Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Role of circulating microRNA146b-5p and microRNA-106a in diagnosing and predicting the severity of inflammatory bowel disease

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Abstract

MicroRNAs have been under the spotlight as a potential noninvasive convenient diagnostic and monitoring tool as well as aiding diagnosis whenever the endoscopic diagnosis is debatable. The aim of this study is to evaluate the possibility of using miRNA 106a and miRNA 146b-5p in diagnosing and assessing the severity of Inflammatory bowel disease. This case–control study recruited 35 ulcerative colitis (UC), 34 Crohn's disease (CD) and 30 healthy controls. Careful clinical assessment, routine laboratory workup and colonoscopy were conducted to confirm the diagnosis and assess disease severity. A blood sample was collected for the quantification of miRNA 106a and miRNA 146b-5p. There was significantly increased expression of both serum markers in the UC group ($p = 0.001$), a trend increase in miRNA 106a and a decrease in miRNA 146b-5p expression with activity. A significantly increased expression of miRNA 146b-5p ($p = 0.001$), but significantly decreased expression of miRNA 106a ($p = 0.001$) was found in the CD group with a trend decrease in serum miRNA 106a and an increase in miRNA 146b-5p expression with activity. A significant different expression of both biomarkers was found between UC and CD ($p = 0.001$ and 0.007 for miRNA 106a and miRNA 146b-5p respectively). MiRNA 106a and miRNA 146b-5p may be promising serum biomarkers for the diagnosis of patients with IBD and differentiation between UC and CD.
Keywords: microRNA; miRNA 106a; miRNA 146b-5p; ulcerative colitis; Crohn's disease; Inflammatory bowel disease.

1. Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a chronic debilitating disorder responsible for the inflammation of the gastrointestinal tract. It is accompanied by major morbidity and mortality that result in a significant burden to the patient and finances of the health care system [1].

Thus far, the pathogenesis of IBD is not fully known; however, evidence suggests that immune response dysregulation [2], environmental factors [3], gut microbiota [4], and genetic susceptibility [5] are incriminated in the disease pathogenesis.

The disease course usually oscillates between relapse and remission. This necessitates effective regular monitoring and evaluation to help in the early detection of relapse and long-term complications, e.g., development of strictures, fistula, or colorectal carcinoma [6].

To date, endoscopy plays an essential role in the diagnosis and evaluation of mucosal lesions in patients with IBD. However, it is very inconvenient and costly to most patients with IBD, who may require repeated endoscopies to evaluate the disease activity, complications and, sometimes, revise the diagnosis, especially if their symptoms are mild. IBD is diagnosed by integrating clinical evaluation with laboratory testing, imaging studies, endoscopy, and histopathology [7,8].

So far, no ideal biomarkers could be used to diagnose and monitor IBD. Despite the wide use of C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) in clinical practice, they are still nonspecific, as their levels are elevated in other inflammatory disorders or remain within normal in patients with IBD despite their activity [9]. The same can be said about fecal calprotectin, which can be

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Receive Date: 14 March 2023, Revise Date: 26 March 2023, Accept Date: 28 March 2023, First Publish Date: 28 March 2023
DOI: https://doi.org/10.21608/ejchem.2023.199933.7727

found in some other intestinal inflammatory diseases such as celiac disease and infectious enteritis [10].

Therefore, there is an urge to develop a new reliable biomarker for convenient and better management of patients with IBD. After the publication of the results of IBD genome studies, there has been an increasing interest to analyze the relationship between IBD and microRNAs, which is one of the most important gene expression regulators [11].

Serum miR-146b-5p is a reliable biomarker for follow-up of IBD [12]. It regulates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) or mammalian target of rapamycin signaling to either stimulate or inhibit autophagy in intestinal cells by the release of anti- or proinflammatory factors, respectively [13]**.**

MiR-106a is crucial for proinflammatory signaling to transiently impair immunosuppressive signaling and might have an important physiological role in stimulating regulatory T cells by transforming growth factor-β [14]**.** It is overexpressed in IBD, and it not only serves as a biological marker for diagnosis and follow-up [11] but also targets miR-106a, which may have a potential therapeutic purpose [14]**.**

The aim of this study is to evaluate the possibility of using miRNA 106a and miRNA 146b-5p in diagnosing and assessing the severity of Inflammatory bowel disease.

2. Subjects and methods

This case–control study included 69 adult patients with a confirmed diagnosis of IBD (UC, n = 35; CD, n = 34) after reviewing their clinical, endoscopic, radiologic, and histopathological data in addition to 30 healthy controls.

The patients were recruited during their follow-up at Kasr Al Ainy Hospital, Cairo University during the period from July 2019 to February 2021. This study was approved by the research ethics committee of Faculty of medicine, Cairo university, Code MD-61-2019. It was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. An informed consent was obtained from all participants.

All patients were subjected to laboratory investigations: complete blood count, CRP by enzyme-linked immunosorbent assay (ELISA), ESR, and serum albumin. Colonoscopy including terminal ileoscopy and biopsy for histopathology was conducted. Imaging studies data including ultrasonography, computed tomography enterography, magnetic resonance enterography, and fistulography were recorded. Data collected were used to confirm the diagnosis and assess the level of activity (Mayo score for UC and Crohn's disease activity index [CDAI] for CD) [15], intestinal and extraintestinal complications.

The healthy control group had normal colonoscopy, colonic biopsy, and routine CBC, ESR, and CRP.

A blood sample was collected from each patient and healthy control participant and then subjected to centrifugation, and the serum was stored in an Eppendorf tube in −80°C freezer until all patients and control samples were collected. RNA was extracted from serum using miRNeasy Mini Kit (Qiagen, Valencia, CA, USA, Catalogue number 217004) following the manufacturer's instructions. NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA) was used to assess RNA quantitation and purity. Reverse transcription was carried out on extracted RNA in a final volume of 20 uL RT reactions using the miScript II RT kit (Qiagen, Valencia, CA, USA Catalogue number 218161) following the manufacturer's instructions. Using the MiScript SYBR Green PCR kit (Qiagen, Valencia, CA, USA Cat. No. 218073) and miScript primer assay for miRNA 106a and 146b-5p, a 25µl per well reaction volume mix was prepared according to manufacturer's instructions. SNORD68 was used as an endogenous control. The expression level of miR-146b, miR-106a were calculated using the ΔCt method (by subtracting the Ct values of SNORD 68 from those of target micro-RNAs) then ΔΔCt was calculated by subtracting the ΔCt of the control samples from the ΔCt of the IBD patient samples. The fold change of miR-146b-5p and miR-106a expression was calculated using the equation $2^{-\Delta\Delta Ct}$ [11].

Descriptive statistics are presented as mean ± standard deviation (SD) and median (min–max) for quantitative data and number (%) for qualitative data.

Analytic statistics was done by using both Fisher's exact test and Chi-square test, non-parametric Mann-Whitney test, non-parametric Kruskal-Wallis test for k Independent-samples, Pearson Correlation test between biomarkers and other study variables among the studied patients. Logistic regression analysis of biomarkers as predictors for Ulcerative colitis and Crohn's disease activity. Diagnostic performance of biomarkers in discrimination of patients with Ulcerative colitis and Crohn's disease was done using ROC curve with area under curve with statistical significance and calculating sensitivity and specificity for the best cut-off value.

Statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA).

Figures were used according to the studied variables to present the mean (standard deviation) of biomarkers using Excel 2019.

3. Results

The demographic and IBD characteristics of the participants are shown in Table 1.

Table 1: Demographic and IBD characteristics of the studied patients (n = 69).

IBD characteristics		Ulcerative colitis (n = 35)	Crohn's disease (n = 34)
Age (*Mean ± SD)*		32 ± 11	33 ± 11
Male N (%)		21 (60)	21 (61.8)
Duration of illness *(years)*	*Median (min–max)*	1 (0–20)	2 (0.1–20)
Presenting symptoms *N (%)*	*Nonbloody diarrhea*	10 (28.6)	21 (61.8)
	Bloody diarrhea	21 (60)	0 (0)
	Constipation	0 (0)	4 (11.8)
	Abdominal pain	1 (2.9)	15 (44.1)
	Perianal fistula and abscess	0 (0)	3 (8.8)
	Weight loss	20 (57.1)	19 (55.9)
	Fever	4 (11.4)	9 (26.5)
Extraintestinal manifestations *N (%)*		14 (40)	25 (73.5)
Therapeutic characteristics			
A) Medications N *(%)*		27 (77.1)	21 (61.8)
5Aminosalicylates5-aminosalicylates N *(%)*		22 (62.9)	12 (35.3)
Steroids *N (%)*		19 (54.3)	14 (41.2)
Immunomodulators *N (%)*		9 (25.7)	9 (26.5)
Biological *N (%)*		6 (17.1)	9 (26.5)
B) Surgical interventions N (%)		0 (0)	7 (20.6)
Disease activity			
Remission		2 (5.7)	7 (20.6)
Mild		11 (31.4)	4 (11.8)
Moderate		10 (28.6)	19 (55.9)
Severe		12 (34.3)	4 (11.8)
Extent of disease by colonoscopy			
Ulcerative colitis N (%)	Proctitis	3 (8.6)	–
	Left side	15 (42.9)	–
	Pancolitis	17 (48.6)	–
Crohn's disease N (%)	Ileal	–	12 (35.3)
	Colonic	–	11 (32.4)
	Ileocolonic	–	11 (32.4)

The majority of patients with CD (70.6%) had a history of intestinal complications, such as fistulas, strictures, intestinal obstruction, and perianal abscess, as shown in Table 2.

Table 2: Intestinal complications of patients with Crohn's disease (n = 34).

Crohn's disease	Frequency	Percentage of patients with CD
History of intestinal complications	24	70.6
Type of intestinal complications		
Strictures	7	20.6
Intestinal perforation	4	11.8
Intestinal obstruction	7	20.6
Perianal fistula	3	8.8
Other fistulas	9	26.5
Colocutaneous	5	14.7
Enteroenteric	3	8.8
Enterovesical	3	8.8
Rectovaginal	1	2.9
Enterocolonic	2	5.9

The fold change of both biomarkers of miR-106a and miR146b5p showed a significant difference among the three groups (P > 0.05), as shown in Table 3.

Using the receiver operating characteristic (ROC) curve, miR106a and miR146b5p can be used to discriminate between patients with UC and healthy individuals, as shown in Table 4.

Using roc curve, it was shown that miR106a and miR146b5p can be used to discriminate between patients with UC & normal, as shown in figure 1

Regarding miR106a, the cutoff level was ≥7.16, with area under the curve (AUC) of 0.943 indicating strong test, 40% sensitivity, and 100% specificity (P < 0.05). Regarding miR146b5p, the cutoff level was ≥5, with an AUC of 0.876 indicating good test, 48.5% sensitivity and 100% specificity (P < 0.05).

In the ROC curve, miR106a and miR146b5p can be used to discriminate between patients with CD and healthy individuals, as shown in Table 5

Using roc curve, it was shown that miR106a and miR146b5p can be used to discriminate between patients with CD & normal, as shown in figures 2 and 3

Table 3: Biomarkers of the study participants (n = 99).

Biomarkers		Ulcerative colitis (n = 35)	Crohn's disease (n = 34)	Control (n = 30)	*p-value*
miR-106a fold change	*Mean ± SD*	7.16 ± 4	0.75 ± 2	1 ± 0.08	*P1 0.001* - P2 0.001* P3 0.001**
miR146b-5p fold change	*Mean ± SD*	5 ± 3	3.1 ± 2.3	1 ± 0.08	*P1 0.001* - P2 0.001* P3 0.001**

**p*-value is significant. - P1 (UC vs Control), P2 (CD vs Control), P3 (UC vs CD)

Table 4: Diagnostic performance of biomarkers in discriminating the patients with UC.

UC	Area	Std. Error	*p*-value	95% Confidence interval		Cutoff	Sensitivity	Specificity
				Lower bound	Upper bound			
miR106a	0.943	0.039	0.001*	0.866	1	≥7.16	40%	100%
miR146b5p	0.876	0.054	0.001*	0.77	0.982	≥5	48.5%	100%

**p*-value is significant

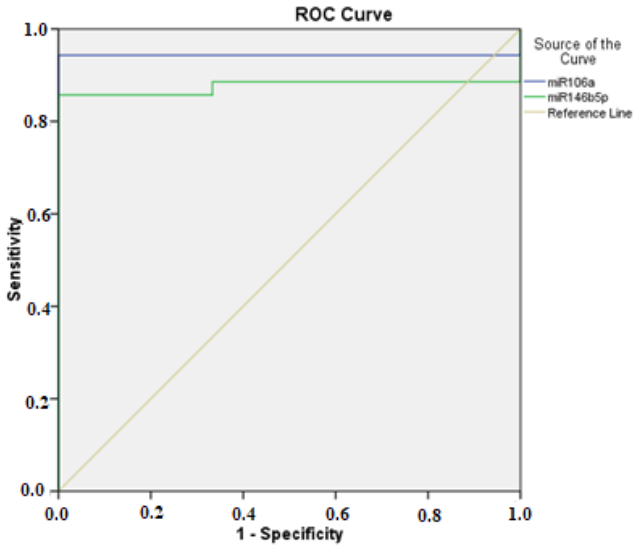

Fig. 1. ROC curve for both biomarkers to diagnose ulcerative colitis.

Table 5: Diagnostic performance of biomarkers in discriminating the patients with CD

Crohn's	Area	Std. error	*p-value*	95% Confidence interval		Cutoff	Sensitivity	Specificity
				Lower bound	Upper bound			
miR106a	0.853	0.061	0.001*	0.734	0.972	≤0.75	85.2%	100%
miR146b5p	0.789	0.067	0.001*	0.659	0.92	≥3.1	41.1%	100%

**p-value* is significant.

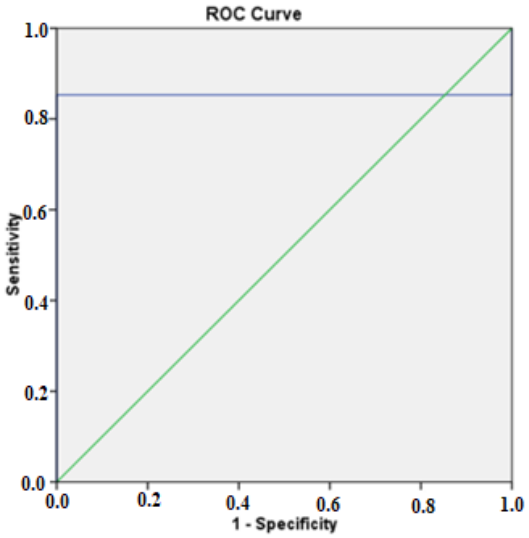

Fig. 2. ROC curve for miR106a to diagnose Crohn's disease.

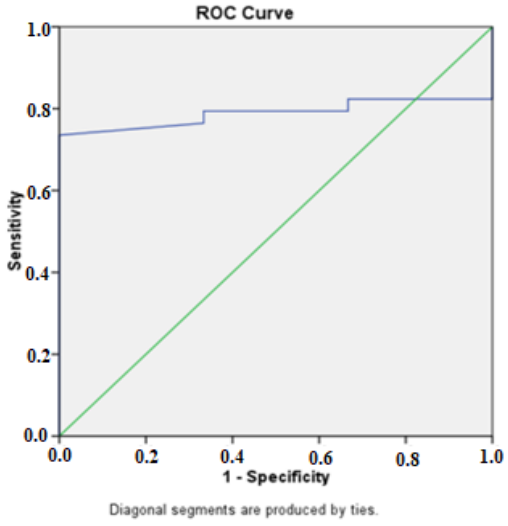

Fig. 3. ROC curve for miR146b5p to diagnose Crohn's disease

The cutoff level of miR106a was ≤0.75, with an AUC of 0.853 indicating good test, 85.2% sensitivity, and 100% specificity ($P < 0.05$). The cutoff level of miR146b5p was ≥3.1, with an AUC of 0.789 indicating good test, 41.1% sensitivity, and 100% specificity ($P < 0.05$).

The miRNA analyzed could not differentiate patients in remission or activity, as shown in Table 6.

4. Discussion

The diagnosis of IBD, i.e., CD or UC, can be very challenging given the wide-ranging types and intensities of presenting symptoms, which are nonspecific and can be associated with several non-IBDs.

The differential expression of miRNA can help differentiate UC from CD, especially if the endoscopic results were inconclusive. The differences in the serum expression of miRNA in the peripheral blood of patients with UC and CD in an active phase in comparison to inactive disease suggest that they may be of value in monitoring the disease activity [16].

miR-146b improves intestinal inflammation through the upregulation of NF-κB as a result of the reduced expression of the siah2 gene, which ubiquitinates tumor necrosis factor (TNF) receptor-associated factor proteins. Therefore, miR-146b expression modulation can be a potentially helpful therapy for intestinal inflammation via the activation of the NF-κB pathway, which in turn inhibits autophagy, improves intestinal epithelial function, reduces intestinal inflammation in dextran sulfate sodium-induced colitis mice, and increases the survival rate of fatal colitis [17].

miR-146 is very crucial in the activation of the NF-ƙB pathway through the NOD2-sonic hedgehog signaling. This pathway is essential for the maintenance of gut homeostasis and development [18,19].

miRNA-106a is one of the first reported miRNAs to be differentially expressed in patients with IBD [20]. miRNA106a could distinguish between UC and CD in biopsied tissues, and its expression is elevated in Crohn's colitis but not in ulcerative colitis [11]. miR-106a regulates the synthesis of macrophage signal-regulatory protein α (SIRPα) and SIRPα-mediated macrophage inflammatory responses redundantly. SIRPα regulates several elements of the leukocyte inflammatory responses, which includes activation, chemotaxis, and phagocytosis [21].

Sanctuary et al. recognized miR-106a, a candidate miRNA, which was increased in IBD in response to TNFα, which targeted the interleukin (IL)-10 3′ untranslated region. miRNA 106a has lately been discovered to increase in the serum of patients with IBD corresponding to disease severity. This suggests that it might be a useful biomarker for both UC and CD [14]. A study confirmed that the elevated miR-106a in murine models when selectively inhibited can enhance Treg function in vitro [14].

This study aimed at assessing the role of microRNA 106a and 146b-5p as biomarkers in the diagnosis of IBD and predicting severity. This study also aimed to explore the potential of both microRNAs in differentiating UC from CD.

There were very limited studies on miRNA profiles that help in differentiating UC from CD whenever the histopathological results failed to clarify the IBD phenotype, especially in the case of isolated colonic CD versus UC [22,23], however, none of these studies included miRNA 106a and miRNA 146b-5p in their investigated miRNA profiles. Very few studies have investigated the role of miRNA 106a and 146b-5P in the diagnosis and evaluation of IBD.

This study revealed that serum miRNA 106a was significantly elevated in patients with UC having p-value of 0.001 (7.16-fold higher than control), whereas the expression of miRNA 106a was significantly decreased in the case of patients with CD having a p-value of 0.001 (0.75-fold compared with control). A significant difference was found in the serum expressions of miRNA 106a between UC and CD (p-value 0.001), which could help differentiate both IBD variants. The results of the UC analysis in the present study agreed with those of Omidbakhsh et al., who reported that the serum miRNA 106a is elevated in both UC and CD compared with those in healthy controls [11]**.** However, the results of the CD analysis in the present study were the exact opposite of their findings, which may be because a higher portion of the study patients was receiving steroids, biological therapy, or both. This might have lowered the miRNA 106a levels as part of their mechanism in controlling the disease. **Iborra et al.** and **Alaa et al.** have reported that serum miRNA 106a increases in patients with IBD (both UC and CD) compared with healthy participants and that the elevation could be correlated to the disease activity [24,25]**. Paraskevi et al.** revealed that serum miRNA 106a levels decrease in UC but increase in CD [26].

Table 6: Relation between biomarkers and remission or activity in ulcerative colitis and Crohn's disease (n = 35).

Biomarkers		**UC**		**CD**		***p-value***
		Remission (n= 2)	**Activity (n=33)**	**Remission (n= 7)**	**Activity (n= 27)**	
miR-106a fold change	*Mean ± SD*	5.6 ± 3	7.2 ± 4	1 ± 2	0.67 ± 1.9	**P1** *0.568* **P2** *0.708*
miR146b-5p fold change	*Mean ± SD*	6.3 ± 4	4.9 ± 3	2.5 ± 1	3.2 ± 2	**P1** *0.524* **P2** *0.739*

P1 (Patients with UC: remission vs. activity), P2 (Patients with CD: remission vs. activity)

In the present study, regarding the expression of miRNA 106a in patients with UC according to the activity of UC, miRNA 106a serum levels were higher in those with active disease than in those in remission (mean fold rise, 7.2 versus 5.6, respectively), indicating a positive association between their serum level and activity of UC; however, this rise was not significant (P = 0.6). These findings were in agreement with the results of Omidbakhsh et al. [11].

In this study, the serum level of miRNA 106a was lower in patients with active CD than in those in remission (mean 0.67-fold change versus mean 1-fold change compared with control) (P = 0.7).

Wu et al. reported that patients with Crohn's colitis had higher levels of miRNA 106a expression in their colonic mucosa than healthy controls. Their expression in CD increases further in those with active CD colitis [27]. In pediatrics CD, Zahm et al. reported that serum miRNA 106a increases in the CD group compared with that in the control group; however, its levels did not correlate with disease activity as determined by the pediatric CDAI score. No patients with ileal CD were recruited in their study. This might explain why their results did not agree with the results of the present study [28].

Regarding serum microRNA 146b-5p, the results of this study revealed a significant difference (p-value of 0.001) between patients with UC or CD and healthy controls where the mean fold change was five-fold higher in the UC group and 3.1-fold higher in the CD group than in the control group. In addition, the miRNA 146b-5p level was significantly higher (P-value 0.007) in the UC group than in the CD group. This roughly agrees with the results of Chen et al., who reported that the serum microRNA146b-5p expression was 2.72-fold higher in the UC group and 2.87-fold higher in the CD group than in the healthy control group [12]. However, no significant difference between UC and CD was found in their study because only 55% of the UC and 56% of the CD group had clinically active disease compared with 94.3% of the UC group and 79.4% of the CD group who had a clinically active disease in the present study.

Batra et al. reported that miRNA 146b increases significantly in both UC and CD tissues. They observed that the serum levels of miRNA 146b decrease with clinical response to anti-TNF (especially infliximab) and glucocorticoids in their pediatric patients with IBD [29]**.**

Regarding miRNA 146b-5p expression, there is no statistically significant difference between patients with active UC than those in remission (4.9-fold versus 6.3-fold increase compared with the control group, respectively, P = *0.5)*, and it was not significantly higher in patients with active CD than in those in remission (3.2-fold versus 2.5-fold increase compared with the control group, respectively, P = *0.7)*. Perhaps, if more of the active cases (UC or CD) not on biological or glucocorticoid therapy were recruited, the correlation would be more pronounced and become significant, as found by **Chen et al.** [12].

5. Conclusion

The results of this study suggest that serum miRNA 106a and 146b-5p may be useful markers for IBD diagnosis and differentiation between UC and CD. Serum miRNA 106a and 146b-5p levels were not related to disease activity.

6. Conflict of interest

The authors have no conflict of interest

7. Acknowledgment

This study was funded by Faculty of Medicine, Cairo University.

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