Comparison of the Expression Levels of SIRT1 and NF-κB Genes in Patients With Inflammatory Bowel Disease and Healthy Individuals

Hengame Yaghoubi[1], Sohrab Halalkhor[2], Hadi Parsian[3], Saman Alhooei[4], Tayebe Azramezani[5], Zahra Geraeili[6]

[1] *Research Committee, Babol University of Medical Sciences, Babol, Iran*

[2] *Department of Biochemistry, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran*

[3] *Cellular and Molecular Biology Research Centre, Babol University of Medical Sciences, Babol, Iran*

[4] *Clinical Research Development Unit of Rouhani Hospital, Babol University of Medical Sciences, Babol, Iran*

[5] *Department of Biochemistry, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran*

[6] *Social Determinants of Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*

Received: 28 Jan. 2024; Accepted: 28 Aug. 2024

Abstract- Invasive methods such as colonoscopy and tissue biopsy are used to diagnose and differentiate inflammatory bowel disease. In this study, we aimed to use the real-time PCR method to examine changes in SIRT1 and NF-κB gene abundance in IBD patients and healthy individuals and to distinguish IBD. This case-control study was conducted on 30 IBD patients and 30 healthy controls. SIRT1 and NF-κB levels in peripheral blood leukocytes of the samples were measured by real-time fluorescent quantitative PCR. According to this study, the expression of the SIRT1 gene in blood leukocytes of IBD patients was lower than that of the healthy group. Using the ROC curve, it was found that SIRT1 gene expression could distinguish IBD patients from healthy individuals with a sensitivity and specificity of 83% and 77%, respectively. According to the results of this study, the expression of the NF- kB gene in the blood white blood cells of IBD patients increased compared to the healthy group. According to the ROC curve, NF- kB gene expression can distinguish IBD patients from healthy individuals with a sensitivity and specificity of 86% and 72%, respectively. The results of this study, together with other methods, may help diagnose and analyze the disease in IBD patients.

Acta Med Iran 2024;62(November-December):333-337.

Keywords: Inflammatory bowel disease; Ulcerative colitis; Sirtuin 1 (SIRT1); Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)

Introduction

Inflammatory bowel disease (IBD) is a term that refers to two chronic inflammatory diseases of the digestive system: ulcerative colitis and Crohn's disease. Although the exact cause of IBD is still unknown, both environmental and genetic factors are thought to play a role in the development of the disease (1). Ulcerative colitis typically affects the rectum and part or all the colon, while Crohn's disease is often associated with transmural inflammation. Inflammation in ulcerative colitis is generally limited to mucosa. In contrast, Crohn's disease can be associated with granulomas, strictures, and fistulas in the intestines that are not seen in ulcerative colitis (2). Ulcerative colitis (UC) is a chronic inflammatory disease that damages the large intestine (3). This particular disease often affects people between the ages of 30 and 40 (4). The disease is characterized by recurrent mucosal infections that typically begin in the anus and spread to the proximal colon (5). In recent decades, there has been a significant increase in the prevalence of inflammatory bowel disease (IBD) in many countries (6). Many countries have seen a significant increase in the prevalence of IBD in recent decades. This has led to a large economic and social burden on health care systems (7). Sirtuins play a key role in NAD-

Corresponding Author: S. Halalkhor
Department of Biochemistry, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran
Tel: +98 9115847956, E-mail address: yaghoubihengame@gmail.com

dependent deacetylation of histone and non-histone proteins (8). SIRT1-dependent deacetylation affects numerous biological processes such as cellular senescence, apoptosis, lipid metabolism, oxidative stress, and inflammation; Therefore, even small changes in SIRT1 expression and function can affect cellular responses. SIRT1 is known for its antioxidant and anti-inflammatory properties (9). Activation of NF-κB by proinflammatory cytokines such as IL-1 and TNF-α leads to the expression of other proinflammatory genes, including cytokines, chemokines, and adhesion molecules. This makes NF-κB a well-known pro-inflammatory signaling pathway (10). Various studies have demonstrated the key role of NF-κB in various biological processes, including cell proliferation, metastasis, DNA damage response, apoptosis, and immune responses through its wide array of target genes. Therefore, NF-κB is associated with human diseases such as inflammation, cancer, and autoimmune diseases (11). Inflammatory bowel diseases (IBD) affect two immune systems–innate immunity and adaptive immunity. Both systems are controlled by the NF-κB inflammatory signaling pathway. Research suggests that NF-κB activation has been observed in the inflamed intestinal tissue of IBD patients (12). Studies suggest that SIRT1 reduces inflammation by inhibiting the expression of inflammatory cytokines through NF-κB. This is achieved through deacetylation of lysine 310, which reduces the level of P65 acetylation and promotes anti-inflammatory effects (13). It is speculated that SIRT1 and NF-κB play a role in the development of inflammatory bowel disease.

Materials and Methods

The participants in this study consisted of people with inflammatory bowel disease who were referred to Hospital for diagnosis or treatment. Patients were examined by a gastroenterologist and underwent colonoscopy. Besides colonoscopy, other criteria that differentiate IBD patients from healthy people include family history, positive FOB tests, digestive problems and erythrocyte sedimentation rate test. The pathologist confirmed his diagnosis and referred him to us to participate in the research project. The research study involved 30 male and female patients between the ages of 20 and 48 who were diagnosed with inflammatory bowel disease, specifically ulcerative colitis. The patients considered in this study were recently analyzed for IBD in the active phase of the disease. These people were not taking medications to treat the disease. In addition, the study included 30 healthy people who were the same age and gender as the patient group and were considered a control group. The method of randomizing the control group is simple random sampling. The criteria for patients to participate in the study were Iranian and a colonoscopy report, while healthy subjects only had to be Iranian. This ensured that the average age and gender of the control group was almost the same as that of the patient group, allowing for a fair comparison. Exclusion criteria for the patient and control groups included a history of chronic upper gastrointestinal diseases such as: Stomach ulcers, pregnancy and the presence of a tumor. A total of 5 ml blood samples were collected from eligible patients. Red blood cells were lysed with ammonium chloride solution and white blood cells were separated by gradient centrifugation. The isolated blood leukocytes were stored at −80° C for further analysis. Total RNA was extracted from isolated blood leukocytes using a YTA RNA extraction kit (Yekta Tehiz Azuma, Tehran, Iran). The extracted total RNA was then converted to DNA using the REVERT AID FIRST STRAND cDNA Synthesis Kit (THERMO SCIENTIFIC, USA). Complement (cDNA) was reversely transcribed. Real- time PCR reactions were then performed on a QIAGEN refurbished QIAGEN ROTOR-GENE Q MDX REAL-TIME PCR system using the SYBR PREMIX EX TAQTM II (TAKARA, Japan) according to the manufacturer's instructions. This procedure consists of 1 cycle of 95° C for 10 minutes, followed by 40 cycles of 95° C for 15 seconds, and 40 cycles of 59° C for 40 seconds. The nature of the reaction was confirmed by analyzing the curve. Finally, contrast-enhanced CT was used to process the data. For this purpose, 2 DCTs of the sample were calculated: DCT=CT (flower gene) CT (internal control). Use B-2MICROGLOBULIN (B2M) as the reference gene and the primer set is as follows:

B2M F: TGCTGTCTCCATGTTTGATGTATCT
B2M R: TCTCTGCTCCCCACCTCTAAGT
SIRT1 F: GAACAGTGAGAAAATGCTGGC
SIRT1 R: CGATTAACCTGCCGAAATAGC
NF-KB F: AACACTGAGGAGACTGCACAC
NF-KB R: ATCCTTGAGCTTGGGTCTGTC

Results

The study examined 30 people with inflammatory bowel disease and 30 healthy controls. Only people of Iranian descent were selected for the study; non-Iranian ethnicities such as Afghans and other ethnicities were excluded. The study included information about participants' characteristics, such as: Age, gender, family

history, type and severity of the disease and the overall population. This information can be found in figures 1 and 2, which show the changes in SIRT1 and NF-κB gene expression in the studied groups, i.e. H. the sick and healthy groups. Based on the results of the Mann-Whitney statistical test, it was found that the average expression of SIRT1 in healthy individuals was 135.1±0.07, while in people with inflammatory bowel disease it was 0.29±0.169. These results show that there is a significant statistical difference between the two groups (*P*=0.001).

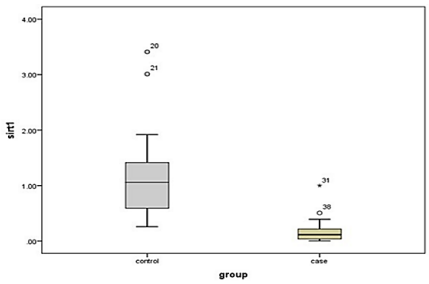

Figure 1. Changes in the expression of SIRT1 genes between groups of people with intestinal inflammation and control

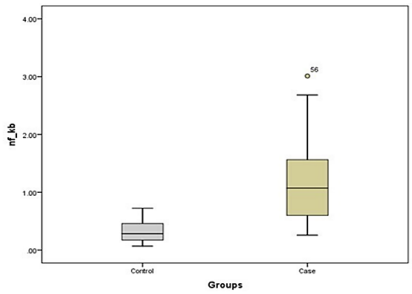

Figure 2. Changes in the expression of NF- κB genes between groups of people with intestinal inflammation and control

Based on the results of the Mann-Whitney statistical test, it was found that the average expression of NF-κB was 13±0.326 in healthy subjects and 178.1±0.06 in subjects with inflammatory diseases. These results suggest that there is a statistically significant difference between the healthy group and those with inflammatory bowel disease(*P*=0.001).

Based on the displayed ROC curves, it can be deduced that SIRT1 values can distinguish between people with inflammatory bowel disease and healthy people with a sensitivity and specificity of 83% and 77%, respectively at a cutoff threshold point=0.185 (AUC: 0.815, *P*=0.001) as shown in Figure 3.

Based on the displayed ROC curves, the NF-κB level was found to be a reliable indicator for distinguishing between patients with inflammatory bowel disease and healthy individuals with a sensitivity of 86% and a specificity of 72%, respectively at a cutoff threshold point=0.437 (AUC: 0.955, *P*=0.001) as shown in Figure 4.

Figure 5 shows the changes in the amount of CRP between the groups examined, namely those with and those without intestinal inflammation. The results of the Kruskal-Wallis statistical test show that the mean CRP in subjects with intestinal inflammation was 6.47±2.66, while in healthy subjects the gene expression level was 1.6±0.56. These results show a statistically significant difference between individuals with intestinal inflammation and those in the control group (*P*=0.001).

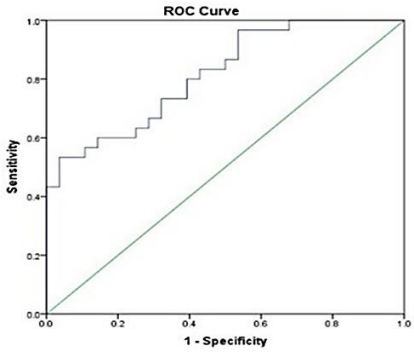

Figure 3. ROC curve of SIRT1 to distinguish patients with intestinal inflammation from healthy people

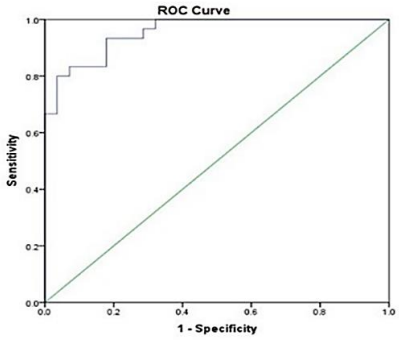

Figure 4. ROC curve of NF-κB to distinguish patients with intestinal inflammation from healthy people

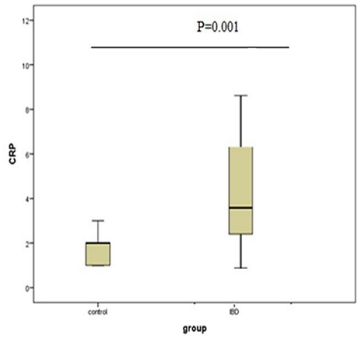

Figure 5. Comparison of CRP changes between the patient and control groups

Discussion

In line with our study, Caruso and colleagues performed a study analyzing SIRT1 gene expression in biopsy samples from 29 ulcerative colitis patients, 28 Crohn's disease patients, and 21 healthy subjects. They used a real-time PCR method to show that SIRT1 levels were reduced in biopsy samples from people with ulcerative colitis and Crohn's disease. Similarly, in our study, we found that SIRT1 gene expression was lower in ulcerative colitis patients compared to healthy subjects. However, due to limitations in our research, we were unable to obtain biopsy specimens from patients with Crohn's disease (14).

Research by Deng and colleagues in 2020 found that miR-4262 plays a key role in regulating immune responses in inflammatory bowel disease (IBD) by affecting the SIRT1 gene. The team used RT-PCR to examine the expression of miR-4262 in 30 IBD patients and 30 normal colon tissues. The results showed an increase in the expression level of miR-4262 in the colon tissue of IBD patients compared to the control group. In addition, the expression of SIRT1 as a target gene of miR-4262 was lower in the colon tissue of IBD subjects compared to the control group. In our study, we found that SIRT1 gene expression was decreased in IBD patients compared to healthy subjects. However, we did not examine the expression of miR-4262 in our study (15).

Consistent with our study, a study by Sharma and colleagues shows that in a DSS-induced colitis model, the expression of TNF-α converting enzyme (TACE) increases, while the increase of TIMP3 enzyme (a TACE inhibitor) decreases SIRT1. In our study, we found that the expression of SIRT1 was significantly decreased in individuals with ulcerative colitis compared with healthy people. However, our study does not mention the role of TNF-α and related enzymes (16).

In 2020, Khazdouz and colleagues conducted a study on 89 IBD patients. They found that taking selenium supplements led to a reduction in the severity of UC. The study also found that SIRT1 expression increased in the group of patients who received selenium supplements compared to the group of patients who received placebo (17).

A study by Hegazy and colleagues showed that patients with ulcerative colitis (UC) had higher expression of NF-κB p65 compared to controls. However, after treating UC patients with sulfasalazine, an antibiotic, levels of NF-κB p65 were reduced, leading to reduced inflammation. Similarly, our study also found higher NF-κB gene expression in people with ulcerative colitis compared to those without the disease. However, a limitation of our study was that no patients with ulcerative colitis were treated (18).

Consistent with our study, the study by Nguyen *et al*., In 2021, 19 patients with Crohn's disease, 8 patients with ulcerative colitis, and 15 healthy subjects demonstrated that the NF-κB signaling pathway in IBD patients is functional. It is significantly increased, which is associated with inflammation of the digestive tract, the death of epithelial cells and the migration of lymphocytes. The results of our studies also indicate an increase in NF-κB gene expression in people with IBD compared to healthy people (19).

In 2018, Chen and colleagues showed that sodium butyrate (SB) attenuates inflammatory responses caused by TNBS consumption and intestinal epithelial barrier dysfunction by inhibiting the AKT and NF-κB-P65 signaling pathways. NF-κB gene expression as a pro-inflammatory factor was found to be higher in ulcerative colitis patients than in healthy people (20).

The aim of this study was to identify non-invasive biomarkers to examine the relationship between SIRT1 and NF-κB gene expression in people with inflammatory bowel disease.

Based on the results of this study, it was found that there is a significant decrease in the expression of the SIRT1 gene and a significant increase in the expression of the NF-κB gene in the blood leukocytes of patients suffering from inflammatory bowel disease compared to healthy ones Control group. The study also found that gene expression levels of SIRT1 and NF-κB can help distinguish between individuals with ulcerative colitis and healthy individuals, with sensitivity and specificity of 83% and 77% for SIRT1 and 86% and 72% for NF -κB. Additionally, CRP levels were significantly higher in people with ulcerative colitis compared to healthy controls.

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