Study on the Regulatory Effect of Blood-Activating and Collateral-Dredging Therapy on Serum Bone Resorption/Angiogenesis/Osteogenesis Proteins in Patients with Steroid-Induced Osteonecrosis of the Femoral Head with Cystic Degeneration: Postprint

Authors: He Xianshun, Wei Yurou, He Mincong, Lin Kun, Tian Jiaqing, Zhan Zhiwei, Tianye Lin, He Xiaoming, He Wei, Wei Qiushi, Wei Qiushi

Date: 2023-11-16T00:00:00+00:00

Abstract

In steroid-induced osteonecrosis of the femoral head (SIONFH), cystic changes exhibit a “double-edged sword” effect. The Huoxue Tongluo method demonstrates excellent efficacy in early-stage SIONFH, but its regulatory effects on proteins involved in bone resorption, angiogenesis, and osteogenesis in cystic changes remain unclear.

Objective: To investigate the effects of the Huoxue Tongluo method on serum proteins related to bone resorption, angiogenesis, and osteogenesis in SIONFH patients with cystic changes.

A total of 60 SIONFH patients admitted to the Hip Joint Research Center of Guangzhou University of Chinese Medicine from January 2019 to January 2021 were enrolled and randomly divided into two groups using a random number table method, with 30 patients in the control group and 30 in the treatment group. Additionally, 30 volunteers with normal physical examination results and no history of steroid use during the same period were selected as the normal group. The treatment group received Huoxue Tongluo capsules (2 g per dose, three times daily) and calcium carbonate (600 mg per dose, once daily), while the control group received an equivalent dose of calcium carbonate alone. The treatment course for both groups was 12 months, with limited weight-bearing on the affected limb. Fasting serum samples were collected, and enzyme-linked immunosorbent assay (ELISA) was used to detect the expression levels of serum receptor activator of nuclear factor- B ligand (RANKL), platelet-derived growth

factor-BB (PDGF-BB), vascular endothelial growth factor A (VEGFA), osteoprotegerin (OPG), and β-catenin (CTNNB1). Follow-up visits were conducted at 6 and 12 months after discharge through outpatient follow-up, with femoral head collapse defined as the endpoint event. Treatment efficacy was evaluated using the visual analog scale (VAS) for hip pain, Harris Hip Score, and necrotic area score.

Baseline levels of RANKL, PDGF-BB, OPG, and CTNNB1 differed significantly among the three groups ($P<0.05$). Intergroup comparison revealed that both the control and treatment groups had higher RANKL and PDGF-BB levels and lower OPG and CTNNB1 levels compared with the normal group ($P<0.05$). Repeated measures ANOVA demonstrated a significant interaction between time and group on RANKL, PDGF-BB, VEGFA, OPG, and CTNNB1 levels ($P<0.05$), with significant main effects of both time and group on these parameters ($P<0.05$). Specifically, RANKL levels in the treatment group were higher than those in the control group at 6 months, PDGF-BB levels were higher in the treatment group at 12 months, and VEGFA, OPG, and CTNNB1 levels were higher in the treatment group at both 6 and 12 months ($P<0.05$). At 12 months post-treatment, the treatment group exhibited lower VAS scores and necrotic area scores but higher Harris Hip Scores compared with the control group ($P<0.05$). Intragroup comparisons showed that at 12 months post-treatment, the treatment group had lower VAS scores and necrotic area scores and higher Harris Hip Scores compared with baseline ($P<0.05$), while the control group had higher VAS scores and Harris Hip Scores compared with baseline ($P<0.05$).

Conclusion: The Huoxue Tongluo method can upregulate the protein expression levels of RANKL, PDGF-BB, VEGFA, OPG, and CTNNB1 in SIONFH patients, effectively promoting bone repair and improving clinical symptoms. It is hypothesized that this therapy promotes bone repair in cystic changes through a ternary repair network of "bone resorption/angiogenesis/osteogenesis".

Full Text

Regulation of Activating Blood and Dredging Collaterals Method on Serum Bone Resorption/Angiogenesis/Osteogenic Proteins in Patients with Steroid-Induced Osteonecrosis of the Femoral Head with Cystic Degeneration

HE Xianshun[12], WEI Yurou[12], HE Mincong[23], LIN Kun[12], TIAN Jiaqing[12], ZHAN Zhiwei[12], LIN Tianye[23], HE Xiaoming[23], HE Wei[23], WEI Qiushi[23]*

[1]The Third Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510378, China
[2]Guangdong Research Institute for Orthopedics and Traumatology of Chinese Medicine, Guangzhou 510378, China

[3]Department of Joint Diseases, The Third Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510378, China

Corresponding author: WEI Qiushi, Associate Chief Physician/Associate Professor/Doctoral Supervisor; E-mail: weiqshi@126.com

Clinical Trial Registration: ChiCTR-OPC-15007030 (Chinese Clinical Trial Registry)

Abstract

Background: Cystic degeneration in steroid-induced osteonecrosis of the femoral head (SIONFH) exhibits a "double-edged sword" effect. While the activating blood and dredging collaterals method demonstrates efficacy in early-stage SIONFH, its regulatory effects on bone resorption/angiogenesis/osteogenic proteins in cystic degeneration remain unclear.

Objective: To investigate the effects of the activating blood and dredging collaterals method on serum bone resorption/angiogenesis/osteogenic proteins in SIONFH patients with cystic degeneration.

Methods: Sixty SIONFH patients admitted to the Hip Joint Research Center of Guangzhou University of Chinese Medicine between January 2019 and January 2021 were enrolled and randomly divided into control and treatment groups (30 patients each). An additional 30 healthy volunteers without steroid use history were recruited as the normal group. The treatment group received Huoxuetongluo Capsule (2 g, three times daily) plus calcium carbonate (600 mg, once daily), while the control group received calcium carbonate alone for 12 months, with restricted weight-bearing on the affected limb. Fasting serum samples were collected to measure nuclear factor B receptor activator ligand (RANKL), platelet-derived growth factor-BB (PDGF-BB), vascular endothelial growth factor A (VEGFA), osteoprotegerin (OPG), and cadherin-associated protein (CTNNB1) levels via enzyme-linked immunosorbent assay. Patients were followed up at 6 and 12 months post-discharge, with femoral head collapse defined as the endpoint. Efficacy was evaluated using the visual analogue scale (VAS) for hip pain, Harris Hip Score, and necrotic area scoring.

Results: Baseline levels of RANKL, PDGF-BB, OPG, and CTNNB1 differed significantly among the three groups ($P<0.05$). Intergroup comparisons revealed that both control and treatment groups had higher RANKL and PDGF-BB but lower OPG and CTNNB1 than the normal group ($P<0.05$). Repeated measures ANOVA demonstrated significant time-group interactions for RANKL, PDGF-BB, VEGFA, OPG, and CTNNB1 ($P<0.05$), with significant main effects of time and group ($P<0.05$). Specifically, RANKL levels were higher in the treatment group at 6 months, PDGF-BB levels were higher at 12 months, and VEGFA, OPG, and CTNNB1 levels were higher at both 6 and 12 months compared to the control group ($P<0.05$). At 12 months, the treatment group

showed lower hip VAS scores and necrotic area scores but higher Harris scores than the control group ($P<0.05$). Intragroup comparisons revealed that the treatment group had improved VAS scores, necrotic area scores, and Harris scores at 12 months compared to baseline ($P<0.05$), while the control group showed increased VAS and Harris scores ($P<0.05$).

Conclusion: The activating blood and dredging collaterals method upregulates serum RANKL, PDGF-BB, VEGFA, OPG, and CTNNB1 protein expression, effectively promoting bone repair and improving clinical symptoms in SIONFH patients. We hypothesize that this therapeutic effect occurs through a "bone resorption/angiogenesis/osteogenesis" triad repair network that facilitates cystic degeneration repair.

Keywords: Femur head necrosis; Steroid-induced osteonecrosis of femoral head; Coxarthrosis; Activating blood dredging collaterals; Bone resorption/angiogenesis/osteogenic protein

Introduction

Steroid-induced osteonecrosis of the femoral head (SIONFH) is a debilitating bone necrosis disease caused by prolonged high-dose glucocorticoid use, predominantly affecting young and middle-aged adults aged 20-50 years. With increasing incidence, this condition is characterized by a prolonged course, high disability rate, and substantial treatment challenges, posing significant obstacles to hip-preserving therapy [1-2]. Recent research has revealed that cystic degeneration in SIONFH carries special clinical significance: on one hand, it reflects active bone repair responses within the necrotic zone; on the other hand, it dramatically reduces hip joint load-bearing capacity, subjecting cartilage to abnormal stress and causing local trabecular fractures, thereby becoming a driving factor for femoral head collapse [2-4]. Consequently, investigating methods to promote bone repair within cystic degeneration has become crucial for treating early-stage SIONFH.

In traditional Chinese medicine (TCM), SIONFH belongs to the "bone erosion" category. Currently, no specific effective medication exists for its treatment domestically or internationally. Our research team has long concluded that blood stasis represents the main syndrome pattern across different etiologies of femoral head necrosis [5-6], establishing the collateral disease theory for this condition [7]. The core therapeutic principle should focus on activating blood and dredging collaterals. Based on the classical Taohong Siwu Decoction, our team developed the hospital preparation Huoxuetongluo Capsule, composed of seven herbs including *Cajanus cajan* leaf, *Angelica sinensis* tail, *Ligusticum chuanxiong*, *Paeonia lactiflora*, prepared *Rehmannia glutinosa*, peach kernel, and safflower. This formula activates blood, dredges collaterals, removes stasis, and relieves pain. Our previous in vivo and in vitro experiments have demonstrated its osteogenic effects [8-11], though its regulatory mechanisms on bone repair

proteins in SIONFH patients with cystic degeneration require further investigation. Previous studies have confirmed that RANKL, PDGF-BB, VEGFA, OPG, and CTNNB1 are important regulators in bone repair processes [12-16]. Therefore, this study employed Huoxuetongluo Capsule to treat SIONFH and explore its effects on bone repair proteins in cystic degeneration, aiming to provide theoretical evidence for TCM-based hip-preserving therapy.

Methods

Sample Size Calculation Using GPower3 software for multi-factor repeated measures ANOVA, the sample size formula was applied:

$$na = \frac{(\sigma_b^2/k)z_{1-\alpha/2} + z_{1-\beta}}{u_A - u_B}, \quad nb = k \cdot na, \quad 1-\beta = \phi\left(\frac{|u_A - u_B|}{\sqrt{na}} - z_{1-\alpha/\tau}\right)$$

With effect size 0.25, α=0.05, power 1-β=0.8, k as group number, and τ as repeated measurement times, the minimum sample size was calculated as 11 per group. Considering a 20% attrition rate, 60 patients were recruited.

Participants Sixty SIONFH patients meeting inclusion/exclusion criteria, admitted to the Hip Joint Research Center of Guangzhou University of Chinese Medicine between January 2019 and January 2021, were randomly divided into control and treatment groups (30 each). Thirty healthy volunteers without steroid use history were recruited as the normal group. This study complied with the Helsinki Declaration; all participants provided informed consent, and the protocol was approved by the hospital ethics committee (Approval No. 2015010) and registered with the Chinese Clinical Trial Registry (ChiCTR-OPC-15007030).

Inclusion Criteria

(1) SIONFH diagnosis based on the *Chinese Clinical Guidelines for Adult Femoral Head Necrosis (2020)* [12] through clinical examination and imaging; (2) Hip imaging showing cystic changes; (3) No prior treatment before blood sampling.

Exclusion Criteria

(1) Allergy to Huoxuetongluo Capsule components; (2) Hip trauma history; (3) Severe congenital hip deformity; (4) Severe bone metabolic disease; (5) Conditions affecting the hip joint, including rheumatoid arthritis, ankylosing spondylitis, joint tuberculosis, or septic arthritis; (6) Poor compliance affecting outcome measurement; (7) Voluntary withdrawal after study commencement; (8) Incomplete protocol adherence; (9) Lost to follow-up.

Interventions The treatment group received Huoxuetongluo Capsule (2 g, three times daily) plus calcium carbonate (600 mg, once daily), while the control group received equivalent calcium carbonate alone. Both groups underwent 12-month treatment with restricted weight-bearing on the affected limb using crutches. The normal group received no intervention.

Blood Sample Collection Fasting venous blood (5 mL) was collected from treatment and control groups at baseline (0 months), 6 months, and 12 months using vacuum tubes. After natural coagulation at 4°C, samples were centrifuged at 1,000 r/min for 15 minutes (radius 10 cm), and serum was extracted, aliquoted, and stored at -80°C. The normal group provided blood samples only at baseline.

Serum Protein Detection Following kit instructions, ELISA was used to detect serum RANKL (Cat# CSB-E05125h), PDGF-BB (Cat# CSB-E08923h), VEGFA (Cat# CSB-E11718h), OPG (Cat# CSB-E04692h), and CTNNB1 (Cat# CSB-E08963h) (all from Cusabio, Wuhan). Equipment included a low-temperature centrifuge (Eppendorf), clean bench (Qingdao Haier), full-wavelength microplate reader (Thermo Scientific), and constant temperature shaker (Taikang, Jiangsu).

Follow-up and Endpoints Outpatient follow-ups were conducted at 6 and 12 months post-discharge, measuring serum proteins, hip imaging, final VAS scores, and Harris Hip Scores at 12 months. Femoral head collapse was defined as the endpoint event. Non-surgical treatment was discontinued when patients achieved significant pain relief, femoral head stability, satisfactory bone repair, or collapse occurred [13].

Efficacy Evaluation Based on the *TCM Efficacy Evaluation Criteria for Femoral Head Necrosis (2019 Edition)* [14], evaluation included:

1. **Hip VAS Score**: A 10-cm scale (0=no pain, 10=unbearable pain) assessed at baseline and 12 months.
2. **Harris Hip Score** [15]: Evaluating pain, function (gait, activities), deformity, and range of motion (flexion, abduction, adduction, internal/external rotation), with scores >90=excellent, 80-89=good, 70-79=fair, <70=poor.
3. **Necrotic Area Score**: On anteroposterior hip radiographs: no cystic change=grade 0 (0 points); <15% necrosis=grade I (1 point); 15-30%=grade II (2 points); >30%=grade III (3 points), assessed at baseline and 12 months.

Quality Control A single-blind assessment was implemented. Clinical and imaging evaluations were performed by two attending physicians or higher, with discrepancies resolved through discussion until consensus.

Statistical Analysis SPSS 25.0 was used for data analysis. Normally distributed continuous data were expressed as mean±SD, compared using one-way ANOVA with LSD-t test for pairwise comparisons. Repeated measures data were analyzed using repeated measures ANOVA. Paired t-tests compared pre- and post-treatment Harris scores and necrotic area scores, while independent samples t-tests compared between groups. Non-normally distributed data were expressed as median (P25, P75), with Wilcoxon test for within-group VAS comparisons and Mann-Whitney U test for between-group comparisons. Categorical data were compared using ^{2} test. P<0.05 indicated statistical significance.

Results

Baseline Characteristics All 60 SIONFH patients completed the study without attrition. No significant differences existed in age, gender, height, or weight among the three groups (P>0.05) .

Serum Protein Levels Baseline RANKL, PDGF-BB, OPG, and CTNNB1 levels differed significantly among groups (F=253.7, 54.1, 30.6, respectively; P<0.05), while VEGFA showed no significant difference (F=1.1, P>0.05). Intergroup comparisons revealed that control and treatment groups had higher RANKL and PDGF-BB but lower OPG and CTNNB1 than the normal group (P<0.05), with no significant differences between control and treatment groups at baseline.

Repeated measures ANOVA (treatment vs. control groups) showed significant time-group interactions for RANKL, PDGF-BB, VEGFA, OPG, and CTNNB1 (P<0.001), with significant main effects of time and group (P<0.001). Specifically, treatment group RANKL levels were higher than control at 6 months, PDGF-BB levels were higher at 12 months, and VEGFA, OPG, and CTNNB1 levels were higher at both 6 and 12 months (P<0.05) .

Treatment Efficacy No significant differences existed in baseline VAS, Harris, or necrotic area scores between groups (P>0.05). At 12 months, the treatment group showed lower VAS scores and necrotic area scores but higher Harris scores compared to the control group (P<0.05). Intragroup comparisons revealed that the treatment group had improved VAS scores, necrotic area scores, and Harris scores at 12 months versus baseline (P<0.05), while the control group showed increased VAS and Harris scores (P<0.05) but no significant change in necrotic area score (P>0.05) .

Discussion

SIONFH represents a global therapeutic challenge in orthopedics. With widespread glucocorticoid use in autoimmune diseases and viral pneumonia

[16], SIONFH has become a leading cause of hip disability in young and middle-aged adults. The associated pain and functional impairment severely affect quality of life and work capacity, with some patients requiring total hip arthroplasty. However, given prosthesis lifespan limitations and complex, costly complications, preserving the native hip joint through enhanced bone repair is paramount.

Cystic degeneration reflects a series of repair responses occurring during osteoclast absorption of necrotic tissue, where intrinsic fibrous granulation tissue may possess osteogenic and angiogenic capabilities [17]. Our previous pathological studies of femoral heads from SIONFH patients undergoing total hip arthroplasty revealed that cystic changes represent granulation tissue in trabecular absorption zones, consistent with prior reports [18]. Thermomechanical mapping demonstrated that cystic degeneration typically occurs in the anterolateral column, coinciding with weight-bearing trabecular locations [19], ultimately compromising mechanical load-bearing capacity and precipitating collapse. Thus, cystic degeneration both reduces mechanical transmission (initiating collapse) and triggers bone repair (promoting osteogenesis) [4,17], making its repair response crucial for hip-preserving therapy.

Current hip-preserving treatments include non-surgical (medication, weight restriction, physical therapy) and surgical approaches, with medication primarily targeting bone metabolism, lipid metabolism, coagulation, and TCM blood-activating stasis-removing principles [1]. The ultimate goal is promoting bone repair, relieving pain, maintaining femoral head stability, restoring hip function, and delaying arthroplasty. Research indicates that RANKL, VEGFA, PDGF-BB, OPG, and CTNNB1 are key regulatory factors in bone repair [20-22].

At baseline, SIONFH patients showed elevated serum RANKL compared to normal controls ($P<0.05$), indicating osteoclast-mediated bone absorption activity within cystic changes. Literature suggests bone repair initiation depends on osteoclast-mediated dissolution of necrotic tissue to clear pathways for repair [23,24]. HE et al. [25] found increased RANKL expression in serum and necrotic zones during early SIONFH, consistent with our findings. Huoxuetongluo Capsule treatment enhanced RANKL elevation during 0-6 months, suggesting enhanced bone absorption and repair activity.

Angiogenic proteins are vital for vascular homeostasis and femoral head perfusion. VEGFA specifically acts on vascular endothelial cells during repair initiation [26], promoting neovascularization in necrotic zones [9]. PDGF-BB, as a vascular growth factor, promotes bone marrow mesenchymal stem cell proliferation, migration, and osteogenic differentiation [27]. XIAO et al. [28] identified differential PDGF-BB and VEGFA expression in SIONFH angiogenic genes, likely reflecting neovascular ingrowth following osteoclast absorption of necrotic trabeculae. Notably, YU et al. [29] demonstrated that osteocytes cannot survive beyond 100 mm from blood vessels, emphasizing that vascular development precedes osteogenesis. Our findings show Huoxuetongluo Capsule upregulated serum PDGF-BB and VEGFA, peaking at 12 months, indicating promotion of

angiogenesis within cystic repair zones to support osteoblasts.

During later repair stages, elevated OPG reflects predominant osteogenesis. OPG competes with RANKL to block RANK/RANKL binding, inhibiting osteoclast differentiation and function [30], thereby facilitating new bone formation. CTNNB1, a key gene in the Wnt/β-catenin osteogenic pathway secreted by osteoclast precursors, promotes bone formation when upregulated. However, previous studies showed weak osteogenic capacity in SIONFH necrotic zones [3,31,32]. Our results demonstrated that Huoxuetongluo Capsule treatment increased serum OPG and CTNNB1 at 6 and 12 months ($P<0.05$), suggesting enhanced osteogenesis during cystic repair. While ZHANG et al. [33] reported initially high β-catenin expression in SIONFH rat models that decreased over time, our human study showed different results, possibly due to species differences and the presence of cystic changes difficult to replicate in animal models. Collectively, we propose that Huoxuetongluo Capsule regulates bone repair proteins sequentially: bone absorption $\rightarrow$ angiogenesis $\rightarrow$ osteogenesis.

Clinically, the treatment group showed improved VAS scores, Harris scores, and necrotic area scores at final follow-up compared to controls ($P<0.05$), demonstrating that Huoxuetongluo Capsule effectively improves symptoms and promotes cystic repair, reducing collapse risk. The control group showed improved Harris and necrotic area scores versus baseline, suggesting calcium carbonate and weight restriction modestly improved bone density [34]. However, control group VAS scores increased, likely because although cystic changes indicate repair activity, they correlate closely with hip pain [17], and incomplete repair may cause discomfort. Moreover, calcium carbonate weakly regulated angiogenic proteins PDGF-BB and VEGFA compared to Huoxuetongluo Capsule ($P<0.001$). From a TCM perspective, blood stasis throughout the disease process causes "obstruction leading to pain" [35]; calcium carbonate maintains bone but cannot activate blood or dredge collaterals, explaining the increased VAS scores. Thus, activating blood and dredging collaterals promotes repair and relieves pain through "unblocking to stop pain."

This study has limitations: small sample size, short follow-up, and single-center design precluded long-term dynamic observation of protein changes. Additionally, the dual role of cystic degeneration warrants further investigation using single-cell sequencing, whole-transcriptome, and spatial transcriptomics to explore molecular mechanisms, with larger multicenter studies needed to validate these findings.

In conclusion, the activating blood and dredging collaterals method upregulates serum RANKL, PDGF-BB, VEGFA, OPG, and CTNNB1 levels, mediating a "bone resorption/angiogenesis/osteogenesis" triad repair network that promotes cystic degeneration repair, improves hip function, reduces pain, and decreases necrotic area, providing experimental evidence for TCM treatment of SIONFH.

Author Contributions

HE Xianshun: manuscript writing; WEI Yurou, HE Mincong, LIN Kun, TIAN Jiaqing, ZHAN Zhiwei: statistical analysis and table preparation; LIN Tianye, HE Xiaoming: data collection and organization; HE Wei, WEI Qiushi: research conception, design, and manuscript revision; WEI Qiushi: quality control and review.

Conflict of Interest: None declared.

References

[1] ZHANG Changqing. Guidelines for hip-preserving treatment of femoral head necrosis (2016 edition) [J]. *Chinese Journal of Geriatric Orthopaedics and Rehabilitation (Electronic Edition)*, 2016, 2(2): 65-70.

[2] COHEN-ROSENBLUM A, CUI Q J. Osteonecrosis of the femoral head [J]. *Orthopedic Clinics of North America*, 2019, 50(2): 139-149. DOI: 10.1016/j.ocl.2018.10.001.

[3] SHEN Yingshan, WU Rishana, ZHUANG Zhikun, et al. Analysis of blood stasis syndrome characteristics in steroid-induced and alcohol-induced femoral head necrosis based on pathological phenotypes [J]. *China Journal of Traditional Chinese Medicine and Pharmacy*, 2021, 36(8): 4868-4872.

[4] WANG Min, YANG Weimin, LI Siming. Clinical significance of femoral head cystic changes in predicting steroid-induced osteonecrosis [J]. *Chinese Journal of Bone and Joint Surgery*, 2019, 12(6): 434-438. DOI: 10.3969/j.issn.2095-9958.2019.06.07.

[5] HE Xiaoming, SHEN Yingshan, PANG Fengxiang, et al. Clinical investigation of diagnostic indicators for blood stasis syndrome in femoral head necrosis patients [J]. *China Journal of Traditional Chinese Medicine and Pharmacy*, 2022, 37(1): 412-416.

[6] WEI Qiushi. Study on syndrome differentiation and standardized indications of traditional Chinese medicine treatment for femoral head necrosis [D]. Guangzhou: Guangzhou University of Chinese Medicine, 2012.

[7] WEI Qiushi, SHEN Yingshan, ZHUANG Zhikun, et al. Establishment and scientific value of collateral disease theory in femoral head necrosis [J]. *Chinese Archives of Traditional Chinese Medicine*, 2021, 39(7): 1-4. DOI: 10.13193/j.issn.1673-7717.2021.07.001.

[8] HE Xiaoming, HE Mincong, YANG Peng, et al. The therapeutic effect of Huoxue Tongluo capsules in ARCO stage II osteonecrosis of the femoral head: a clinical study with an average follow-up period of 7.95 years [J]. *Frontiers in Pharmacology*, 2021, 12: 773758. DOI: 10.3389/fphar.2021.773758.

[9] WU Rishana, CHEN Xiaojun, SHEN Yingshan, et al. Intervention effect of Huoxuetongluo Capsule on steroid-induced femoral head necrosis model rats through OPG/RANKL/PDGF-BB pathway [J]. *Traditional Chinese Drug Research and Clinical Pharmacology*, 2022, 33(4): 433-440. DOI: 10.19378/j.issn.1003-9783.2022.04.003.

[10] WU Zhongshu, WEI Yurou, CHEN Xiaojun, et al. ERα-Wnt/β-catenin signaling pathway in Huoxuetongluo Capsule-promoted osteogenesis of bone marrow mesenchymal stem cells [J]. *Chinese Journal of Tissue Engineering Research*, 2022, 26(25): 3937-3943.

[11] WEI Q S, HONG G J, YUAN Y J, et al. Huoxue Tongluo capsule, a vasoactive herbal formula prevents progression of asymptomatic osteonecrosis of femoral head: a prospective study [J]. *Journal of Orthopaedic Translation*, 2019, 18: 65-73. DOI: 10.1016/j.jot.2018.11.002.

[12] Chinese Medical Doctor Association Orthopedics Branch Bone Circulation and Necrosis Committee, Chinese Medical Association Orthopedics Branch Bone Microsurgery Group, International Association of Bone Circulation China Region. Chinese clinical guidelines for adult femoral head necrosis (2020) [J]. *Chinese Journal of Orthopaedics*, 2020, 40(20): 945-954. DOI: 10.3760/cma.j.cn121113-20200909-00477.

[13] WEI Qiushi, HE Xiaoming, HE Wei, et al. Clinical efficacy and influencing factors of non-surgical hip-preserving treatment for ARCO stage II femoral head necrosis [J]. *Chinese Journal of Bone and Joint Surgery*, 2022, 15(6): 424-430. DOI: 10.3969/j.issn.2095-9958.2022.06.05.

[14] CHEN Weiheng, HE Wei, ZHAO Dewei, et al. TCM efficacy evaluation criteria for femoral head necrosis (2019 edition) [J]. *The Journal of Traditional Chinese Orthopedics and Traumatology*, 2019, 31(6): 3-6.

[15] HARRIS W H. Traumatic arthritis of the hip after dislocation and acetabular fractures: treatment by mold arthroplasty. An end-result study using a new method of result evaluation [J]. *The Journal of Bone and Joint Surgery (American Volume)*, 1969, 51(4): 737-755.

[16] CHEN Qing, FAN Zhongjie, YANG Meiying, et al. Clinical discussion on glucocorticoid application in COVID-19 patients [J]. *Chinese Journal of Respiratory and Critical Care Medicine*, 2021, 20(4): 235-240.

[17] WEI Qiushi, HE Wei, ZHANG Qingwen, et al. Study on different imaging manifestations of pre-collapse stage femoral head necrosis [J]. *Chinese Journal of Reparative and Reconstructive Surgery*, 2021, 35(9): 1098-1104.

[18] LIU Guangbo, MA Haiyang, LU Qiang, et al. Distribution characteristics of cystic changes in femoral head osteonecrosis [J]. *Journal of Chinese PLA Medical School*, 2019, 40(12): 1109-1113, 1137. DOI: 10.3969/j.issn.2095-5227.2019.12.001.

[19] TIAN Jiaqing, HE Mincong, WEI Yurou, et al. Distribution patterns and pathological characteristics of cystic changes in steroid-induced femoral head necrosis [J]. *Chinese Journal of Tissue Engineering Research*, 2023, 27(31): 4937-4942.

[20] KIM J M, LIN C J, STAVRE Z, et al. Osteoblast-osteoclast communication and bone homeostasis [J]. *Cells*, 2020, 9(9): 2073. DOI: 10.3390/cells9092073.

[21] ZHANG Zhixiu, LIU Jing, ZENG Peiyun, et al. Research progress on H-type vessels in osteoporosis [J]. *Chinese Journal of Osteoporosis*, 2022, 28(4): 575-579. DOI: 10.3969/j.issn.1006-7108.2022.04.020.

[22] HUANG Y S, WAN S, YANG M. Circ_{0067680} expedites the osteogenic differentiation of human bone marrow-derived mesenchymal stem cells through miR-4429/CTNNB1/Wnt/β-catenin pathway [J]. *Biology Direct*, 2021, 16(1): 16. DOI: 10.1186/s13062-021-00302-w.

[23] GRANCHI D, CIAPETTI G, GÓMEZ-BARRENA E, et al. Biomarkers of bone healing induced by a regenerative approach based on expanded bone marrow-derived mesenchymal stromal cells [J]. *Cytotherapy*, 2019, 21(8): 870-885. DOI: 10.1016/j.jcyt.2019.06.002.

[24] MA Chao. Evaluation of trabecular microstructure changes during SIONFH progression in rats and intervention effects of Huoxuetongluo method based on Micro-CT [D]. Guangzhou: Guangzhou University of Chinese Medicine, 2021.

[25] HE M C, ZHANG J, CHEN X J, et al. Osteoclastic activity was associated with the development of steroid-induced osteonecrosis of femoral head [J]. *Artificial Cells, Nanomedicine, and Biotechnology*, 2020, 48(1): 1036-1046. DOI: 10.1080/21691401.2020.1774596.

[26] LIU Xiaonan, YU Bin. Research progress on the role of H-type vessels in bone development and bone diseases [J]. *Chinese Journal of Osteoporosis*, 2023, 29(5): 751-757. DOI: 10.3969/j.issn.1006-7108.2023.05.025.

[27] LI Dianqi. Mechanism of PDGF-BB in regulating osteoblasts/osteoclasts and experimental study on promoting bone healing [D]. Wuhan: Wuhan University, 2016.

[28] XIAO Zhen, LI Shenghua. Identification of angiogenesis-related genes in steroid-induced femoral head necrosis and screening validation of targeted traditional Chinese medicine components [J]. *Chinese Traditional and Herbal Drugs*, 2023, 54(5): 1526-1539. DOI: 10.7501/j.issn.0253-2670.2023.05.020.

[29] YU H Y, VANDEVORD P J, MAO L, et al. Improved tissue-engineered bone regeneration by endothelial cell mediated vascularization [J]. *Biomaterials*, 2009, 30(4): 508-517. DOI: 10.1016/j.biomaterials.2008.09.047.

[30] BOYCE B F, XING L P. Functions of RANKL/RANK/OPG in bone modeling and remodeling [J]. *Archives of Biochemistry and Biophysics*, 2008, 473(2): 139-146. DOI: 10.1016/j.abb.2008.03.018.

[31] WEI Qiushi, YANG Fan, CHEN Xiaojun, et al. Analysis of pathological and microstructural characteristics of necrotic regions in bone specimens from steroid-induced and alcohol-induced femoral head necrosis patients [J]. *Chinese Journal of Reparative and Reconstructive Surgery*, 2018, 32(7): 866-872. DOI: 10.7507/1002-1892.201806064.

[32] SHEN Yingshan. Scientific value of collateral disease theory based on phenotypic differences of "blood stasis syndrome" in different etiologies of femoral head necrosis [D]. Guangzhou: Guangzhou University of Chinese Medicine, 2021.

[33] ZHANG C, ZOU Y L, MA J, et al. Apoptosis associated with Wnt/β-catenin pathway leads to steroid-induced avascular necrosis of femoral head [J]. *BMC Musculoskeletal Disorders*, 2015, 16: 132. DOI: 10.1186/s12891-015-0606-2.

[34] KLUMPP R, TREVISAN C. Aseptic osteonecrosis of the hip in the adult: current evidence on conservative treatment [J]. *Clinical Cases in Mineral and Bone Metabolism*, 2015, 12(Suppl 1): 39-42. DOI: 10.11138/ccmbm/2015.12.3s.039.

[35] SHEN Yingshan, CHEN Zhenqiu, HE Wei, et al. Application of non-surgical hip-preserving therapy based on "activating blood and dredging collaterals method" in patients with systemic multiple osteonecrosis [J]. *Lishizhen Medicine and Materia Medica Research*, 2020, 31(6): 1389-1392.

Received: 2023-05-19; *Revised:* 2023-10-18
Edited by: ZOU Lin

Note: Figure translations are in progress. See original paper for figures.