R. Cabrera-Rubio, M. Raverdeau, C. McEntee, P.D. Cotter, E. Elinav, L.A. O'Neill, S.C. Corr, Loss of microRNA-21 influences the gut microbiota, causing reduced susceptibility in a murine model of colitis, *Journal of Crohn's and Colitis*, **2018**, *12*, 835-848. [Crossref], [Google Scholar], [Publisher]

[12] S.R. Whiteoak, R. Felwick, T. Sanchez-Elsner, J.R. Fraser Cummings, MicroRNAs in inflammatory bowel diseases: paradoxes and possibilities, *Inflammatory Bowel Diseases*, **2015**, *21*, 1160-1165. [Crossref], [Google Scholar], [Publisher]

[13] R. Rameshshanker, N. Arebi, Endoscopy in inflammatory bowel disease when and why, *World Journal of Gastrointestinal Endoscopy*, **2012** *4*, 201. [Crossref], [Google Scholar], [Publisher]

[14] S.P. Travis, D. Schnell, P. Krzeski, M.T. Abreu, D.G. Altman, J.F. Colombel, B.G. Feagan, S.B. Hanauer, M. Lémann, G.R. Lichtenstein, P.R. Marteau, Developing an instrument to assess the endoscopic severity of ulcerative colitis: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS), *Gut*, **2012**, *61*, 535-542. [Crossref], [Google Scholar], [Publisher]

[15] L. Masi, I. Capobianco, C. Magrì, I. Marafini, V. Petito, F. Scaldaferri, MicroRNAs as innovative biomarkers for inflammatory bowel disease and prediction of colorectal cancer, *International Journal of Molecular Sciences*, **2022**, *23*, 7991. [Crossref], [Google Scholar], [Publisher]

[16] L. Sun, Y. Han, H. Wang, H. Liu, S. Liu, H. Yang, X. Ren, Y. Fang, MicroRNAs as potential biomarkers for the diagnosis of inflammatory bowel disease: a systematic review and meta-analysis, *Journal of International Medical Research*, **2022**, *50*, 3000605221089503. [Crossref], [Google Scholar], [Publisher]

[17] S.S. Krishnachaitanya, M. Liu, K. Fujise, Q. Li, MicroRNAs in inflammatory bowel disease and its complications, *International Journal of Molecular Sciences*, **2022**, *23*, 8751. [Crossref], [Google Scholar], [Publisher]

[18] F. Wu, M. Zikusoka, A. Trindade, T. Dassopoulos, M.L. Harris, T.M. Bayless, S.R. Brant, S. Chakravarti, J.H. Kwon, MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2α, *Gastroenterology*, **2008**, *135*, 1624-1635. [Crossref], [Google Scholar], [Publisher]

[19] A. Paraskevi, G. Theodoropoulos, I. Papaconstantinou, G. Mantzaris, N. Nikiteas, M. Gazouli, Circulating MicroRNA in inflammatory bowel disease, *Journal of Crohn's and Colitis*, **2012**, *6*, 900-904. [Crossref], [Google Scholar], [Publisher]

[20] K. Schönauen, N. Le, U. von Arnim, C. Schulz, P. Malfertheiner, A. Link, Circulating and fecal microRNAs as biomarkers for inflammatory bowel diseases, *Inflammatory Bowel Diseases*, **2018**, *24*, 1547-1557. [Crossref], [Google Scholar], [Publisher]

[21] A. Atanassova, A. Georgieva, Circulating miRNA-16 in inflammatory bowel disease and some clinical correlations–a cohort study in Bulgarian patients, *European Review for Medical & Pharmacological Sciences*, **2022**, *26*. [Crossref], [Google Scholar], [Publisher]

[22] T. Tian, Y. Zhou, X. Feng, S. Ye, H. Wang, W. Wu, W. Tan, C. Yu, J. Hu, R. Zheng, Z. Chen, MicroRNA-16 is putatively involved in the NF-κB pathway regulation in ulcerative colitis through adenosine A2a receptor (A2aAR) mRNA targeting, *Scientific Reports*, **2016**, *6*, 30824. [Crossref], [Google Scholar], [Publisher]

[23] A. Habib, A. Minisi, M. Awad, A. Essa, A. Khalifa, S. Shehab-Eldeen, Serum microRNA 106 and microRNA 223 as novel biomarkers in inflammatory bowel disease, *Medical Journal of Viral Hepatitis*, **2020**, *5*, 19-24. [Crossref], [Google Scholar], [Publisher]

[24] M. Fasseu, X. Tréton, C. Guichard, E. Pedruzzi, D. Cazals-Hatem, C. Richard, T. Aparicio, F. Daniel, J.C. Soulé, R. Moreau, Y. Bouhnik, Identification of restricted subsets of mature microRNA abnormally expressed in inactive colonic mucosa of patients with inflammatory bowel disease, *PloS One*, **2010**,

5, 13160. [Crossref], [Google Scholar], [Publisher]

[25] A.M. Zahm, M. Thayu, N.J. Hand, A. Horner, M.B. Leonard, J.R. Friedman, Circulating microRNA is a biomarker of pediatric Crohn disease, *Journal of Pediatric Gastroenterology and Nutrition*, **2011**, *53*, 26-33. [Crossref], [Google Scholar], [Publisher]

[26] R. Duttagupta, S. DiRienzo, R. Jiang, J. Bowers, J. Gollub, J. Kao, K. Kearney, D. Rudolph, N.B. Dawany, M.K. Showe, T. Stamato, Genome-wide maps of circulating miRNA biomarkers for ulcerative colitis, *PloS One*, 2012, *7*, 31241. [Crossref], [Google Scholar], [Publisher]

[27] H. Wang, S. Zhang, Q. Yu, G. Yang, J. Guo, M. Li, Z. Zeng, Y. He, B. Chen, M. Chen, Circulating microRNA223 is a new biomarker for inflammatory bowel disease, *Medicine*, **2016**, *95*, 2703. [Crossref], [Google Scholar], [Publisher]

[28] A. Omidbakhsh, M. Saeedi, M. Khoshnia, A. Marjani, S. Hakimi, Micro-RNAs-106a and-362-3p in peripheral blood of inflammatory bowel disease patients, *The Open Biochemistry Journal*, **2018**, *12*, 78. [Crossref], [Google Scholar], [Publisher]

[29] K.D. Taganov, M.P. Boldin, K.J. Chang, D. Baltimore, NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses, *Proceedings of the National Academy of Sciences*, **2006**, *103*, 12481-12486. [Crossref], [Google Scholar], [Publisher]

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