# CHANGES IN AND SIGNIFICANCE OF SERUM LEVELS OF NGAL, NGALR, MMP-9 AND CHE IN PATIENTS WITH ULCERATIVE COLITIS

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***ABSTRACT***

***Objective****: To investigate the changes in and significance of serum levels of neutrophil gelatinase-related apolipoproteins (NGAL), neutrophil gelatinase-related apolipoprotein receptor (NGALR), matrix metalloproteinase-9 (MMP-9) and choline esterase (ChE) in patients with ulcerative colitis.*

***Methods:*** *From April 2017 to September 2018, 67 patients with ulcerative colitis treated in our hospital were randomly selected as subjects. Thirty-seven healthy people who underwent a physical examination in our hospital at the same time were selected as the control group. According to the Truelove classification, patients were divided into three groups: mild (24), moderate (35) and severe (8). Serum levels of NGAL, NGALR, MMP-9, ChE, serum total bilirubin (STB), and the inflammatory factors C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) were compared among groups. The Pearson correlation test was used to analyze correlations among serum levels of NGAL, NGALR, MMP-9 and ChE in patients with ulcerative colitis. An ROC curve was established to analyze the diagnostic value of NGAL, NGALR, MMP-9 and ChE in ulcerative colitis.*

***Results:*** *Serum levels of NGAL, NGALR, MMP-9, TNF-α and CRP in the study group were significantly increased, ChE and STB levels were significantly decreased, and there was a significant correlation among all indicators (P<0.05). As the severity of the disease increased, serum levels of NGAL, NGALR, MMP-9, TNF-α and CRP increased gradually, while ChE and STB decreased gradually. Serum NGAL, NGALR, MMP-9 and ChE have significant diagnostic value for ulcerative colitis, and the detection of all indicators combined is of greater diagnostic value.*

***Conclusion:*** *Serum levels of NGAL, NGALR and MMP-9 in patients with ulcerative colitis were significantly higher than those in healthy subjects, whereas ChE and STB levels were significantly lower than those in healthy subjects. The level of each indicator correlated with the severity of the disease and had significant diagnostic value for the occurrence of ulcerative colitis.*

***Keywords:*** *Ulcerative colitis, NGAL, NGALR, MMP-9, ChE.*

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## Introduction

Approximately 12 per 100,000 people in China suffer from ulcerative colitis, a chronic non-specific positive intestinal disease. The predisposing age for exhibiting ulcerative colitis is between 20 and 40 years, and there is no significant difference in its incidence between men and women[1]. With the development of the social economy and the resulting change in people's lifestyles, the incidence of ulcerative colon cancer is increasing year by year. As a result of the advancement of medical technology, the clinical diagnosis of ulcerative colitis is also improving. At present, the pathogenesis of ulcerative colitis is not clear, but is believed to be related to environment, heredity, infection, inflammatory mediators and immune factors[2]. Some studies have found that clinical manifestations of ulcerative colitis can vary with the location, extent and degree of the disease.

The recurrence rate of ulcerative colitis is high and there is no treatment for its etiology. It is therefore important to find relevant indicators and closely monitor the changes that occur in relation to ulcerative colitis for clinical diagnosis and prognostic evaluation[3]. In this study, 67 patients with ulcerative colitis who were treated in our hospital from April 2017 to September 2018 were selected as subjects to observe changes and significance of neu-

trophil gelatinase-related apolipoproteins (NGAL), neutrophil gelatinase-related apolipoprotein receptor (NGALR), matrix metalloproteinase-9 (MMP-9) and choline esterase (ChE) in the serum of patients with ulcerative colitis.

## Materials and methods

### *Basic information*

The research conducted during this study was approved by the hospital ethics committee and accorded with all medical ethics.

From April 2017 to September 2018, 67 patients with ulcerative colitis treated in our hospital were randomly selected as subjects.

*Inclusion criteria were:*

• All patients met the relevant diagnostic criteria for ulcerative colitis[4];

• Condition was diagnosed as ulcerative colitis through enteroscopy or pathological examination;

• Patients and their families who participated in the study gave informed consent and signed informed consent;

• And patient medical records were complete and cooperated with the investigator.

*Exclusion criteria were:*

• Patients with severe liver and kidney function and/or heart dysfunction;

• Patients with a history of mental illness;

• Patients during pregnancy or lactation;

• And patients with infectious diseases.

Among the 67 patients chosen, there were 35 males and 32 females, aged 21 to 67 years old, with an average age of 47.57±3.61 years. According to the Truelove classification, patients were then divided into three categories, with 24 cases in the mild group (13 males and 11 females; 20-63 years old, mean age 46.29±3.74 years), 35 cases in the moderate group (18 males and 17 females; 23-65 years old, mean age 47.47±4.46 years) and eight cases in the severe group (five males and three females; 22-64 years old, mean age 46.69±3.78 years).

In addition, 37 healthy subjects who underwent a physical examination in our hospital at the same time were selected as the control group (19 males and 18 females; 22-66 years old, mean age 48.26±4.31 years). There was no significant difference in clinical data among the groups (P>0.05).

### *Methods and observation indicators*

All the research subjects were forbidden to drink or eat for at least 8 h before 5 mL of fasting venous blood was extracted from the median elbow the next morning. Blood samples were centrifuged at 3000 r/min (Beijing Era Beili Centrifuge Co., LTD., model: GTR16-2), and then the supernatant was taken and stored in a -80°C refrigerator (Wuxi Shengze Physicochemical Equipment Co. LTD, model: GY-A050N) for subsequent experiments. Serum levels of NGAL, NGALR and MMP-9 were determined for each group using an enzyme-linked immunosorbent assay (ELISA). Serum ChE and STB levels were analyzed using an automatic biochemical analyzer (Jinan Gelite Technology Co., Ltd., model: GRT-6008). The levels of C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) in each group were determined via ELISA. A receiver operating characteristic (ROC) curve was established to analyze the diagnostic value of NGAL, NGALR, MMP-9 and ChE in ulcerative colitis.

### *Statistical methods*

An independent sample t test was used to compare the measurement data between two groups, and a one-way ANOVA was used to compare among three or more groups. The counting data were compared using a $\chi^2$ test. A Pearson correlation test was used to analyze correlations among serum levels of NGAL, NGALR, MMP-9 and ChE in patients with ulcerative colitis. For all statistical analysis, $P<0.05$ was considered statistically significant and the SPSS22.0 software package was used.

## Results

### *Serum levels of NGAL, NGALR and MMP-9 in each group*

In comparison to the control group, serum levels of NGAL, NGALR and MMP-9 in the mild, moderate and severe groups were significantly increased ($P<0.05$; Table 1).

| Group | Cases (*n*) | NGAL (ng/mL) | NGALR (ng/mL) | MMP-9 (pg/mL) |
|---|---|---|---|---|
| Control | 37 | 43.54±8.53 | 16.22±6.38 | 5.55±1.04 |
| Research | | | | |
| Mild | 24 | 74.28±17.62 | 36.74±10.28 | 7.48±1.64 |
| Moderate | 35 | 112.13±14.35 | 51.15±10.06 | 10.12±3.03 |
| Severe | 8 | 132.68±25.76 | 69.16±15.16 | 13.59±3.99 |
| *F* | | 168.59 | 116.46 | 40.27 |
| *P* | | <0.001 | <0.001 | <0.001 |

**Table 1:** Serum levels of neutrophil gelatinase-related apolipoproteins (NGAL), neutrophil gelatinase-related apolipoprotein receptor (NGALR) and matrix metalloproteinase-9 (MMP-9) in a control group and in three research groups representing three levels of severity of ulcerative colitis ($\bar{x}$±s).

***Serum levels of ChE and STB in each group***

The serum levels of ChE and STB were lower in the mild, moderate and severe groups than in the control group (P<0.05; Table 2).

| Group | Cases (*n*) | ChE (IU/L) | STB (μmol/L) |
|---|---|---|---|
| Control | 37 | 8463.14±1624.33 | 12.09±3.45 |
| Research | | | |
| Mild | 24 | 8545.31±2216.32 | 9.62±4.26 |
| Moderate | 35 | 6606.27±2046.25 | 8.04±2.76 |
| Severe | 8 | 6315.21±1752.62 | 6.97±3.09 |
| *F* | | 13.93 | 10.44 |
| *P* | | <0.001 | <0.001 |

**Table 2:** Serum levels of choline esterase (ChE) and serum total bilirubin (STB) in a control group and in three research groups representing three levels of severity of ulcerative colitis ($\bar{x}\pm s$).

***Serum levels of inflammatory factors in each group***

In comparison to the control group, serum levels of TNF-α and CRP in the mild, moderate and severe groups were significantly increased (P<0.05; Table 3).

| Group | Cases (*n*) | TNF-α (mg/L) | CRP (mg/L) |
|---|---|---|---|
| Control | 37 | 11.32±2.86 | 1.27±0.55 |
| Research | | | |
| Mild | 24 | 18.69±3.14 | 5.06±1.02 |
| Moderate | 35 | 27.14±4.86 | 16.65±2.74 |
| Severe | 8 | 48.11±5.46 | 41.62±6.57 |
| *F* | | 234.64 | 725.13 |
| *P* | | <0.001 | <0.001 |

**Table 3:** SSerum levels of the inflammatory factors tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) in a control group and in three research groups representing three levels of severity of ulcerative colitis ($\bar{x}\pm s$).

***Correlations among serum levels of NGAL, NGALR, MMP-9, ChE, STB, TNF-α and CRP in patients with ulcerative colitis***

Pearson correlation analysis showed that serum levels of NGAL, NGALR, MMP-9, ChE, TNF-α and CRP in patients with ulcerative colitis were all correlated (P<0.05; Table 4).

| Indicators | NGAL | NGALR | MMP-9 | ChE | STB | TNF-α | CRP |
|---|---|---|---|---|---|---|---|
| NGAL | — | 0.372 | 0.139 | -0.011 | -0.279 | 0.259 | 0.047 |
| NGALR | 0.372 | — | 0.344 | -0.009 | -0.751 | 0.545 | 0.236 |
| MMP-9 | 0.139 | 0.344 | — | -0.002 | -0.417 | 0.455 | 0.234 |
| ChE | -0.011 | -0.009 | -0.002 | — | 0.003 | -0.936 | -0.001 |
| STB | -0.279 | -0.751 | -0.417 | 0.003 | — | -0.003 | -0.105 |
| TNF-α | 0.259 | 0.545 | 0.455 | -0.936 | -0.003 | — | 0.115 |
| CRP | 0.047 | 0.236 | 0.234 | -0.001 | -0.105 | 0.115 | — |

**Table 4:** Correlations among serum levels of neutrophil gelatinase-related apolipoproteins (NGAL), neutrophil gelatinase-related apolipoprotein receptor (NGALR), matrix metalloproteinase-9 (MMP-9), choline esterase (ChE), serum total bilirubin (STB), tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) in patients with ulcerative colitis.

***Diagnostic value of serum levels of NGAL, NGALR, MMP-9 and ChE in ulcerative colitis***

The ROC curve results showed that the areas under the curves of NGAL, NGALR, MMP-9, ChE and NGAL+NGALR+ MMP-9 +ChE were 0.911, 0.843, 0.817, 0.796 and 0.953, respectively. All indices were of significant value in the diagnosis of ulcerative colitis, and the combination of all indices had the greatest diagnostic value (Table 5).

| Indicators | Area under the curve | Sensitivity (%) | Specificity (%) |
|---|---|---|---|
| NGAL | 0.911 | 89.99 | 90.14 |
| NGALR | 0.843 | 82.64 | 79.42 |
| MMP-9 | 0.817 | 64.52 | 85.31 |
| ChE | 0.796 | 75.24 | 71.73 |
| Combined detection | 0.953 | 96.24 | 95.51 |

**Table 5:** Diagnostic value of serum levels of neutrophil gelatinase-related apolipoproteins (NGAL), neutrophil gelatinase-related apolipoprotein receptor (NGALR), matrix metalloproteinase-9 (MMP-9) and choline esterase (ChE) in ulcerative colitis.

## Discussion

Ulcerative colitis is a chronic non-specific intestinal inflammatory disease for which the etiology has not been fully identified. With the continuous improvement of people's lives, the incidence of ulcerative colitis is gradually increasing, and it has become a research hotspot and difficult problem in the field of digestive system diseases[5]. Some scholars believe that the pathogenesis of ulcerative colitis is closely related to immunology, genetics, microbiology and molecular biology, with the intestinal environment and normal intestinal flora also playing important roles[6]. Ulcerative colitis is characterized by repeated attacks, which seriously affect the physical and mental health of patients[7]. It is therefore of great importance to find relevant indicators for early diagnosis, in addition to timely treatment for patients with ulcerative colitis.

Studies have found that the occurrence and development of ulcerative colitis result from the interaction of multiple factors[8]. As a member of the lipocalin family, NGAL is a β-fold barrel formed by eight antiparallel β-folds through interchain hydrogen bonding. It can transport arachidonic acid, prostaglandin, fatty acid, iron, etc., to produce different functions. Under normal circumstances, NGAL levels are low, and when tumors occur, NGAL expression levels increase significantly. Studies have confirmed that NGAL expression is significantly in-

creased in breast cancer, esophageal cancer and other malignant tumors, and the expression level of NGAL is significantly related to the degree of differentiation and deterioration of tumor cells[9]. NGALR is a receptor for NGAL and, in combination with NGAL, it can transport iron into cells, having important effects on cell growth, differentiation and apoptosis[10]. MMP-9 is a member of the MMP family, and can participate in the degradation of extracellular matrix, regulate cell adhesion, and directly or indirectly participate in inflammatory reactions. Schwegmann et al.[11] showed that the expression level of MMP-9 is significantly increased in ulcerative colitis, with expression being highest in severely inflamed tissues.

The normal intestinal mucosal barrier is composed of a mechanical barrier, a chemical barrier, an immune barrier and a biological barrier, all of which play an important role in anti-infection, injury and immune defense[12]. Studies have shown that patients with ulcerative colitis have increased intestinal mucosal permeability, and a decline in antioxidant capacity may be an important factor in mucosal damage[13]. Most of the bilirubin in serum is derived from the oxidation of hemoglobin produced by the destruction of senescent red blood cells. Direct bilirubin is glucuronidated in the liver, whereas indirect bilirubin is not glucuronidated in the liver. STB is the sum of direct bilirubin and indirect bilirubin. According to Shi et al.[14], under normal conditions, STB is an important endogenous antioxidant, and high levels of STB can significantly reduce the incidence of diabetes, colorectal cancer and the like.

ChE is an enzyme that catalyzes the hydrolysis of acylcholine, including acetylcholine esterase (AChE) and pseudocholine esterase (PChE). The plasma concentration of ChE can reflect its synthesis rate and is therefore an important index for evaluating liver synthesis function. Some studies suggest that ChE can inhibit the release of inflammatory factors such as TNF-α by macrophages, thereby reducing the systemic inflammatory response[15].

This study found that the serum levels of NGAL, NGALR, MMP-9, TNF-α and CRP were significantly increased in the ulcerative colitis groups in comparison to the control group, and the levels of ChE and STB were significantly reduced for those respective groups. In addition, there were significant correlations among all the indicators ($P<0.05$). With an increase in patient severity, the serum levels of NGAL, NGALR, MMP-9, TNF-α and CRP gradually increased, and levels of ChE and STB gradually decreased. Serum levels of NGAL, NGALR, MMP-9 and ChE all have a significant diagnostic value for ulcerative colitis, and combining the various indicators has an even greater diagnostic value.

In summary, serum levels of NGAL, NGALR and MMP-9 are significantly higher in patients with ulcerative colitis than in healthy subjects, and serum levels of ChE and STB are significantly lower in patients with ulcerative colitis than in healthy subjects. Each index level significantly correlates with the severity of the patient's condition, and has significant diagnostic value for the occurrence of ulcerative colitis.

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