Evaluation of Mirna-155 and Mirna-223 in Blood in Patients with Ulcerative Colitis

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ABSTRACT: Ulcerative colitis is common and complex conditions that are difficult to cure. MiRNAs are a class of small, non-coding, single-stranded RNA molecules which play a key role in autoimmune and inflammatory diseases especially in IBD. Despite the efficacy of biological treatment, a significant proportion of patients with IBD do not show an adequate response. For this reason it is crucial to identify biomarkers useful to predict clinical response. In present study, we aimed to evaluate the microRNA-155 (miR-155) and microRNA-223 (miR-223) as prognostic marker in disease response to therapy in patients with UC. This study included 80 patients with UC (40 patients who response to conventional treatment and 40 who resist and they are on biological treatment) and 40 healthy controls. Real-time polymerase chain reaction (RT-PCR) assay was performed for evaluation miRNA-155 and miRNA-223. According to the results of this study, There was significant variation in mean fold change of miR-155 among groups (p <0.001), the level was highest in patients on conventional treatment followed by patients on biological therapy and then by control group. With respect to miR-223, the difference in mean fold change was significant among study groups (p <0.001), the level was highest in patients on biological treatment followed control group and then by patients on conventional therapy. Receiver operator characteristic (ROC) curve analysis was carried, the cutoff value of miRNA-155 was ≥1.43 with 20 % sensitivity level, 95 % specificity level and 53.3 % accuracy level. The cutoff vale of miRNA-223 was >4.51 with 25 % sensitivity level, 92.5 % specificity level and 59.1 % accuracy level. In conclusion, this study have evaluated the therapeutic response to corticosteroid, or IFX therapy and blood expression of miRNA by screening the responses to anti-TNF-α and conventional therapy, this study demonstrated that the level of miRNA-223 associated with the response to IFX which is highly increased in biological therapy (negative response) comparing to the conventional therapy (positive response) group after IFX therapy while the present study demonstrated that miRNA-155 fold change upregulated in patients with ulcerative colitis and on conventional therapy (positive response) in comparison with healthy control group.

INTRODUCTION

Inflammatory bowel diseases (IBD) including ulcerative colitis (UC) and Crohn’s disease (CD) are common and complex conditions that are difficult to cure. UC often starts from the rectum and extends to part or whole colon in a continuous manner with mucosal damage and clinical manifestations are bloody diarrhea and abdominal pain.1

MiRNAs are a class of small (18-25 nucleotides in length), non-coding, single-stranded RNA molecules that can negatively regulate target gene expression at the post-transcriptional level through binding to the 3'untranslated regions of the target mRNAs and promoting mRNA degradation or translational repression.2 MiRNAs play a key role in autoimmune and inflammatory diseases, especially in IBD. MiRNAs have been found to be involved in the regulation of the nuclear transcription factor NF-κB pathway, intestinal epithelial barrier function, and autophagic function. NF-κB is considered to be an important regulators of the immune system and inflammatory diseases.3 Dysregulation of the symbiosis between the miRNA and the microbiota is linked with a range of disease, which include inflammatory bowel disease (IBD), colon cancer, and neurological disorders.4

MiR-155 has shown a central regulatory role in innate and acquired immune systems. miR-155-5p is expressed in response to inflammatory mediators such as LPS, TLR ligands, and IFN-b and is induced in antigen-presenting cells, including plasmacytoid dendritic cells and macrophages.5 MiR-155 has been found to markedly promote cell proliferation and proinflammatory secretions, regulate the immune balance in colonic mucosa of IBD, thus contribute to the pathogenesis of experimental colitis.6
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MIp-223 is a crucial regulator of innate immunity, including myeloid differentiation and the function of neutrophils and macrophages.\textsuperscript{7} Intestinal macrophages and dendritic cells (DCs) lacking miR-223 exhibit a pro-inflammatory phenotype, and monocytes deficiency of miR-223 promotes an increase in monocyte-derived DCs, resulting in more severe colitis.\textsuperscript{8} Upregulation of miRNA-223 has previously been demonstrated within the inflamed colonic region and serum samples obtained from UC patients. IKK\(\alpha\), capable of phosphorylation and degradation of the in-hibitor of NF-\(\kappa\)B, was identified as a target gene for miRNA-223, and the expression of IKK\(\alpha\) exhibited a significant downregulation in the colonic mucosal samples from UC patients.\textsuperscript{9}

MATERIALS AND METHODS

During the period from January 2022 to June 2022, a case-control study was designed on 120 subjects. Of them, 80 ulcerative colitis patients including 40 (21 males and 19 females) who response to conventional treatment (immuran) (positive response) and 40 (males 19 , females 21 ) who resist to conventional treatment and they are on biological (negative response). The patients were attended to the consultant clinic for Gastroenterologist specialist in Diwaniyah city in addition to those who were followed-up at the Gastrointestinal Tract and Liver Disease Center in Baghdad, Iraq and 40 (13 females and 27 males) healthy controls were enrolled. Three milliliters (ml) of blood was withdrawn from each participant by vein puncture and collected in EDTA tube and mixed immediately with trizol, then kept at -20 for detection miRNA-155 and miRNA-223.

RNA Isolation and Reverse-Transcriptase PCR: Total RNA were extracted from serum samples or blood samples by using (TRizol\textsuperscript{\textregistered} reagent kit. Bioneer. Korea) and done according to company instructions. For the reverse transcription (RT) of miR-155, miR-223 we prepared a reaction with the RT master mix using the Transcriptor First Stand cDNA Synthesis Kit (Promega / Korea).

Quantitative-PCR: The Fast Start Universal SYBR Green Master was used to confirm the miRNA expression changes of miR-155, miR-223 in the serum (primer sequences for miR-155: Forward primer: TGGTAATTGTGTAATACCC; reverse primer: GTGAGTTTTCCTACGCACACTGGGATTACGCACCATAT) and (primer sequences for miR-223 forward primer: GCCGGCCTGCTAGTTGTC; reverse primer: GTCGAGGGTGCGAGGT). The expression of miRNA was calculated relative to U6 (primers sequences for U6, forward primer: 5-GTTCGGTACCGTGTTGCTTACC; reverse primer: 5-CTAACCTACATCAAAAACAACAAACAAC). A comparative threshold cycle method was used to compare each condition.

Ethical approval: All protocol investigations will be done in accordance with the Human Ethical Clearance Committee guidelines for Clinical Researches at the faculty of Medicine, University of Al-Qadisiyah.

Statistical analysis: Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. One way analysis of variance (ANOVA) was used to evaluate difference in mean of numeric variables among more than two groups provided that these numeric variables were normally distributed. One way ANOVA was followed by post hoc LSD test to evaluate individual differences in mean values between any two groups among groups. In order to detect the cutoff value that predict a positive finding, receiver operator characteristic (ROC) curve analysis was used with its corresponding area under the curve (AUC), accuracy level, sensitivity, specificity and level of significance (P). The risk was calculated based on odds ratio with corresponding 95 % confidence interval and etiological and preventive fractions. The level of significance was considered at P-value of equal or less than 0.05.

Results: 1-Demographic characteristics of patients and control subjects

General characteristics of patients and control subjects are shown in table 1. There was no significant difference in mean age among study groups, 35.60 ±10.42 years, 36.13 ±12.88 years and 34.30 ±10.01 years, respectively (\(p = 0.754\)). There was also no significant variation in the proportions of males and females among study groups, 21 (52.5 %) versus 19 (47.5 %), 19 (47.5 %) versus 21 (52.5 %) and 27 (67.5 %) versus 13 (32.5 %), respectively (\(p = 0.173\)). Smoking was less frequently seen in patients’ groups in comparison with control group, 1 (2.5 %) and 3 (7.5 %) versus 10 (25.0 %), respectively (\(p = 0.004\)). In addition, family history is limited to patients’ groups, 1 (2.5 %) and 2 (5.0 %) in comparison with control group 0 (0.0 %); however, the difference was no significant (\(p = 0.359\)).

Table 1: General characteristics of patients and control subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conventional therapy (n = 40)</th>
<th>Biological treatment (n = 40)</th>
<th>Control (n = 40)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ±SD 35.60 ±10.42</td>
<td>36.13 ±12.88</td>
<td>34.30 ±10.01</td>
<td>0.754 O</td>
</tr>
<tr>
<td></td>
<td>Range 18 -65</td>
<td>16 -61</td>
<td>20 -75</td>
<td>NS</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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| Male, n (%) | 21 (52.5 %) | 19 (47.5 %) | 27 (67.5 %) | 0.173 C NS |
| Female, n (%) | 19 (47.5 %) | 21 (52.5 %) | 13 (32.5 %) |
| Negative, n (%) | 39 (97.5 %) | 38 (95.0 %) | 40 (100.0 %) |

n: number of cases; SD: standard deviation; O: one way ANOVA; NS: not significant; *: significant at $p \leq 0.05$; ***: significant at $p \leq 0.05$

Duration of disease compared between those on conventional therapy and those on biological treatment is shown in table 2. The frequency distribution of all patients was as following, 55 (68.8 %), 18 (22.5 %) and 7 (8.8 %) as <5 years, 5-10 years and > 10 years, respectively. The proportion of less than 5 years was significantly higher and that of 5-10 years and > 10 years were significantly lower in patients on conventional therapy in comparison with biological treatment ($p < 0.001$).

Table 2: Duration of disease compared between those on conventional therapy and those on biological treatment

<table>
<thead>
<tr>
<th>Disease Duration</th>
<th>Total n = 80</th>
<th>Conventional therapy n = 40</th>
<th>Biological treatment n = 40</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5 years, n (%)</td>
<td>55 (68.8 %)</td>
<td>36 (90.0 %)</td>
<td>19 (47.5 %)</td>
<td>&lt; 0.001 C ***</td>
</tr>
<tr>
<td>5-10 years, n (%)</td>
<td>18 (22.5 %)</td>
<td>4 (10.0 %)</td>
<td>14 (35.0 %)</td>
<td></td>
</tr>
<tr>
<td>&gt;10 years, n (%)</td>
<td>7 (8.8 %)</td>
<td>0 (0.0 %)</td>
<td>7 (17.5 %)</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; C: chi-square test; ***: significant at $p \leq 0.001$

Comparison of mean fold change of miRNA-155 among study groups

With respect to miR-155, the difference in mean fold change was significant among study groups ($p <0.001$), the level was highest in patients on conventional treatment (positive response) followed by patients on biological therapy (negative response) and then by control group, as shown in table 3 and figure 1.

Table 3: Comparison of mean fold change of miR155 among study groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conventional therapy (Positive response) n = 40</th>
<th>Biological treatment (negative response) n = 40</th>
<th>Control n = 40</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-155 (fold change)</td>
<td>4.74 ±2.01 A</td>
<td>2.30 ±1.35 B</td>
<td>1.00 ±0.00 C</td>
<td>&lt; 0.001 O ***</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>0.53 -11.33</td>
<td>0.61 -3.9</td>
<td>1 -1</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; miR: micro-RNA; SD: standard deviation; O: one way ANOVA; ***: significant at $p \leq 0.001$

Comparison of mean fold change of MiRNA-223 among study groups

With respect to miR-223, the difference in mean fold change was significant among study groups ($p <0.001$), the level was highest in patients on biological treatment (negative response) followed by control group and then by patients on conventional therapy (positive response), as shown in table 4 and figure 2.
Table 4: Comparison of mean fold change of miR-223 among study groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conventional therapy (positive response)</th>
<th>Biological treatment (negative response)</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-223 (fold change)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>0.42 ±0.13</td>
<td>1.69 ±1.08</td>
<td>1.00 ±0.00</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Range</td>
<td>0.12 - 0.87</td>
<td>0.63 - 2.99</td>
<td>1.1</td>
<td>***</td>
</tr>
</tbody>
</table>

n: number of cases; miR: micro-RNA; SD: standard deviation; O: one way ANOVA; ***: significant at p ≤ 0.001

Figure 2: Comparison of mean fold change of miR223 among study groups

Receiver operator characteristic (ROC): Receiver operator characteristic (ROC) curve analysis was carried out in order to find the cutoff value predictor for biological treatment of both miRNA-155, miRNA-223 that can predict and the results are shown in table 5 and figures 3 and 4. The cutoff value of miRNA-155 was ≤1.43 with 20 % sensitivity level, 95 % specificity level and 53.3% accuracy level. The cutoff value of miRNA-223 was >4.51 with 25 % sensitivity level, 92.5% specificity level and 59.1 % accuracy level.

Table 5: The results of receiver operating characteristic curve (ROC) analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>miR-155</th>
<th>miR-223</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoff</td>
<td>≤1.43</td>
<td>&gt;4.51</td>
</tr>
<tr>
<td>AUC</td>
<td>0.533</td>
<td>0.591</td>
</tr>
<tr>
<td>95 % CI</td>
<td>0.418 to 0.646</td>
<td>0.475 to 0.699</td>
</tr>
<tr>
<td>p</td>
<td>0.61</td>
<td>0.159</td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Specificity %</td>
<td>95</td>
<td>92.5</td>
</tr>
<tr>
<td>Accuracy %</td>
<td>53.3</td>
<td>59.1</td>
</tr>
</tbody>
</table>

Figure 3: Receiver operating characteristic curve (ROC) analysis for miR-155 to find the cutoff value predictor for biological treatment
DISCUSSION

Since this is a case-control study, the non-significant association between patients and the control group regarding age and sex factors is necessary for such study in order to exclude the impact of age and sex factors on the biases of studied parameters.

Accumulating data have shown that susceptible age is late adolescence to young adults, mostly in the 20's and 30's. Moreover, it was found that 30's was the most susceptible age for onset of ulcerative colitis. Moreover, IBD is a disease of young adults with the peak incidence between the ages of 20 to 39. However, around one-third of the IBD population are 60 years or older, of whom up to 15% were diagnosed after the age of 60 years. Additionally, almost 25–35% of IBD patients are ≥60 years old. About 20% of them have been diagnosed at a younger age, and now they transitioned into older age. All these evidences consistent with the result of this study which revealed that the onset of UC occurs mainly in younger individuals, usually in the third decade of life among the patient groups. In relation to gender studies have noted either no preference regarding sex, or a slight predilection for men. Also, it was demonstrated that the CD is frequent amongst men with no gender difference for UC which compatible with the result of this study.

Sex-related differences have been found in the epidemiology of CD but were not so marked in UC. In a pooled analysis of population-based studies on 95,605 and 112,004 incident cases of CD and UC, respectively, found that age at UC onset varied with sex. Indeed, the incidence of UC is similar between males and females until age 45. After this age, females showed a 13% to 32% lower likelihood of being diagnosed with UC than males. Disease duration has been evaluated with the hypothesis that patients with shorter disease duration will have a better response to early treatment. Intuitively though, treating patients earlier, when inflammatory disease predominates over fibrosis, is appealing. On the other hand, worse response to treatment in patients with longer disease duration may be due to several factors, including a selection bias of patients with more severe disease and also a greater proportion of advanced fibrosing organ damage. This is consistent with the results of this study which demonstrated that the less duration seen in conventional group who don’t need for the biological treatment and had a better response to conventional therapy. Moreover, in this study on the impact of disease duration on treatment response and outcomes in (negative response) patients group, it was observed that 35% of them have a disease duration 5-10 years which indicated that the long disease duration is independently associated with increased risk of treatment failure. These findings suggest that early need for biologic therapy may be an independent negative prognostic factor in patients with UC, regardless of inflammatory burden, disease extent.

In a retrospective cohort study of 190 infliximab-treated patients with corticosteroid-dependent or -refractory patients, Murthy and colleagues observed a lower risk of infliximab failure and colectomy with longer disease duration; however, 35% of their cohort included patients with corticosteroid-refractory, hospitalized acute severe UC, patients who are intrinsically at significantly higher risk of colectomy.

The results of this study disagree with another study which demonstrated that UC is primarily a luminal disease with mucosal inflammation, although long-term inadequate control of disease may result in mural changes and submucosal fibrosis. It was demonstrated either no association between disease duration and response to therapy or higher risk of treatment failure in patients with short disease duration at the time of starting biologic therapy.

With respect to miR-155, the difference in mean fold change was significant among study groups ($p <0.001$), the level was highest in patients on conventional treatment (positive response) followed by patients on biological therapy (negative response).
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and then by control group. MiR-155 is an miRNA that downregulates more than 25 target genes and regulates various physiological and pathological processes, including immunity, inflammation, and cancer. MiR-155 support a positive correlation between miR-155 upregulation and NF-kB activation which may act either directly or indirectly to increase the rate of translation of TNF-α transcripts, and miR-155 is involved in the activation of the TNF-a pathway. These facts suggest that miR-155 is involved in the regulation of various inflammatory reactions.19

During inflammatory responses, miR-155 is rapidly upregulated by NF-kB within the first 12 hour of inflammatory response. Subsequently, by targeting SHIP1, miR-155 activates the IKK signalosome complex in a PI3K/Akt-dependent manner, forming a positive feedback loop necessary for signal amplification. Thus, miR-155 can cross-regulate inflammatory responses.20

A report indicated that the expression of 7 miRNAs (miR-16, miR-19a, miR-21, miR-101, miR-155, miR-223, and miR-594 was elevated in the colonic mucosa of both UC and CD compared to healthy controls 21. It has been frequently demonstrated that the miR-155 expression is markedly increased in inflamed colonic mucosa of UC patients thus, it is not unexpected that this present study found miR-155 to be more highly expressed in UC than in control which is consistent with results of this study.

Several studies have reported on the role of other miRNAs in IBD in general, both exploring mechanistic roles and the potential role of miRNA as biomarkers and drug targets. It was stated that in the DSS-induced colitis model, loss of miR-155 was found to ameliorate colitis by reducing TNF-α and interleukin-6, -12 and -17 in the tissue. 22

In patients and animal models of colitis, the systemic level of TNF-α, IFN-γ, IL-6, and IL-12 is significantly increased, which might be responsible for inflammatory status in IBD. Additionally, it was discovered fewer symptoms (minor change in body weight and no diarrhea or blood in feces), decreased numbers of Th1/17 cells, macrophages, and DCs as well as the reduced amount of inflammatory cytokines such as TNF-α, IFN-γ, IL-6, and IL-12 in miR-155 deficient animal model of IBD. Moreover, miR-155 might have a crucial role in the development of dextran sulfate sodium (DSS)-induced colitis in mice.23

It was found that miR-155 can directly increase TNF-α levels by augmenting transcript stability through binding to its 3’UTR, and in vivo studies showed that miR-155 probably directly targets transcript coding for several proteins involved in lipopolysaccharide (LPS) signaling while enhancing TNF-α translation.24 Other study have also shown that miR-155 is significantly up-regulated in blood samples from UC patients and is the highest among all the up-regulated miRNAs. 25

MiR-155 was elevated at a statistically significant level in the UC endoscopically involved colonic tissue in comparison to the endoscopically uninvolved colonic tissue.26 The results of the all above studies consistent with the results of the present study.

However, some authors did not find increased miR-155 levels in the blood from IBD patients, suggesting that more studies are needed to determine whether miR-155 is a putative blood-related biomarker. 27 Keeping in mind the present study support blood-related biomarker value for miRNA-155.

The NF-kB pathway is an important mediator of inflammatory responses, and overexpression of miR-155 has been shown to trigger NF-kB activation in mouse macrophages. MiR-155 positively correlated with NF-kappa B activation and contributed to development of cancer and inflammation.28 Blockade of the miR-155/NF-kB axis is a promising treatment strategy for IBD.29 miR-155-mediated reduction of FOXO3a has been shown to positively regulate nucleotide-binding domain-like receptor protein 3 (NLRP3) inflammasome. This finding is supported by data showing that induced NF-kB expression leads to higher NLRP3 expression. Blockade of miR-155 in lipopolysaccharide (LPS)-activated RAW 264.7 cells resulted in downregulation of inflammatory cytokines by reducing pNF-κB and NLRP3-related proteins. 29

Based on these findings, in this study the use of the biological drug was evaluated, and miR-155 is correlated with clinical response, and its expressions was efficiently inhibited with IFX treatment (negative response) than with longer-term GCs conventional treatment (positive response). This result disagree with another study which found that TNF-α treatment increases the expression of miR-155 in HT-29 colonic epithelial cells.30 While a study examining miR-155 in the serum during the induction of anti-TNF-α therapy in adult patients with IB could not find differences in the expression of this miRNA, and no data are available on their local effects during biological therap.31

Furthermore, it was reported that inhibition of miR-155 in intestinal myofibroblasts from UC patients resulted in decreased cytokine production and higher expression of suppressor of cytokine signaling 1 (SOCS1), whereas silencing of SOCS1 in intestinal control myofibroblasts greatly increased the production of pro-inflammatory cytokines. All these data together explain the low level of miRNA 155 in biological group of ulcerative colitis patients.

Serum-monitoring biomarkers could provide an alternative method of measuring disease activity and potentially allow for earlier assessment of response and optimization of therapy.

The results of the present show that the expression of miR-223 is inversely correlated with the disease activity of UC. Besides that, this study have evaluated the therapeutic response to corticosteroid, or IFX therapy and blood expression of miRNA by screening the responses to anti-TNF-α and conventional therapy, this study demonstrated that the level of miRNA associated with the response to IFX which is highly increased in negative response comparing to the positive response group after IFX therapy.
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MicroRNA-233 is a crucial regulator of innate immunity, including myeloid differentiation and the function of neutrophils and macrophages. Recently, miR-223 has been shown to participate in the regulation of the immune response and the production of cytokines in the pathogenesis of IBD.

NF-κB pathway plays a central role in regulating the release of cytokines in patients with UC and participates in the inflammation and immune response in the intestinal tract of UC. It indicates that NF-κBp65 highly expresses in intestinal mucosal epithelium, crypt epithelial cells and lamina propria monocytes of patients with UC. NF-kappab p65 antisense oligonucleotides blocks NF-κB pathway and down-regulates NF-κB-dependent IL-1beta and IL-8 mRNA expressions, which attenuates the productions of pro-inflammatory cytokines in lamina propria mononuclear cells from patients with UC.

The effects of miR-223 on NF-κB are multiple. NF-κB is composed of p65 and p50 subunits which are suppressed by the inhibitory subunit IκBα (NF-κB polypeptide gene enhancer in B cells inhibitor alpha). IKKα (IkB kinase alpha), a negative regulator of IκBα, has been identified as a target of miR-223. By comparing 30 UC patients and 20 non-IBD controls, Valmiki and his colleagues show that miR-223 levels were 8.63 folds of that of controls while IKKα was 40% less in UC patients than in controls.

The decreased effect of IKKα on IκBα should lead to activation of NF-κB. The NF-κB activators Traf6/Tab1 (TNF receptor-associated factor 6/TGF-beta activated kinase 1) was identified as targets of miR-223. Transfection of miR-223 into HUVECs blocked nuclear translocation ofNF-κB as well as inhibition of p38 MAPK, JNK and ERK phosphorylation. This suggest that miR-223 could reduce proinflammatory status in IBD through inhibiting NF-κB pathway.

One of the pro-inflammatory cytokines activated by NF-κB is tumor necrosis factor-α (TNF-α), whose feedback stimulates NF-κB by binding to TNF receptors (TNFR1 or TNFR2), resulting in a kinase cascade. The TNF-α level is increased in the blood, stool and intestinal tissue from patients with CD and UC, and therefore it has been a target for medical treatment. Infliximab and adalimumab are therapeutic antibodies that block the binding of TNF-α to its cell-surface receptors and limit downstream cell signaling pathways.

In IBD patients, the increased NF-κB expression in mucosal macrophages is accompanied by an increased capacity of these cells to produce and secrete TNF-α, IL-1 and IL-6. This finding nicely reflects the central function of NF-κB in monocytes, which is the induction and control of pro-inflammatory cytokines. Many of the already established immunosuppressive drugs in IBD like anti-TNF-α antibodies are known to mediate their anti-inflammatory effects at least partly via inhibition of NF-κB activity.

Moreover, in the present study the level of miRNA 223 in patients group (positive and negative response) was highly significant in comparison to the control group (p=0.001). The results of this study agree with another study which demonstrated increased miR-223 levels in colonic tissues and blood samples in both IBD patients and animal models. miR-223 levels were highly increased.

In previous study with IL-10−/− mice, miR-19a, miR-146a, miR-155, miR-223, and miR-375 were selectively dysregulated in whole blood of mice with mild intestinal pathology.

Recently, >800 fecal miRNAs have been investigated in stool samples of patients with UC compared to controls. Findings suggest that patients with active UC have distinct fecal miRNA profiles compared to non-IBD patients, particularly higher levels of miRNA-223. Moreover, analyzed both serum and fecal miRNAs in IBD and found increased levels of and miR-223 in both sera and feces from the IBD patients compared to controls. MiR-223 in feces can well distinguish IBD patients in the active stage and remission stage, and the sensitivity and specificity are 80 and 93%, respectively.

An assessment of miRNA expression in terminal ileum biopsies from 6 patients with chronically active terminal ileal CD and 6 control individuals revealed that miR-16, miR-21, miR-223, and miR-594 were overexpressed in chronically active terminal ileal CD tissues. All above studies consistent with the result of this study regarding the elevated level of miRNA-223 among patients group in comparison to the control group.

Additionally, the expression of seven miRNAs showed in remarkable changes after treatment in responders but not in non-responders in a small cohort of pediatric IBD with diverse treatments including anti-TNF mAbs. However, the association of miRNA polymorphisms with anti-TNF treatment response did not detect any correlations between studied miRNA polymorphisms and patients’ response to anti-TNF mAbs. Therefore, the profile of serum or mucosa miRNAs as promise biomarkers in analyzing the therapeutic response to anti-TNF mAbs in IBD patients remains to be investigated in future clinical practice.

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