

# Expression of serum CYR61 mRNA and protein in patients with tibial fracture and its relationship with prognosis

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**Objective:** To explore the relationship between the expression of serum CYR61 mRNA and protein and prognosis in patients with tibial fracture. **Methods:** Eighty-six patients with tibiofibular fractures (fracture group) admitted to Xi'an Central Hospital from July 2016 to July 2018 and 84 healthy controls (control group) who underwent physical examination in our hospital were selected as the study subjects. The imaging healing score (RUST) and prognosis were divided into two subgroups, 74 in the normal healing group and 10 in the abnormal healing group. The serum CYR61 mRNA and protein expression levels were compared and the relationship between serum CYR61 mRNA and protein expression levels and fracture prognosis was analyzed. **Result:** The relative expression levels of CYR61 mRNA and protein in the normal healing group were  $(2.35\pm 0.49)$  and  $(0.33\pm 0.10)$ , respectively  $(2.02\pm 0.29)$  and  $(0.23\pm 0.07)$  in the abnormal healing group and  $1.88\pm 0.37$  in the control group.  $(0.26\pm 0.06)$ , the difference between the three groups was statistically significant ( $P<0.05$ ), and the serum CYR61 mRNA and CYR61 protein in the normal healing group were significantly higher than the control group ( $P<0.05$ ), and the serum CYR61 mRNA in the abnormal healing group. CYR61 protein was significantly lower than the normal healing group ( $P<0.05$ ). The RUST of the normal healing group was  $(11.35\pm 1.51)$ , which was higher than that of the abnormal healing group  $(7.17\pm 0.95)$ , and the difference was statistically significant ( $P<0.05$ ). Serum CYR61 mRNA and protein expression levels were significantly positively correlated with prognosis ( $P<0.05$ ). ROC curve analysis showed that the AUC of serum CYR61 mRNA and protein were 0.735 and 0.778, respectively, and the Cut-off values were 2.363 and 0.338, respectively. The sensitivities were 60.80% and 54.10%, respectively, and the specificities were 80.00% and 90.00%, respectively. They were 0.408 and 0.441, respectively, thus indicating that serum CYR61 mRNA and protein expression levels have predictive value for prognosis. **Conclusion:** The higher the mRNA and protein expression levels of serum CYR61 in early stage, the better the prognosis of fracture healing in patients with tibiofibular fractures. To a certain extent, serum CYR61 can be used as a reference for predicting the prognosis of tibiofibular fractures.

**Key words:** Fracture; Serum cysteine-rich 61; mRNA; Protein; Prognosis

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With the development of modern medicine, at present, there are many treatment methods for humeral fractures, including cast immobilization, bone traction, open reduction and internal fixation, surgery, etc., and have achieved good therapeutic effects; however, due to massive blood loss and

blood supply damage Invasion of bacteria, etc., some patients will have symptoms such as compartmental compartment syndrome during treatment, resulting in poor bone prognosis and difficult healing, which will adversely affect the quality of life of patients <sup>1</sup>. Related studies <sup>2</sup> found

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that early vascular formation of fractures is of great significance for fracture healing, and largely determines the fracture healing rate and prognosis of fractures. Cysteine rich 61 (CYR61) is one of the most representative members of the CCN family, which has the effects of inducing angiogenesis and promoting chondrogenesis<sup>3</sup>. At present, there are many studies on CYR61 and tumors, but there are relatively few studies on fracture healing. Based on this, this study selected 84 patients with tibiofibular fractures admitted to our department in the past 3 years and 84 healthy subjects who underwent physical examination in our hospital for the purpose of exploring the relationship between CYR61 mRNA and protein expression and prognosis. In order to provide a theoretical basis for the clinical application of this indicator, the results of the study are reported below.

## MATERIALS AND METHODS

### General information

With the approval of the Medical Ethics Committee, 84 patients with tibiofibular fractures admitted to Xi'an Central Hospital from July 2016 to July 2018 were retrospectively selected as the study group. According to the radiographic union score for tibial fractures (RUST) and the actual prognosis of patients with fractures were divided into two subgroups, including 74 cases of normal healing group, 49 males and 25 females, aged 19-42 years old. The average age was (32.76±5.47) years old; the fracture site of the tibia: 38 cases on the left side and 36 cases on the right side; the cause of the fracture: 23 cases of high-altitude fall, 37 cases of traffic accidents, 26 cases of falls; AO classification<sup>4</sup>: A1 type 26 For example, there are 31 cases of A2 type and 29 cases of A3 type. 10 cases of abnormal healing group, 7 males and 3 females, aged 20 to 43 years, mean age (33.15±5.31) years old; fractures of the tibia: 5 cases on the left side, 5 cases on the right side; causes of fracture: 3 cases of falling from high altitude 4 cases of traffic accidents, 3 cases of falls; AO classification: 3 cases of A1 type, 4 cases of A2 type, 3 cases of A3 type. Inclusion criteria: (1) Complete clinical data; (2) Meet the diagnostic criteria for tibiofibular fracture

s in the editorial committee of the Chinese Journal of Trauma and Orthopaedics<sup>5</sup>, and confirmed by the MRI, CT and other imaging examinations as fractures of the tibia; (3) Fracture sites All were fractures for the first time, and the time from fracture-to-fracture fixation was ≤ 3 days. Exclusion criteria: (1) Pathological fractures caused by non-trauma; (2) Malignant tumors such as liver cancer and esophageal cancer; (3) Systemic metabolic diseases such as diabetes; (4) Long-term use of glucocorticoids, alkylation Immunosuppressive drugs such as agents. Another 84 healthy people who underwent physical examination in our hospital were selected as the control group, 46 males and 26 females, aged 19-42 years, mean age (32.86±5.21) years old. There were no significant differences in age, gender, fracture site and fracture cause between the normal healing group and the abnormal healing group (P>0.05). There was no significant difference in gender and age between the observation group and the control group (P>0.05). The general data of hemoglobin, albumin and ferritin in each group were not significantly different (P>0.05), as shown in Table 1.

Grouping	Hemoglobin	Albumin	Ferritin
Normal healing group (n=74)	10.98±1.93	3.85±0.57	104.75±27.43
Abnormal healing group (n=10)	11.23±1.13	3.83±0.46	95.17±24.41
Control group (n=84)	11.45±1.40	4.00±0.61	101.63±33.33
t value	1.607	1.429	0.526
P value	0.204	0.242	0.592

### Method

(1) Treatment methods for fractures. After admission to the hospital, patients with tibiofibular fractures are routinely examined for blood and imaging, and appropriate treatment plans are developed according to the patient's condition; debridement and anti-infection interventions are performed according to the patient's condition, and the fracture site corresponding to the condition is selected. (2) Prognosis assessment. Patients with fractures were reviewed 24 weeks after the fracture, and 2 experienced orthopedic surgeons evaluated

the fracture healing of the patients using the RUST system. The average scores of the two physicians were scored for bone healing; and the healing was The RUST score is  $\geq 7$  points, the tenderness of the affected area is not obvious, and the abnormal displacement of the bone is regarded as healing, otherwise it is regarded as a healing disorder.

#### Observation indicators

(1) qRT-PCR was used to detect the mRNA level expression of CYR61. On the 4th day after the fracture, 10 mL of fasting venous blood was taken from the patient and the control group. After centrifugation, serum samples were separated and stored at  $-80^{\circ}\text{C}$  for examination. The total RNA in the serum samples was extracted using the RNA pure total RNA extraction kit, followed by reverse transcription using random primers, reverse transcription of mRNA into cDNA, and PCR amplification using cDNA as a template. PCR amplification was performed with GAPDH as an internal reference, and the relative expression of CYR61 mRNA was analyzed by  $2^{-\Delta\Delta t}$ . The primer sequence detected by qRT-PCR was: CYR61 upstream primer: 5' - TGGAGTTATATTCACAGGGTCTG - 3'; CYR61 downstream primer: 5' - GCAGCTCAACGAG-GACTG - 3'; GAPDH upstream primer: 5'-GAAGGTGAAGGTCG-GAGTC- 3'; GAPDH downstream primer: 5'-GAAGATGGTGATGGGATTTC-3. (2) Western Blot was used to detect the protein expression level of CYR61. Serum samples were taken and the total protein in the serum was extracted using the total protein extraction kit; serum proteins were then separated by western technique, and CYR61 was detected by CYR61 murine polyclonal antibody and its corresponding secondary antibody. GAPDH was used as a control in the assay; Semi-quantitative analysis was performed using Image J, and the ratio of the target band to the GAPDH band was used as the relative expression level of CYR61. In the above tests, the primers and kits used were purchased from Shanghai Bioengineering Technology Co., Ltd. and operated in strict accordance with the kit instructions.

#### cal processing

The data was processed by the statistical software SPSS22.0. The count data was expressed by (n, %), and the line 2 test; the measurement data was represented by  $(\bar{x} \pm s)$ , and the line test was performed.  $P < 0.05$  was considered statistically significant. Statistical software SPSS 22.0 was used to process data, and count data was analyzed by  $\chi^2$  test; measurement data was represented by  $(\bar{x} \pm s)$ , single factor analysis; correlation was analyzed by Spearman correlation; predictive value was determined by receiver operating characteristic (ROC) analysis.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Comparison of serum CYR61 mRNA and protein expression levels, and RUST levels

The expression levels of CYR61 mRNA and protein in serum of each group were significantly different ( $P < 0.05$ ). The serum CYR61 mRNA and CYR61 protein in the normal healing group were significantly higher than those in the control group ( $P < 0.05$ ). The serum CYR61 mRNA and CYR61 protein in the abnormal healing group were significant. Lower than normal healing group ( $P < 0.05$ ). The RUST score of the normal healing group was significantly higher than that of the abnormal healing group ( $P < 0.05$ ). See Table 2 for details.

Grouping	CYR61 mRNA	CYR61 protein	RUST (minutes)
Normal birth healing group (n=74)	2.35±0.49 <sup>①</sup>	0.33±0.10 <sup>①</sup>	11.35±1.51
Abnormal healing group (n=10)	2.02±0.29 <sup>②</sup>	0.23±0.07 <sup>②</sup>	7.17±0.95 <sup>②</sup>
Control group (n=84)	1.88±0.37	0.26±0.06	-
F/t value	24.352	17.674	8.503
P value	<0.001	<0.001	<0.001
Note: ① Compared with the control group, $P < 0.05$ ; ② Compared with the healing group, $P < 0.05$ .			

Correlation analysis between serum CYR61 mRNA and protein expression levels and prognosis

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Serum CYR61 mRNA and protein expression levels were significantly positively correlated with prognosis ( $P < 0.05$ ). See Table 3 for details.

Expression	r value	P value
CYR61 mRNA	0.264	0.015
CYR61 protein	0.313	0.004

#### Predictive value of serum CYR61 mRNA and protein expression levels for prognosis

ROC curve analysis showed that the AUC of serum CYR61 mRNA and protein were 0.735 and 0.778, respectively, and the Cut-off values were 2.363 and 0.338, respectively. The sensitivities were 60.80% and 54.10%, respectively, and the specificities were 80.00% and 90.00%, respectively. They were 0.408 and 0.441, respectively, thus indicating that serum CYR61 mRNA and protein expression levels have predictive value for prognosis. See Table 4 for details.

Detection Indicator	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	Youden index
CYR61 mRNA	0.735	2.363	60.80	80.00	0.408
CYR61 protein	0.778	0.338	54.10	90.00	0.441

#### Discussion

Tibial fracture is a common clinical fracture, including humeral shaft fracture and tibial plateau fracture. The clinical manifestation of humeral shaft is swelling of the calf, pain, and symptoms such as deformity and abnormal activity. Clinical manifestations of tibial plateau fracture for the knee joint swelling and pain, movement disorders and other symptoms<sup>6</sup>. It is mainly caused by direct or indirect violence such as heavy hits, kicks, impact injuries or wheel rolling injuries, falling from high places, rotating violent sprains, slipping, etc.; and, with the advancement of the transportation industry, its incidence rate upward trend. At present, post-debridement and fixed treatment is a common

clinical treatment. However, some patients often suffer from poor prognosis and delayed fracture healing due to fracture of the fractured skin, muscle contusion, excessive blood loss and improper treatment<sup>7</sup>. Therefore, it has been suggested that a substance can be found as an early predictor of fracture prognosis, and targeted prevention and rehabilitation of high-risk patients with possible adverse prognosis can be achieved, thereby reducing the occurrence of poor prognosis such as delayed fracture healing<sup>8</sup>.

The formation of blood vessels such as veins, arteries, and capillaries in fracture patients is the basis of bone repair, and CYR61 plays an important role in promoting angiogenesis in fracture patients as an angiogenic marker<sup>9</sup>. Biological studies have shown that the human CYR61 gene is located at 1q22-3 and contains five exons and four introns; whereas the CYR61 protein contains 381 amino acids and consists of four different protein structural modules, and these four different protein structures can not only bind to other proteins in the extracellular matrix, but also produce different biological effects, and can work together to produce more complex biological effects<sup>10</sup>. Therefore, CYR61 can participate in chondrocytes and Migration, proliferation and differentiation of bone cells and vascular endothelial cells. Previous studies have shown that CYR61 can activate Wnt signaling pathway, which promotes the differentiation of mesenchymal stem cells into osteoblasts, and may be an important regulator of osteogenesis during stromal cell adhesion, migration, proliferation and differentiation, suggesting that CYR61 is promoting. Play an important role in cartilage formation<sup>11</sup>. The formation mechanism of blood vessels is more complicated, and its formation is positively correlated with the formation of bone. The main reason is that the blood vessels provide sufficient nutrient supply, which is beneficial to the body to form a suitable bone growth environment; and in the process of blood vessel formation, secreted Vascular endothelial growth factor promotes the activity of chondrocytes and osteoblasts and accelerates the formation of new bone<sup>12</sup>. Fan Weiming's research on the mechanism

of CYR61 indicates that CYR61 protein plays an important role in activating ERK signaling pathway and promoting osteoblastogenesis. Therefore, in this study, the serum CYR61 mRNA and CYR61 protein in the normal healing group were significantly higher than those in the control group. The serum CYR61 mRNA and CYR61 protein in the abnormal healing group were significantly lower than those in the normal healing group. The RUST score of the normal healing group was significantly higher than that of the abnormal healing group. Serum CYR61 mRNA and protein expression levels were significantly positively correlated with prognosis. ROC curve analysis showed that serum CYR61 mRNA and protein expression levels have a certain predictive value for prognosis. It is worth noting that although this study shows the predictive value of CYR61 for fracture prognosis and related cut-off values, due to the limited sample data of this study, it may cause certain deviations, so further research is needed to determine the follow-up.

## CONCLUSION

In summary, serum CYR61 mRNA and protein are up-regulated in patients with tibiofibular fractures, and the higher the mRNA and protein expression levels of early serum CYR61, the better the prognosis of fracture healing; in addition, to some extent, serum CYR61 Can be used as a reference indicator for early prediction of prognosis of tibiofibular fractures.

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