Expression of TGF-β1 and VEGF in patients with Achilles tendon rupture and the clinical efficacy

JUNCHENG CUI, ZHIWEI CHEN and WENTE WU

Department of Orthopaedics, First Affiliated Hospital of University of South China, Henyang, Hunan 421001, P.R. China

Received April 16, 2019; Accepted July 10, 2019

DOI: 10.3892/etm.2019.7968

Abstract. Expression of transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor (VEGF) in patients with Achilles tendon rupture, and the predictive values and significance in clinical efficacy were explored. Forty-two patients with Achilles tendon rupture, surgically treated in the First Affiliated Hospital of University of South China, were selected and the clinical efficacy was evaluated based on the American Orthopaedic Foot and Ankle Society (AOFAS) scoring system. RT-qPCR was adopted to detect the expression of serum TGF-β1 and VEGF in the patients before and after treatment, and Spearman's correlation was used to analyze the correlation of TGF-β1 and VEGF with the clinical efficacy after treatment. Patients were divided into an excellent efficacy group and a good/general efficacy group according to the predictive efficacy. In the two groups, the expression levels of TGF-β1 and VEGF before treatment were observed, and the predictive values of TGF-β1 and VEGF in clinical efficacy using the receiver operating characteristic (ROC) curves were obtained. The 42 patients showed significantly higher expression of TGF-β1 and VEGF at 3 months after treatment, and significantly decreased expression at 6 months after treatment, compared to the results before treatment (both $P<0.001$). After treatment, the efficacy was excellent in 11 patients, good in 25 and general in 6. Spearman's correlation analysis revealed that the expression of TGF-β1 and VEGF decreased with the improvement of efficacy after treatment ($P<0.001$), and the excellent efficacy group showed significantly lower expression of TGF-β1 and VEGF than that in the good/general efficacy group ($P<0.01$). Moreover, according to ROC curves, the areas under the curves (AUCs) of TGF-β1 and VEGF were 0.651 and 0.645, respectively. In conclusion, TGF-β1 and VEGF can be considered as observational indexes and predictors for clinical efficacy in patients with Achilles tendon rupture, before and after treatment.

Correspondence to: Dr Wente Wu, Department of Orthopaedics, First Affiliated Hospital of University of South China, 69 Chuanshan Road, Hengyang, Hunan 421001, P.R. China
E-mail: v8u2jw@163.com; wuwente@sina.com

Key words: transforming growth factor-β1, vascular endothelial growth factor, Achilles tendon rupture

Introduction

Achilles tendon, as the most powerful tendon in the body, is responsible for the plantar flexion of ankle joint and important for people's daily walking and life (1). Achilles tendon rupture is a common ankle injury. Statistics have shown that the annual incidence rate of acute Achilles tendon rupture is ~1.8‰ which increases with age, and the patients are mostly young and middle-aged male athletes or actors (2). The disease is caused by a number of factors, mainly the sudden acceleration or deceleration of movement and inappropriate modes of exercise (3). Clinically, conservative and surgical treatments are controversial therapeutic schemes for Achilles tendon rupture (4,5). However, a mate analysis has shown that the incidence rate of re-rupture after surgical treatment is significantly lower than that after conservative treatment, which indirectly indicates that the former is more effective than the latter (6). Although the two schemes are controversial, most scholars advocate surgical treatment for the important role of Achilles tendon in human body motion (7).

Currently, the Achilles tendon rupture after treatment is mainly evaluated based on the doctors' clinical experience and the efficacy evaluation criteria of the American Orthopaedic Foot and Ankle Society (AOFAS), due to the lack of effective observational indexes (8). However, young clinicians are inexperienced and the AOFAS scoring is subjective, although it is the most important criterion for evaluating Achilles tendon rupture. Therefore, it is vital to find a biomarker for this problem. Transforming growth factor-β1 (TGF-β1) is a multifunctional protein that regulates cell proliferation, differentiation and wound healing (9). Studies have shown that injection of different concentrations of TGF-β1 can promote tendon formation, growth and repair, suggesting that TGF-β1 expression is closely related to tendon recovery (10). The reduction of blood supply is one of the reasons of poor healing of Achilles tendon, therefore, it is of great significance to promote blood vessel production during Achilles tendon healing (11). As a signal protein, vascular endothelial growth factor (VEGF) belongs to the platelet-derived growth factor family of cystine knot growth factor (10), with the function of regulating angiogenesis (12). A study showed that TGF-β1

and VEGF were differentially expressed in a rabbit model of Achilles tendon injury (13). However, there are few studies on whether the expression of TGF-β1 and VEGF in the human body is the same, and whether TGF-β1 and VEGF can be used as prognostic indicators.

Thus, in the present study, the expression of TGF-β1 and VEGF in patients with Achilles tendon rupture were investigated, before and after treatment, and their potential predictive values were explored, in order to provide new references for clinicians.

Subjects and methods

Information of the study subjects. Forty-two patients with Achilles tendon rupture, treated in the First Affiliated Hospital of University of South China (Hengyang, China) from August 2016 to September 2017, were selected as the observation group, including 32 males and 10 females, with an average age of 34.5±6.7 years, and a course of disease of 3.51±1.42 days. There were 22 cases caused by football, 10 by basketball and 10 by other factors. Also, 30 normal subjects undergoing physical examination in the hospital were selected as the normal group, including 20 males and 10 females, with an average age of 35.1±7.20 years. The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of University of South China, and the patients who participated in this research signed an informed consent and had complete clinical data.

Inclusion criteria: Patients with depression and tenderness at Achilles tendon; patients with positive Thompson's test; patients diagnosed with Achilles tendon rupture by nuclear magnetic resonance; patients with closed wounds; patients who cooperated with treatment; patients with complete clinical data. Exclusion criteria: Patients with congenital cardiovascular diseases; patients with immunodeficiency diseases; patients with arthritis, gout, infectious diseases and malignant tumors; patients unable to receive operation for their own reasons.

Therapeutic regimens and postoperative treatment. Patients were treated according to the therapeutic regimens described in the study by Ismail *et al* (14). After operation the affected limbs were fixed with long leg casts, with the knee bent and the ankle joint at a plantar flexion of 30˚. After 6 weeks, the affected limbs were fixed with short leg casts for active/passive flexion and extension of the ankle joint. The patients received partial weight-bearing exercises with crutches after 8 weeks, and normal weight-bearing exercises after 12 weeks. Intense exercises were avoided for 6 months.

Main kits. EasyPure Genomic DNA kit and TransScript Green Two-Step qRT-PCR SuperMix (EE101-01 and AQ201-01, respectively; both from TransGen Biotech Co., Ltd.) were used.

Expression of serum TGF-β1 and VEGF. Fasting peripheral venous blood (5 ml) was collected from subjects and patients, let to stand for 30 min, and centrifuged at 1,500 x g, at 25˚C for 10 min in order to collect the supernatant for subsequent experiments. Total RNA was extracted using the EasyPure Genomic DNA kit. One microliter of the extracted Total RNA, 4 μl of 5X TransScript® Tip Green qPCR SuperMix and 1 μ gDNA Remover (both from Thermo Fisher Scientific, Inc.) were added. RNase-free water was also added to a final volume of 20 μl. After mixing, and incubating at 42˚C for 15 min, and then heating to 85˚C for 5 sec, an ultraviolet spectrophotometer (Thermo Fisher Scientific, Inc.; GENESYS™ 140/150) was used and agarose gel electrophoresis was performed for purity, concentration and integrity detection. 5X TransScript® II All-in-One SuperMix for qPCR and gDNA Remover kits (both from Thermo Fisher Scientific, Inc.) were used for reverse transcription, in strict accordance with the manufacturer's instructions. Then, PCR amplification was performed. Upstream and downstream primers for TGF-β1 were 5'-TGC GCCTGCAGAGATTCAAG-3' and 5'-AGGTAACGCCAGG AATTGTTGCTA-3', respectively. Those of VEGF were 5'-GCACGTTGGCTCACTTCCAG-3' and 5'-AGGTAACGC CAGGAATTGTTGCTA-3', respectively. The reaction system was as follows: 1 μl of cDNA, 0.4 μl of upstream and downstream primers, respectively, 10 μl of 2X TransScript® Tip Green qPCR SuperMix, 0.4 μl of Passive Reference Dye (50X) (both from Thermo Fisher Scientific, Inc.), and Nuclease-free water were added to a final volume of 20 μl. The reaction conditions were as follows: Pre-denaturation at 94˚C for 30 sec, denaturation at 94˚C for 5 sec, and annealing and extension at 60˚C for 30 sec for a total of 40 cycles. Each sample was provided with three identical wells, and the experiment was carried out 3 times. β-actin was used as an internal reference, and its upstream and downstream primers were 5'-CTCCATCCTGGCCTCGCTG-3' and 5'-GCTGTC ACCTTCACCGTTCC-3', respectively. $2^{-\Delta Cq}$ was used to analyze the data (15).

Observational indexes

Main observational indexes. The expression of serum TGF-β1 and VEGF was compared between the observation and normal group, and the TGF-β1 and VEGF expression levels in the observation group were observed before treatment, and at 3 and 6 months after treatment. The patients were divided into the excellent efficacy group and the good/general efficacy group according to the predictive efficacy at 6 months after treatment, and the expression levels of TGF-β1 and VEGF before treatment were compared between the two groups. Receiver operating characteristic (ROC) curves were plotted to analyze the predictive values of TGF-β1 and VEGF for the efficacy.

Secondary observational indexes. AOFAS scoring system with 100 points in total was adopted to evaluate the efficacy at 6 months after treatment, including pain, function and foot line (16). Grading: 90-100 points, excellent efficacy; 75-89 points, good efficacy; 50-74 points, general efficacy, and <50 points, poor efficacy. Patients were separated into the excellent efficacy group, good efficacy group and general efficacy group, according to the AOFAS scores after treatment, and the expression of TGF-β1 and VEGF was compared between the three groups. The correlation of TGF-β1 and VEGF with efficacy was analyzed, and the clinical data were compared between the observation and normal group.

Statistical analysis. SPSS 20.0 (Guangzhou Pomine Information Technology Co., Ltd.) was used to statistically analyze the data, and GraphPad Prism 7 (Cabit Information

Table I. Comparison of clinical data.

Factors	Normal group (n=30)	Observation group (n=42)	t/χ^2 value	P-value
Sex			0.791	0.374
Male	20 (66.67)	32 (76.19)		
Female	10 (33.33)	10 (23.81)		
Age (years)	35.1±7.20	34.5±6.70	0.363	0.718
BMI (kg/m^2)	22.88±1.74	22.51±1.82	0.866	0.389
Medical history				
Hypertension	4 (13.33)	6 (14.29)	0.013	0.908
Diabetes	2 (6.67)	2 (4.76)	0.121	0.728
COPD	0 (0.00)	2 (4.76)	1.469	0.225
Place of residence			0.159	0.690
City	15 (50.00)	23 (54.76)		
Countryside	15 (50.00)	19 (45.24)		
Level of education			0.411	0.521
≥ Senior high school	12 (40.00)	20 (47.62)		
< Senior high school	18 (60.00)	22 (52.38)		
History of smoking			0.266	0.606
Yes	22 (73.33)	33 (78.57)		
No	8 (26.67)	9 (21.43)		
History of alcoholism			0.150	0.600
Yes	4 (13.33)	7 (16.67)		
No	26 (86.67)	35 (83.33)		
Pathogenesis				
Football		22 (52.38)		
Basketball		10 (23.81)		
Others		10 (23.81)		
Course of disease (days)		3.51±1.42		

BMI, body mass index; COPD, chronic obstructive pulmonary disease.

Technology Co., Ltd.) to create the graphs. Enumeration data were expressed as ratio (%) and were compared by Chi-square test. Measurement data were expressed as the mean ± standard deviation. The data between groups were compared using the independent-samples t-test, while comparisons within groups, before and after treatment, were carried out using the paired t-test. ROC curve analysis was adopted to analyze the predictive values of TGF-β1 and VEGF expression in clinical efficacy before treatment, and Spearman's correlation was used to analyze the correlation of TGF-β1 and VEGF with efficacy. One-way ANOVA was carried out for the comparisons between multiple groups (F analysis), and LSD-t test was adopted for post hoc pairwise comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of clinical data. Comparison of clinical data between the normal and observation group showed that there was no statistically significant difference in age, sex, body mass index (BMI), medical history, place of residence, level of education, history of smoking or alcoholism (all $P>0.05$) (Table I).

Expression of serum TGF-β1 and VEGF in the normal and observation group. According to the results, the expression of TGF-β1 and VEGF in the normal group before treatment was 1.122±0.187 and 1.092±0.163, respectively, while that in the observation group was 1.636±0.331 and 1.533±0.281, respectively. The expression of TGF-β1 and VEGF in the observation group was significantly higher than that in the normal group (both $P<0.001$) (Fig. 1).

Expression of TGF-β1 and VEGF in the observation group before and after treatment. Comparisons of the expression of TGF-β1 and VEGF in patients before treatment, and at 3 and 6 months after treatment indicated that there was a significant difference in the expression of TGF-β1 and VEGF before and after treatment ($P<0.001$). The results showed significantly higher expression of TGF-β1 and VEGF at 3 months after treatment and slightly decreased expression at 6 months after

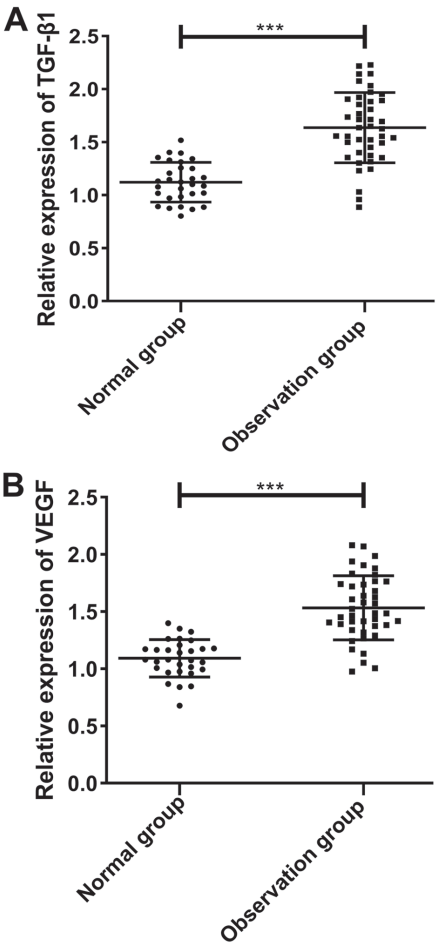

Figure 1. Expression of serum TGF-β1 and VEGF in the normal and observation group. (A) The expression of TGF-β1 in the observation group was significantly higher than that in the normal group (t=7.672, P<0.001). (B) The expression of VEGF in the observation group was significantly higher than that in the normal group (t=77.19, P<0.001). ***P<0.001.

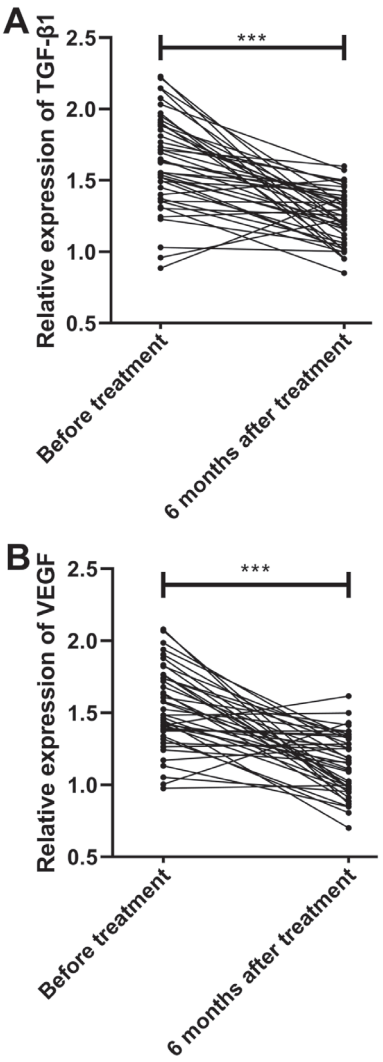

Figure 2. Expression of TGF-β1 and VEGF in the observation group before treatment and at 6 months after treatment. (A) Expression of TGF-β1 before treatment and at 6 months after treatment (t=6.889, P<0.001). (B) Expression of VEGF before treatment and at 6 months after treatment (t=7.043, P<0.001). ***P<0.001. TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor.

treatment, compared to the results before treatment (both P<0.001) (Fig. 2 and Table II).

Correlation of TGF-β1 and VEGF expression with the clinical efficacy after treatment. After treatment for 6 months, the AOFAS score in the observation group was 84.29±7.91 points. There were 11 patients with excellent efficacy, 25 patients with good efficacy and 6 patients with general efficacy. The comparison of the expression of serum TGF-β1 and VEGF between the excellent efficacy, good efficacy and general efficacy groups showed a significant difference (all P<0.05), and the analysis with Spearman's correlation showed that the expression of TGF-β1 and VEGF decreased with the improvement of efficacy ($r_{TGF-β1}$=-0.734, $P_{TGF-β1}$<0.001; r_{VEGF}=-0.767, P_{VEGF}<0.001) (Fig. 3 and Table III).

Predictive values of TGF-β1 and VEGF in clinical efficacy before treatment. According to the predictive efficacy, the patients were further divided into the excellent efficacy group and the good/general efficacy group. The comparison of the expression of TGF-β1 and VEGF between the two groups indicated that the excellent efficacy group showed significantly lower expression of TGF-β1 and VEGF than that of the good/general efficacy group (P<0.01). According to ROC curves, the areas under the curves (AUCs) of TGF-β1 and VEGF were 0.651 and 0.645, respectively (Fig. 4 and Table IV).

Table II. Expression of TGF-β1 and VEGF before and after treatment.

Time	TGF-β1	VEGF
Before treatment	1.636±0.331	1.533±0.281
At 3 months after treatment	2.225±0.340[a]	2.013±0.262[a]
At 6 months after treatment	1.238±1.190[a,b]	1.138±0.211[a,b]
F-value	108.735	135.136
P-value	<0.001	<0.001

[a]P<0.001, compared with the results before treatment; [b]P<0.05, compared with the results at 3 months after treatment. TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor.

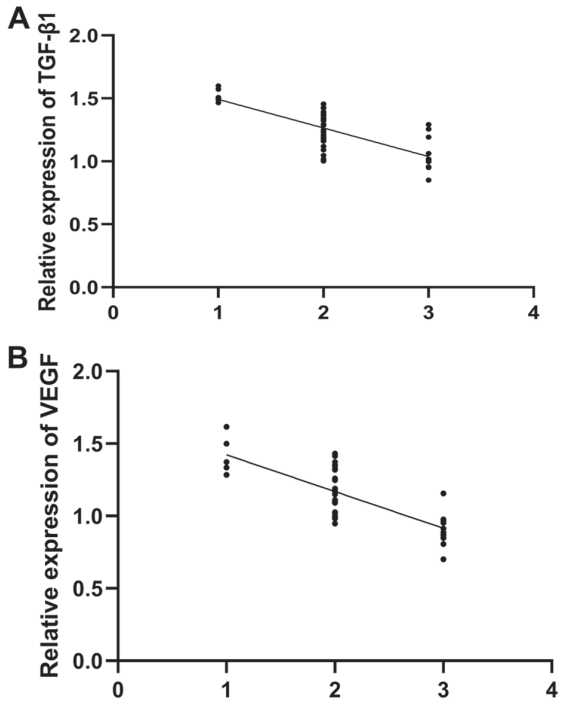

Figure 3. Correlation of clinical efficacy with TGF-β1 and VEGF. (A) TGF-β1 was negatively correlated with efficacy. (B) VEGF was negatively correlated with efficacy. 1, general; 2, good; 3, excellent. TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor.

Table III. Correlation of clinical efficacy with the expression of TGF-β1 and VEGF.

Efficacy	TGF-β1	VEGF
Excellent (n=11)	1.055±0.137	0.902±0.116
Good (n=25)	1.250±0.132[a]	1.180±0.151[a]
General (n=6)	1.522±0.052[a,b]	1.398±0.133[a,b]
F-value	26.993	27.024
P-value	<0.001	<0.001

[a]P<0.05, compared with the excellent efficacy group; [b]P<0.05, compared with the good efficacy group. TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor.

Table IV. ROC parameters.

Parameters	TGF-β1	VEGF
AUC	0.651	0.645
Standard error	0.086	0.088
95% CI	0.483-0.819	0.473-0.817
Sensitivity	61.29%	61.29%
Specificity	81.81%	75.00%
Youden index	43.11%	36.29%
Cut-off value	>1.631	>1.475

ROC, receiver operating characteristic; TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor; AUC, area under the curve.

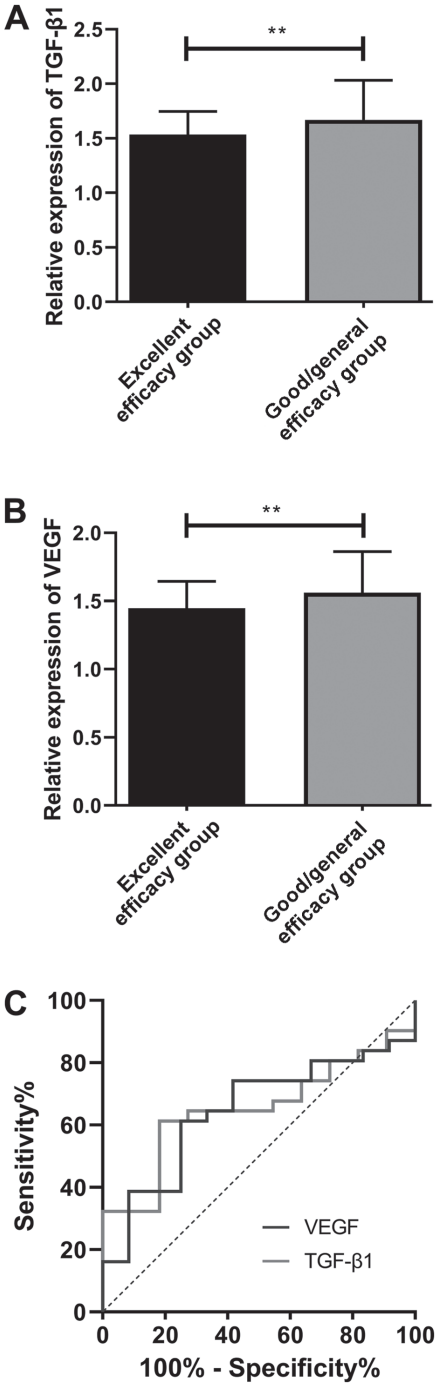

Figure 4. Predictive values of TGF-β1 and VEGF in clinical efficacy before treatment. (A) Before treatment, the expression of TGF-β1 in the excellent efficacy group was lower than that in the good/general efficacy group. **P<0.01. (B) Before treatment, the expression of VEGF in the excellent efficacy group was lower than that in the good/general efficacy group. **P<0.01. (C) The best cut-off value of TGF-β1 was 1.631 when the sensitivity was 61.29% and the specificity was 81.81%. The best cut-off value of VEGF was 1.475 when the sensitivity was 61.29% and the specificity was 75.00%. TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor.

Discussion

Achilles tendon is the most common ruptured tendon of lower limbs. According to Ganestam *et al* (17), a total of 33,160 patients suffered from Achilles tendon rupture from 1994 to 2013 in Denmark, with males (aged 40-50 years)

accounting for >75%. The treatment of Achilles tendon rupture is essential, as it affects the patients' daily living and especially the careers of injured athletes (18). At present, the treatment of the disease is controversial (19). Some people advocate conservative treatment, while others surgical treatment, both of which have positive effects (20). However, a study has shown that surgical treatment reduces the incidence of re-rupture of Achilles tendon (21), therefore surgical treatment is considered to be slightly superior to conservative treatment.

Achilles tendon rupture is currently treated by numerous surgical treatments, one of which is the minimally invasive percutaneous treatment with rivet with thread (22). Also, Kessler suture (23), Krachow suture (24), and minimally invasive suture (25) are adopted according to the degree of rupture. A study has shown that the minimally invasive Achilles tendon repair causes little damage to tissues and blood vessels around the Achilles tendon rupture, and is widely used in the treatment of the disease (26). The suture with Achillon device, that was used in the present study, is a therapeutic scheme originally proposed by Kakiuchi (27) in 1995. With the advantages of small incision and conveniental operation, it is more effective than Kessler suture. Suture with Achillon device is markedly effective in the treatment of Achilles tendon rupture, however, its evaluation for postoperative efficacy is mainly based on AOFAS score and the experience of clinicians, so it has limitations. Therefore, the identification of biomarkers for observation is particularly important.

TGF-β1 and VEGF are important growth factors, as TGF-β1 promotes cell growth and development, wound healing, and modulation of immune responses (28), and VEGF is a powerful angiogenesis regulatory factor with an important influence on revascularization (29). A relevant study has shown that TGF-β1 and VEGF are highly expressed in an animal model of Achilles tendon injury (30), however, no clinical study has been carried out. Therefore, the expression and significance of TGF-β1 and VEGF in the treatment of Achilles tendon rupture were explored in this study. The results showed that the expression of TGF-β1 and VEGF in the observation group was significantly higher than that in the normal group, indicating that the expression of TGF-β1 and VEGF increases after injury. This is probably because after Achilles tendon injury, patients' vascular tissues at the injured part are damaged, which causes excessive secretion of TGF-β1 and VEGF in the body. A study by Lyras *et al* (31) has shown that the expression of VEGF in an animal model of Achilles tendon injury decreases after surgical treatment. According to another study (32), exogenous VEGF for the treatment of rats with Achilles tendon injury improves the tensile strength of Achilles tendon, and increases the expression of TGF-β1. The expression of TGF-β1 and VEGF in the observation group before treatment, and at 3 and 6 months after treatment was compared and the findings showed that the patients had significantly higher expression of TGF-β1 and VEGF after 3 months of treatment, but slightly decreased expression after 6 months of treatment. This indicates that the expression of TGF-β1 and VEGF in patients after treatment increases in a certain period of time. It may be due to the fact that the body releases a large number of inflammatory factors after Achilles tendon rupture, while TGF-β1 and VEGF are not only angiogenesis and growth factors, but also important inflammatory factors, thus, TGF-β1 and VEGF increase after injury. In addition, as inflammatory factors, TGF-β1 and VEGF can promote angiogenesis and cell repair in the injured area. When the patient's inflammatory response is alleviated, the expression of TGF-β1 and VEGF decreases, and the Achilles tendon is healed. In this study, the expression of TGF-β1 and VEGF at 6 months after treatment was significantly lower than that before treatment. Additionally, according to correlation analysis, the expression of TGF-β1 and VEGF decreased with the improvement of efficacy, indicating that TGF-β1 and VEGF can be used as potential indicators for the clinical observation of efficacy after treatment.

Differences in individuals lead to differences in postoperative recovery, so it is particularly important to predict the clinical efficacy by observing serological indicators before treatment, in order to promote the patients' recovery. In the present study, the patients were grouped based on the predictive efficacy after treatment to observe the expression of TGF-β1 and VEGF before treatment. The results showed that the expression in the excellent efficacy group was lower than that in the good/general efficacy group, indicating that TGF-β1 and VEGF may be potential predictors of clinical efficacy. According to the results of the ROC curve analysis, the AUCs of TGF-β1 and VEGF were >0.5, suggesting that the two indicators could be potential predictors of the efficacy in Achilles tendon rupture.

This study was focused on efficacy prediction, and did not confirm that the two indexes can be adopted as observation indexes for Achilles tendon rupture. However, it is undeniable that the results of this study confirmed through the relevant research that the two indexes do have certain clinical value. In the present study, there are still some limitations. The AUCs of TGF-β1 and VEGF were only just >0.5, suggesting that their clinical significance is not high, and PCR detection is expensive, so it may increase the economic burden of patients.

In conclusion, TGF-β1 and VEGF can be used as observational indexes and predictors for clinical efficacy in patients with Achilles tendon rupture before and after treatment.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JC and ZC conceived and designed the study. JC acquired the patients' data. ZC and WW analyzed and interpreted the data regarding the Achilles tendon rupture. JC wrote the article. WW reviewed the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of University of South China (Hengyang, China). Patients who participated in this research signed an informed consent and had complete clinical data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Demirel M, Turhan E, Dereboy F and Yazar T: Augmented repair of acute tendo Achilles ruptures with gastrosoleus turn down flap. Indian J Orthop 45: 45-52, 2011.
2. Frankewycz B, Krutsch W, Weber J, Ernstberger A, Nerlich M and Pfeifer CG: Rehabilitation of Achilles tendon ruptures: Is early functional rehabilitation daily routine? Arch Orthop Trauma Surg 137: 333-340, 2017.
3. Aisaiding A, Wang J, Maimaiti R, Jialihasi A, Aibek R, Qianman B, Shawutali N, Badelihan A, Bahetiya W, Kubai A, *et al*: A novel minimally invasive surgery combined with early exercise therapy promoting tendon regeneration in the treatment of spontaneous Achilles tendon rupture. Injury 49: 712-719, 2018.
4. Yang X, Meng H, Quan Q, Peng J, Lu S and Wang A: Management of acute Achilles tendon ruptures: A review. Bone Joint Res 7: 561-569, 2018.
5. Lantto I, Heikkinen J, Flinkkila T, Ohtonen P, Siira P, Laine V and Leppilahti J: A prospective randomized trial comparing surgical and nonsurgical treatments of acute Achilles tendon ruptures. Am J Sports Med 44: 2406-2414, 2016.
6. Weatherall JM, Mroczek K and Tejwani N: Acute achilles tendon ruptures. Orthopedics 33: 758-764, 2010.
7. Alcelik I, Diana G, Craig A, Loster N and Budgen A: Minimally invasive versus open surgery for acute Achilles tendon ruptures a systematic review and meta-analysis. Acta Orthop Belg 83: 387-395, 2017.
8. Macaulay A, Nandyala SV, Miller CP, Ghorbanhoseini M, Walley KC and Kwon JY: Potential for Bias and the American Orthopaedic Foot and Ankle Society Ankle-Hindfoot Scoring System. Foot Ankle Spec 11: 416-419, 2018.
9. Hinz B: The extracellular matrix and transforming growth factor-β1: Tale of a strained relationship. Matrix Biol 47: 54-65, 2015.
10. Hou Y, Mao Z, Wei X, Lin L, Chen L, Wang H, Fu X, Zhang J and Yu C: The roles of TGF-beta1 gene transfer on collagen formation during Achilles tendon healing. Biochem Biophys Res Commun 383: 235-239, 2009.
11. Filardo G, Presti ML, Kon E and Marcacci M: Nonoperative biological treatment approach for partial Achilles tendon lesion. Orthopedics 33: 120-123, 2010.
12. Ferrara N and Adamis AP: Ten years of anti-vascular endothelial growth factor therapy. Nat Rev Drug Discov 15: 385-403, 2016.
13. Pedowitz D and Kirwan G: Achilles tendon ruptures. Curr Rev Musculoskelet Med 6: 285-293, 2013.
14. Ismail M, Karim A, Shulman R, Amis A and Calder J: The Achillon achilles tendon repair: Is it strong enough? Foot Ankle Int 29: 808-813, 2008.
15. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
16. Sayyed-Hosseinian SH, Hassankhani GG, Bagheri F, Alavi N, Shojaie B and Mousavian A: Validation of the persian version of the American Orthopedic Foot and Ankle Society Score (AOFAS) questionnaire. Arch Bone Jt Surg 6: 233-239, 2018.
17. Ganestam A, Kallemose T, Troelsen A and Barfod KW: Increasing incidence of acute Achilles tendon rupture and a noticeable decline in surgical treatment from 1994 to 2013. A nationwide registry study of 33,160 patients. Knee Surg Sports Traumatol Arthrosc 24: 3730-3737, 2016.
18. Mai HT, Alvarez AP, Freshman RD, Chun DS, Minhas SV, Patel AA, Nuber GW and Hsu WK: The NFL Orthopaedic Surgery Outcomes Database (NO-SOD): The Effect of common orthopaedic procedures on football careers. Am J Sports Med 44: 2255-2262, 2016.
19. Huang J, Wang C, Ma X, Wang X, Zhang C and Chen L: Rehabilitation regimen after surgical treatment of acute Achilles tendon ruptures: A systematic review with meta-analysis. Am J Sports Med 43: 1008-1016, 2015.
20. Deng S, Sun Z, Zhang C, Chen G and Li J: Surgical treatment versus conservative management for acute Achilles tendon rupture: A systematic review and meta-analysis of randomized controlled trials. J Foot Ankle Surg 56: 1236-1243, 2017.
21. Maffulli G, Buono AD, Richards P, Oliva F and Maffulli N: Conservative, minimally invasive and open surgical repair for management of acute ruptures of the Achilles tendon: A clinical and functional retrospective study. Muscles Ligaments Tendons J 7: 46-52, 2017.
22. Gulati V, Jaggard M, Al-Nammari SS, Uzoigwe C, Gulati P, Ismail N, Gibbons C and Gupte C: Management of achilles tendon injury: A current concepts systematic review. World J Orthop 6: 380-386, 2015.
23. Walden G, Liao X, Donell S, Raxworthy MJ, Riley GP and Saeed A: A clinical, biological, and biomaterials perspective into tendon injuries and regeneration. Tissue Eng Part B Rev 23: 44-58, 2017.
24. Mait JE, Hayes WT, Blum CL, Pivec R, Zaino CJ, Jauregui JJ, Saha S, Uribe JA and Urban WP: A biomechanical comparison of different tendon repair techniques. J Long Term Eff Med Implants 26: 167-171, 2016.
25. Carmont MR: Achilles tendon rupture: The evaluation and outcome of percutaneous and minimally invasive repair. Br J Sports Med 52: 1281-1282, 2018.
26. Braunstein M, Baumbach SF, Boecker W, Carmont MR and Polzer H: Development of an accelerated functional rehabilitation protocol following minimal invasive Achilles tendon repair. Knee Surg Sports Traumatol Arthrosc 26: 846-853, 2018.
27. Kakiuchi M: A combined open and percutaneous technique for repair of tendo Achillis. Comparison with open repair. J Bone Joint Surg Br 77: 60-63, 1995.
28. Inoue M, Nakajima M, Oi Y, Hojo T, Itoi M and Kitakoji H: The effect of electroacupuncture on tendon repair in a rat Achilles tendon rupture model. Acupunct Med 33: 58-64, 2015.
29. Tempfer H, Kaser-Eichberger A, Lehner C, Gehwolf R, Korntner S, Kunkel N, Wagner A, Gruetz M, Heindl LM, Schroedl F, *et al*: Bevacizumab improves Achilles tendon repair in a rat model. Cell Physiol Biochem 46: 1148-1158, 2018.
30. Yuksel S, Guleç MA, Gultekin MZ, Adanır O, Caglar A, Beytemur O, Onur Küçükyıldırım B, Avcı A, Subaşı C, İnci Ç, *et al*: Comparison of the early period effects of bone marrow-derived mesenchymal stem cells and platelet-rich plasma on the Achilles tendon ruptures in rats. Connect Tissue Res 57: 360-373, 2016.
31. Lyras DN, Kazakos K, Verettas D, Polychronidis A, Tryfonidis M, Botaitis S, Agrogiannis G, Simopoulos C, Kokka A and Patsouris E: The influence of platelet-rich plasma on angiogenesis during the early phase of tendon healing. Foot Ankle Int 30: 1101-1106, 2009.
32. Zhang F, Liu H, Stile F, Lei MP, Pang Y, Oswald TM, Beck J, Dorsett-Martin W and Lineaweaver WC: Effect of vascular endothelial growth factor on rat Achilles tendon healing. Plast Reconstr Surg 112: 1613-1619, 2003.