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Serum miR-34a as a potential biomarker for diagnosis of inflammatory bowel diseases in Egyptian patients

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Abstract

Background: IBD is a multifactorial disease. Although dysbiosis of commensal bacteria and breakdown of the intestinal barrier are considered as major pathological mechanisms in the development of IBD, other important factors such as genetic aberrations also contribute to its development.

Results: Our results revealed that serum miR-34a RQ values were significantly lower and serum *MACF1* RQ values were significantly higher in IBD patients compared to healthy controls. In addition, serum miR-34a in relation to pathological activity and disease severity in the IBD group revealed a significant difference ($p>0.05$).

Conclusion: Serum miR-34a RQ and serum *MACF1* RQ value-based biomarker panels can act as a potential biomarker for IBD diagnosis and prognosis.

Keywords: IBD, Serum miR-34a RQ, Serum *MACF1* RQ, Dysbiosis, Intestinal barrier

Background

Ulcerative colitis (UC) and Crohn's disease (CD) are two chronic, relapsing, and remitting inflammatory bowel diseases (IBD) that have no definitive medication treatment and can cause severe long-term morbidity [1]. Only the colon is affected by UC, which is mostly limited to the mucosal and, to a lesser extent, submucosal compartments. It could include all layers of the intestine, and this CD could also contain various digestive system components, ranging from the oral cavity to the anus [2]. These diseases have long been regarded as a problem in Western civilization, and Western lifestyles have played a significant role in disease development [3, 4]. It is also increasingly being seen as an emergent worldwide illness [5]. Irritable bowel disease is linked to a significant increase in the risk of colorectal cancer (CRC), especially after years of active disease [6, 7]. *MACF1* (also known as macrophin 1 or trabeculin-alpha) is a human actin-crosslinking protein that is encoded by *MACF1*, which is found on chromosome 1p34 and spans over 270 kb [8]. It is encoded by *MACF1*, which has at least 102 exons and spans over 270 kb. *MACF1* appears to serve an important role in organisms. The potential role of *MACF1* was investigated using *MACF1* knockout animals or conditional knockout in specific organs. The findings revealed that *MACF1* is required for embryonic development as well as the maintenance of the neural system, bone, colon, cardiomyocyte function, and skin integrity [9]. *MACF1* has also been linked to a variety of malignancies, including breast cancer in women, colon cancer, and liver cancer [10, 11]. It is also linked to lung cancer, renal cell carcinoma, and some tumors like glioblastoma [12]. Through the Wnt/catenin pathway, *MACF1* 1 is involved in colon cancer cell proliferation and progression. As a result, we anticipated that inhibiting *MACF1*

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might slow the evolution of CRC. Using MIDB [13], bioinformatic investigation indicated that *MACF1* is a target of miR-34a-5p. MicroRNAs (miRs) are 22-nucleotide noncoding RNAs that regulate the amounts of numerous proteins in basic biological processes [14]. The miR-34 family is made up of miRs, which are encoded by two separate genes: miR-34a is transcriptionally independent, whereas miR-34b and miR-34c share a first copy linked in the human genome on chromosome 1, miR-34a, and miR-34b and miR-34c homologs on chromosome 11. In vertebrates, the miR-34 family is highly conserved [15]. Several microRNAs have also been discovered as important regulators of inflammation resolution during the healing process [16–18]. The first aim of this work is to identify a novel molecular signature and a selected target gene involved in IBD with high accuracy to detect IBD through in silico data analysis. The second aim is to characterize the expression of serum non-coding RNA profile and the associated target gene to evaluate their usefulness as diagnostic biomarkers.

Methods

This case-control study was conducted on 51 subjects subdivided into two groups: group 1: a total of 37 inflammatory bowel disease patients; group 2: control group: a total of 14 normal healthy subjects. Patients who complain from any other inflammatory diseases were excluded from the study. Patients were selected from within the National Tropical Hepatology and Tropical Medicine Research Institute (NHTMRI), and their consent to conduct this study was obtained.

Sample collection and processing

The venous blood (5 mL) was withdrawn from each subject under complete aseptic conditions and was left to clot for 30 min. The serum was separated by centrifugation at 4000gf or 15 min. All samples were kept at −80°C until use.

Relative expression of miR-34a and MACF1 using quantitative real-time polymerase chain reaction miRNA expression analysis

The quantification of miR-34a levels was performed using the SYBR-green fluorescent-based primer assay (hsa-miR-34) primer assay, and the Hs_SNORD_68 primer assay was used as a housekeeper gene for normalization. The qPCR was performed in the 5-plex Rotor Gene PCR System (Qiagen, Hilden, Germany). The 20-ul reaction mixture/reaction consist of 2x QuantiTectsyber green PCR mastermix, 10x miscript universal primer, 2μL primer assay, and 50pg–3ng cDNA. Both targets were amplified in duplicates for each sample. The thermal protocol consists 15 min for HotStarTaq DNA polymerase

Table 1 Demographic, clinicopathological, and laboratory parameters

Demographic, clinicopathological and laboratory parameters	IBD (*n*=37)	Healthy control (*n*=14)	*p*
Age			
≥ 46.65 years (*n*=29)	19 (51.4%)	10 (71.4%)	>0.05[(a)]
<46.65 years (*n*=22)	18 (48.6%)	4 (28.6%)	
Sex			
Male (*n*=30)	22 (59.5%)	8 (57.1%)	>0.05[(a)]
Female (*n*=21)	15 (40.5%)	6 (42.9%)	
Hemoglobin[†]	19.35	43.57	<0.01[(b)]**
Pathology			
Active	6 (16.2%)	—	—
Inactive	31 (83.8%)		
Mayo score			
1	5 (13.5%)	—	—
2	2 (5.4%)		
3	2 (5.4%)		
4	4 (10.8%)		
6	15 (40.5%)		
7	4(10.8%)		
8	2(5.4%)		
9	3(8.1%)		
Severity			
Mild	13 (35.1%)	—	—
Mild to moderate	21 (56.8%)		
Moderate to severe	3 (8.1%)		

[†] Values are expressed as mean rank

[a] Chi-square test

[b] Mann-Whitney test

p>0.05, non-significant, **p<0.01, highly significant

Table 2 Serum miR-34a and MACF1RQ values in inflammatory bowel disease patients

Parameter	IBD (*n*=37)	*p*
miR-34a		
Median	0.69	<0.01[(a)]**
Mean rank	19.27	
Positivity rate "*n* of cases≤1.36 (%)"	36 (97.3%)	<0.01[(b)]**
MACF1		
Median	3.23	<0.01[(a)]**
Mean rank	33.00	
Positivity rate "*n* of cases≥1.2 (%)"	37 (100%)	<0.01[(b)]**

[a] Mann-Whitney test

[b] Chi-square test

** *p*<0.01, highly significant

activation at 95°C followed by 40 cycles of denaturation at 94°C for 15 min, primer annealing for 30 s at 55°C, and extension at 70°C for 30 s. The 2ΔΔCt method was conducted for the analysis of miR-34a expression levels, using SNOR-68 as an endogenous reference control for normalization purposes [19].

Gene expression analysis for mRNA

The quantification of *MACF1* gene expression levels was amplified from cDNA using a QuantiTectqPCR Assay for Human genes; [Hs_ *MACF* primer assays (Qiagen, Germany). The ACTB_1_SG QuantiTect Primer Assay cat no: 249900 was used as a housekeeper gene. The PCR amplification was conducted using the QuantiTect SYBER Green master mix. The 20-ul reaction mixture/reaction consist of a 10-µl syber green PCR mastermix, 2-ul primer assay (10µM), RNAase H_2O, and cDNA. The thermal protocol consists 10 min for HotStarTaq DNA polymerase activation at 95°C followed by 40 cycles of denaturation at 94°C for 15 min, primer annealing for 30 s at 55°C, and extension at 70°C for 30 s. The 2ΔΔCt method was conducted for the analysis of *MACF1* gene expression levels, using ACTB as an endogenous reference control for normalization purposes [20].

Statistical analysis

All analyses were done using the Statistical Package for the Social Sciences (SPSS software version 20, Chicago, IL, USA) on a personal computer. Mann–Whitney and Kruskal–Wallis tests were used for statistical comparison of the non-parametric data variables between groups. Chi-square analysis was used to find out the relation between various qualitative data. Variables were cross-tabulated in all possible combinations against each other. The correlation coefficients (*r*) were calculated using the Spearman's correlation. Receiver operating characteristic (ROC) curve determined the best value that gave maximum sensitivity and specificity.

Results

Demographic, clinicopathological, and laboratory parameters of study subjects

Different demographic and laboratory parameters of subjects of the two groups are shown in Table 1. Moreover,

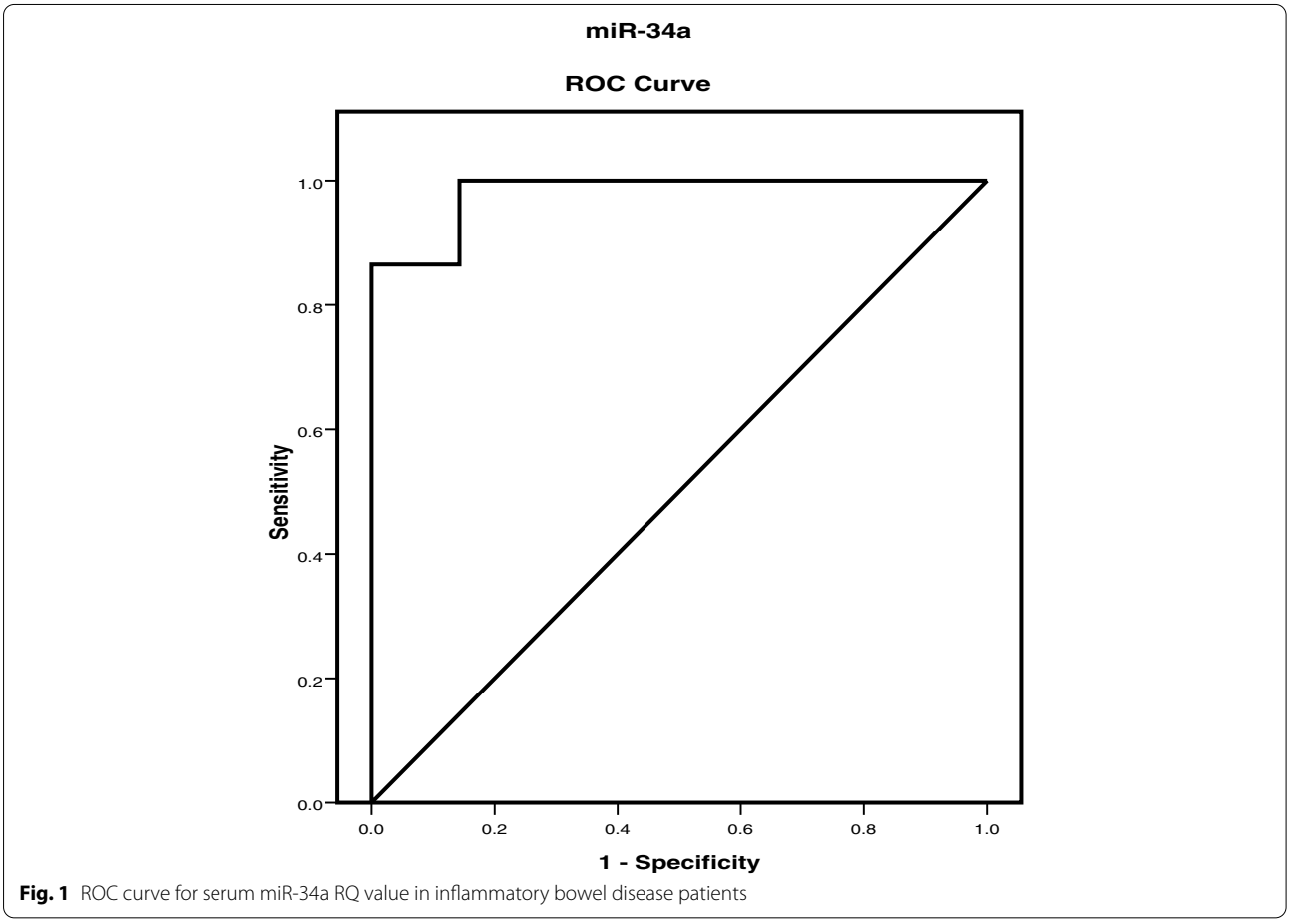

Fig. 1 ROC curve for serum miR-34a RQ value in inflammatory bowel disease patients

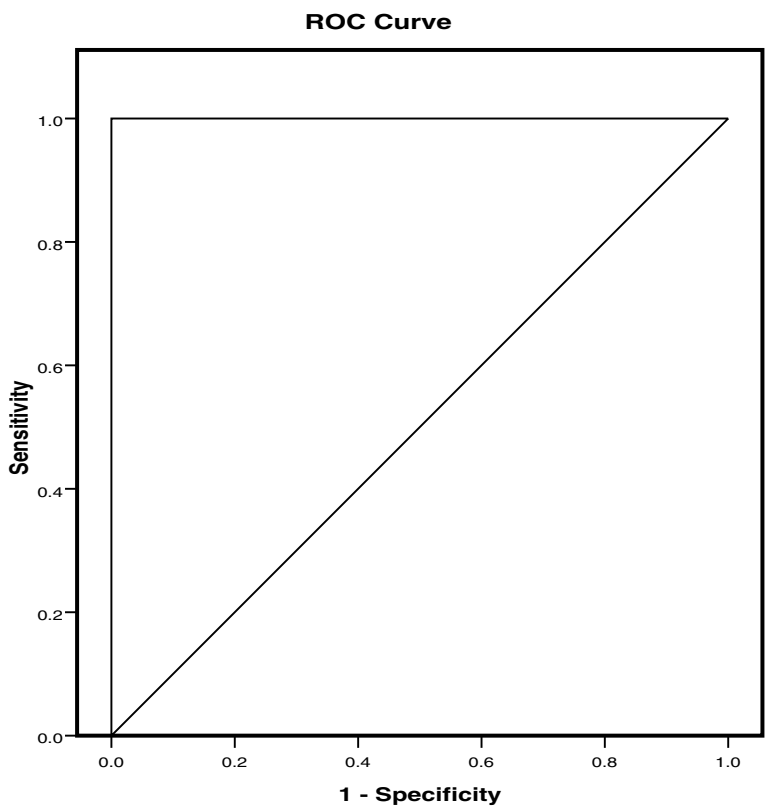

Fig. 2 ROC curve for serum MACF1RQ value in inflammatory bowel disease patients

Table 3 Sensitivity and specificity of miR-34a and MACF1

	AUC	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
miR-34a	0.981	≤1.36	97.3%	85.7%	94.7%	92.3%	94.1%
MACF1	1.000	≥1.2	100%	100%	100%	100%	100%

the characteristics of IBD patients were investigated including pathological activity and assessment of disease severity by Mayo score.

Serum miR-34a and MACF1 RQvalues in inflammatory bowel disease patients

Results showed that serum miR-34a RQ values were significantly lower and serum *MACF1* RQ values were significantly higher in IBD patients compared to healthy controls (p<0.01, Table 2). ROC curve was done to determine the best cutoff values for miR-34a and *MACF1* discriminating the IBD group from healthy control group (Figs. 1 and 2) (Table 3). The positivity rates of serum miR-34a and *MACF1* were estimated among the groups of the study (p<0.01, Table 2). A correlation analysis was performed between the two groups regarding miR-34a, *MACF1* RQ values, and hemoglobin level (p<0.05, Table 4). In addition, serum miR-34a in relation to pathological activity and disease severity in the IBD group revealed a significant difference (p>0.05, Table 5).

Discussion

Although the Mayo score is used to diagnose and characterize IBD, coloscopy is one of its most essential characteristics. The discovery of valid non-invasive diagnostic biomarkers is required because sigmoidoscopy and colonoscopy are criticized for their high costs and worsening

Table 4 MiR-34a, MACF1 RQ values, and hemoglobin level

Spearman rho	miR-34a	MACF1	Hemoglobin
miR-34a			
r	1.000	-0.560	0.337
p	—	<0.01**	<0.05*
MACF1			
r	-0.560	1.000	-0.695
p	<0.01**	—	<0.01**

r correlation coefficient

* $p<0.05$, significant, ** $p<0.01$, highly significant

Table 5 Positivity rate of miR34a

Clinicopathological variables	miR-34a			
	Mean rank	*p*	Positivity rate *n* (%)	$p^{(c)}$
Pathology				
Active (*n*=6)	29.83	<0.01**[a]	5 (83.3%)	>0.05
Inactive (*n*=31)	16.90		31 (100%)	
Severity				
Mild (*n*=13)	7.00	<0.01**[b]	13 (100%)	>0.05
Mild to moderate (*n*=21)	24.19		20 (95.2%)	
Moderate to severe (*n*=3)	34.67		3 (100%)	

[a] Mann-Whitney test

[b] Kruskal-Wallis test

[c] Chi-square test

$p>0.05$=non-significant, ** $p<0.01$, highly significant

of inflammatory bowel disease. For the first time, we used serum to investigate a network of genetic and epigenetic indicators in IBD (has-miR-34a, *MACF1* mRNA). We believe that this strategy has a better likelihood of success than the more traditional single-marker approach. MiR-34a is a tumor suppressor gene that also has an antiproliferative function. It is found in the second exon of the 33-kb transcript and is considered a tumor suppressor gene as well as has antiproliferative activity [21, 22]. However, it has a role in the inflammatory process and prevents epithelial to mesenchymal transition which is one of the risk factors for a progression of CRC [23].

Females are more likely to develop immune-mediated illnesses. Other immune-mediated illnesses, such as sarcoidosis, type 1 diabetes, and IBD, show substantially less gender-specific abnormalities [24, 25]. In agreement with the current study that revealed no significant difference in sex between IBD patients and the healthy group ($p>0.05$, Table 1). Consistent with our study, El-Daly et al [26]. reveal suppression of miR-34a in four stages of colitis-related carcinogenesis, and there was a dependent decrease in miR-34a expression associated with a tumor stage. Also, HOU et al. [27] indicated that miR 34a 3p expression is reduced in FLS from RA patients. Our study revealed a significant difference in serum miR-34a expression in relation to pathological activity and disease severity in the IBD group. In addition, there is a significant positive correlation between miR-34a and HB level in IBD patient [28]. In K562 cells, a steady level of miR-34a increased the level of fetal HB as previously observed by Yanlei Ma et al. [29]. Our study showed *MACF1* 1RQ values were significantly higher in IBD patients compared to healthy controls ($p<0.01$, Table 2). Since ROC curve analysis reveals miR-34a (Fig. 1) values had higher sensitivity (97.3%, 100%) and lower specificity (85.7%, 1%), it could be used to confirm the diagnosis rather than decline it (good positive test). The study limitations include the following: it was performed at a single center in Egypt with a relatively limited sample size. Moreover, in vitro functional analysis is needed to elucidate the biological mechanisms of RNA-RNA cross-talk in IBD is still needed which is currently running in our center.

Conclusion

We approached a novel access that enables reliable incorporation of differential *MACF1* mRNA expression with the selected epigenetic regulators. This approach has been shown to create an interesting biomarker panel (hsa-miRNA-34a and *MACF1* mRNA) for IBD diagnosis and prognosis. These findings extend our knowledge about competing for endogenous hypothesis and provide new tools to clarify disease processes and offer new targets for IBD diagnosis.

Authors' contributions

All authors had the same contribution in this work. The author(s) read and approved the final manuscript.

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Availability of data and materials

The data of this study are available upon reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol conforms to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. Written informed consent was obtained from all the participants of this study and was approved by the Ethics Committee for human subject research at National Hepatology and Tropical Medicine Research Institute (serial no: 8-2020).

Consent for publication

Participants provided consent for the study findings to be published.

Competing interests

The authors declare they have no competing interests.

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