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Original Article

Serum microRNA 106 and microRNA 223 as novel biomarkers in inflammatory bowel disease

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Abstract:
Background: Inflammatory bowel disease (IBD) involves mainly Crohn's disease (CD) and ulcerative colitis (UC). Distinguishing between the two diseases may be problematic in 15% of patients due to inconclusive findings on histopathology or endoscopy. miRNAs are non-coding RNAs composed of 18-23 nucleotides and are widely detected in the cells involved in variable pathological and physiological processes. The aim of this study is to assess if serum microRNA 106 and microRNA 223 (miR-223) can be reliable biomarkers in IBD patients. *Methods:* Serum levels of microRNA 106 and microRNA 223 (miR-223) were determined in 80 IBD patients by using the Transcript or First Stand cDNA, expression levels of serum miRNA in IBD patients and the control group and between active and inactive diseases were detected. *Results:* There was a significant increase in expression levels of serum miRNA 106 and miRNA 223 in IBD patients (UC and CD) compared to the control group (P<0.001, both). Also, There was a significant increase of expression levels of serum miRNA 106 and serum miRNA 223 with increased disease activity of IBD (P <0.05, both). *Conclusions:* The levels of miRNA 106 and miRNA 223 are higher in the peripheral blood of IBD patients and the elevation is more significant in the active disease. The miRNA 106 and miRNA 223 may be used as a novel biomarker in the diagnosis of IBD and evaluating the disease activity.

Keywords: miRNA, inflammatory bowel disease, Crohn's disease and ulcerative colitis.

Introduction

Inflammatory bowel disease including mainly Crohn's disease and ulcerative colitis. IBD usually develop due to complex interaction between immunologic, genetic, and environmental factors leads to significant changes in gene expression[1-3].UC involves the colonic mucosa, causing continuous intense inflammation, however, the CD might involve the whole gastrointestinal tract; via immune reactive response mainly in the terminal ileum[4,5]. The diagnosis of IBD is relying on clinical, laboratory, radiologic and endoscopic findings. Usually, the diagnosis of CD or UC is clear. However, if the disease is only colonic, distinguishing between the 2 diseases will be inconclusive in up to 15% of cases due to indecisive findings on histopathology or endoscopy[6-8]. Due to the propensity for intermittent exacerbations and relapses efficient and frequent evaluation are essential for IBD patients[9]. Endoscopy has a crucial role in assessing mucosal lesions. However, this maneuver is usually costly and has some complications[10]. Up till now, no ideal biomarkers to diagnose and monitor IBD. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are mainly used in follow-up. However, mostly they are nonspecific, as their values increase in different inflammatory disorders or remain at normal values in active IBD patients[11]. Fecal calprotectin is considered as a non-invasive biomarker for evaluating disease relapse among IBD patients. However, it is still nonspecific and is positive in other intestinal inflammatory disorders, as celiac disease, infectious enteritis[12,13]. So, there is increasing demand in biomarkers with more specific diagnostic criteria for IBD and could distinguish between different phenotypes[14]. Micro RNAs are non-coding RNAs composed of 18-23 nucleotides and are extensively present in the cells involved in variable physiological and pathological processes[15]. MicroRNAs are involved in the regulation of different biologic processes, such as intestinal barrier function, endoplasmic reticulum stress, immune regulation, and gut microbiota interactions, to generate chronic mucosal inflammation[16, 17]. microRNAs may be involved in regulation of specific genes. It is well known that micro RNAs have important roles in the cell differentiation and organogenesis. Disturbances in expression of micro RNAs may lead to disease development[18]. micro RNAs have been found in the peripheral blood of some immunologic disorders such as rheumatoid arthritis and systemic lupuserythematosus[19]. Recent data detected abnormal expression of micro RNA profiles in the tissues and peripheral blood of IBD patients compared with healthy controls[20,21]. However, the data are conflicting in some studies as regards the relationships between micro RNA levels and disease activity[22]. The aim of this study is to assess

if serum microRNA 106 and microRNA 223 can be reliable novel biomarkers in IBD patients and their relation to disease activity.

Patients and Methods

Our study involved 80 IBD patients (40 patients with ulcerative colitis and 40 patients with Chron's disease) and 40 sex and age matched healthy individuals as a control group recruited from Riyadh Care Hospital (Outpatient and inpatient) from February to December, 2018. The diagnosis of IBD was based on the clinical, laboratory, radiological, endoscopic and histological investigations. All involved subjects signed informed consents. The research was consistent with The Declaration of Helsinki for human research ethics. The age of our subjects ranged between 18 and 68 years old. Montreal classification was used to define the site of the disease[23]. Clinical disease activity was measured by the Crohn's Disease Activity Index (CDAI) for CD and the Mayo clinical score for UC[24,25]. Clinical activity was defined as CDAI ≥150 for active CD or Mayo clinical score >2 for active UC. Crohn's Disease Endoscopic Index of Severity (CDEIS) scores for CD and UCEIS scores for UC were determined after performing colonoscopy to assess the endoscopic activity[26,27]. CDEIS >3 for CD and UCEIS >1 for UC are the cut off values of disease activity in both diseases[28,29]. Infectious and malignant etiologies of colitis were excluded. Thorough history taking, including current and past medications and careful examination including, BMI were done. Serum samples for CBC, quantitative CRP &ESR were collected.

Isolation of microRNA from the periheral blood

The mirVana miRNA Isolation Kit (Ambion, Austin, TX) was used to isolate the total RNA according to instructions of manufacturer. The reverse transcription (RT) of miR-106 and 223 was manufactured through a reaction with the RT master mix by using the Transcript or First Stand cDNA Synthesis Kit(Roche, Germany, Penzberg).

Statistical analysis

All data were presented as the mean ± SD. The Kruskal-Wallis test, followed by the "kruskalmc" function ("pgirmess" package), was the standard for intergroup comparisons. P <0.05 was statistically significant. The Kruskal-Wallis test and Pearson correlation coefficient(r) analysis were applied via SPSS 13.0 software (SPSS, Inc., USA).

Results

Table (1) shows the demographic data of our studied patients. **Table (2)** shows a significant increase of expression levels of serum miRNA 106 in IBD patients compared to the control group, (P <0.001).

Table 1. The demographic data of our studied groups

	Ulcerative Colitis	Crohn's disease	Control group
Number	40	40	40
Gender(male/female)	24/16	19/21	23/17
Age(mean±SD)years	40.7±12.8	36.2±10.6	42±7.8
Duration of the disease (mean±SD)years	10·5 ±8·5	13·7 ±5·8	NA
Exent of UC			
E1: procistgmoiditis	16	-	-
E2: left-sided	14	-	-
E3: pancolitis	20	-	-
Site of CD			
L1: ileal	-	8	-
L2: colonic	-	16	-
L3: ileocolonic	-	18	-
L4: upper disease	-	0	-
Medications (n)			
5 - ASA	21	8	-
Antibiotics	0	5	-
Steroids	18	15	-
Azathioprine	4	5	-
6-Mercaptopurine	5	4	-
Methotrexate	6	6	-
Biologics	8	12	-
Disease behavior			
- Active n (%)	27(67.5%)	23(57.5%)	-
- Quiescent n (%)	13(32.5%)	17(42.5%)	-

Table 2. Comparison of expression levels of serum miRNA 106 in IBD patients and the control group

Parameter	Ulcerative colitis group (n=40)	Crohn's disease group (n=40)	Control group (n =40)	P value
Fold change of serum miRNA 106 (Mean ± SD)	2.38±1.13	4.66±1.92	1	**P 1** <0.001 **P2** <0.001 **P3** <0.001

P 1*= UC versus control,* ***P2****= CD versus control,* ***P3****= UC versus CD.*

Moreover, a significant increase in serum miRNA 106 was found in CD versus UC. **Table (3)** shows a significant increase of expression levels of serum miRNA 223 in IBD patients compared to the control group, (P <0.001). In addition, a significant inc-rease in serum miRNA 223 was found in CD versus UC. **Table (4)** shows the sensitivity and specificity of serum miRNA 106 & serum miRNA 223 in patients of UC and CD. **Table (5)** shows a significant increase of the expression levels of serum miRNA 106 and serum miRNA 223 with increased activity of IBD (UC and CD), (P <0.05).

Table 3. Comparison of expression levels of serum miRNA 223 in IBD patients and in the control group

Parameter	Ulcerative colitis group (n=40)	Crohn's disease group (n=40)	Control group (n =40)	P value
Fold change of serum miRNA 223 (Mean ± SD)	1.78±1.16	3.18±1.18	1	**P 1** <0.001 **P2** <0.001 **P3** <0.001

P 1*= UC versus control,* ***P2****= CD versus control,* ***p3****= UC versus CD*

Table 4. Sensitivity and specificity of serum miRNA 106 & serum miRNA 223

	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
miRNA 106	0.70 (0.58–0.88)	72.8 (54.8–82.8)	68.7 (47.6–81.9)	73.2 (55.3–83.6)
miRNA 223	0.65 (0.59–0.87)	69.0 (49.9–78)	62.5 (47.6–72.9)	69.7 (58.6–79.9)

AUC: Area under the curve

Table 5. Comparison of expression levels of serum miRNA 106 & serum miRNA 223 inactive and inactive IBD patients and in the control group.

Parameter	Fold change of serum miRNA 106 (Mean ± SD)	P-value	Fold change of serum miRNA 223 (Mean ± SD)	P-value
Control group(no=40)	1	-	1	-
Inactive UC(no=13)	2.01±1.03	P1<0.001	1.59±1.26	P7<0.05
Active UC(no=27)	2.78±1.26	P2<0.001 P3<0.05	2.58±1.22	P8<0.001 P9<0.001
Inactive CD(no=17)	3.26±1.02	P4<0.001	2.38±1.10	P10<0.001
Active CD(no=23)	4.96±1.99	P5<0.001 P6<0.001	4.02±1.18	P11<0.000 P12<0.001

P1 *value to show the difference between the inactive UC and the control group as regards serum levels of miRNA106;* ***P2*** *value to show the difference between the active UC and the control group as regards serum levels of miRNA106;* ***P3*** *value to show the difference between the active UC and the inactive UC group as regards serum levels of miRNA106;* ***P4*** *value to show the difference between the inactive CD and the control group as regards serum levels of miRNA 106;* ***P5*** *value to show the difference between the active CD and the control group as regards serum levels of miRNA 106;* ***P6*** *value to show the difference between the inactive CD and active CD as regards serum levels of miRNA106;* ***P7*** *value to show the difference between the inactive UC and the control group as regards serum levels of miRNA 223;* ***P8*** *value to show the difference between the active UC and the control group as regards serum levels of miRNA223;* ***P9*** *value to show the difference between the active UC and the inactive UC group as regards serum levels of miRNA223;* ***P10*** *value to show the difference between the inactive CD and the control group as regards serum levels of miRNA 223;* ***P11*** *value to show the difference between the active CD and the control group as regards serum levels of miRNA 223;* ***P12*** *value to show the difference between the inactive CD and active CD as regards serum level of miRNA223.*

Discussion

Differentiation between ulcerative colitis and Crohn's disease is very serious, however, sometimes may be very hard in many cases in particular isolated colonic phenotype of Crohn's disease and ulcerative colitis[30,31]. Both ESR and CRP are the most frequently used biomarkers determining the activity of IBD, however, unfortunately they are of low specificity in these patients as they are highly expressed in many other pathological conditions[32,33]. Fecal calprotectin is another non-invasive commonly used marker for IBD, but, still also nonspecific and is present in some other inflammatory diseases[12,13]. A large number of studies showing the pathological significance of dys regulated miRNAs in many pathological disorders as cancers of the esophagus, breast, bile duct, prostate, pancreas and other immune disorders such rheumatoid arthritis and systemic lupus erythematosus[19,34-38]. Many studies showed variable expression patterns of miRNA in the peripheral blood in IBD patients (UC &CD) if compared to control groups[39-42]. Other studies indicated the association between expression patterns of specific miRNA and different IBD activity stages that supports their potential usefulness as biological biomarkers for the diagnosis of IBD and differentiation of the different major types and stages of activity. In our study, we compared the expression levels of the peripheral blood of miRNA-106 and miRNA -223 in a number of IBD patients (40 ulcerative colitis and 40 Crohn's disease patients) to the levels of 40 individuals as a control group and the different expression levels in the different activity stage groups. Significantly higher expression levels of miRNA-106 & miRNA -223 in IBD (UC & CD) patients compared to the control group and the expression levels are higher with increased activity of the disease. Our results are in agreement with the studies of Wu et al.[43,44] and Zahm et al.[45] on IBD patients that have revealed that peripheral blood miRNAs can distinguish between active IBD subtypes and healthy controls. Also, studies of Wu et al., and Fasseu et al. on peripheral blood and biopsies of the sigmoid colon from patients of IBD (UC and CD) have revealed that miR106 levels are significantly higher in these patients when compared to control groups[43,46]. Wu et al. also observed that mi-RNA expression levels are increased in biopsies of patients with active CD and ileal CD (Crohn' sileitis) and colonic CD (Crohn's colitis)[44]. Paraskevi and colleagues showed that miR-106a was significantly increased in peripheral blood of active CD patients when compared to healthy controls[47]. Studies of Zahm and colleagues on the peripheral blood of active pediatric CD patients have indicated that miR-106a expression was significantly higher compared with healthy controls[45]. Our study is also in agreement with the study of Duttagupta et al.[48] that revealed that expression levels of miR-106a in the blood are elevated in Crohn's disease and ulcerative colitis. In agreement with our study, Polytarchou et al recently recognized that miR-223 was increased in the peripheral blood of UC patients compared with the healthy control group[49]. Another study by Wang et al., indicated increased peripheral blood miRNA in patients of ulcerative colitis and Crohn's and levels are more correlated with the factors of activity than ESR and CRP[50].

Conclusion

Our present study revealed that the expression levels of miRNA 106 and miRNA 223 are higher in the peripheral blood of IBD patients and the elevation is correlated with the disease activity, so they may have an important role as a novel biomarker in the diagnosis of IBD and evaluating the disease activity.

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