ORIGINAL RESEARCH

Correlation Between Traditional Chinese Medicine Evidence and Lipid Metabolism-Regulating Kinase in Elderly Patients with Osteoporosis

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ABSTRACT

Objective • Previous studies have indicated that an increase in the number of fat cells is associated with a decrease in bone density and bone mass. During this process, the differentiation of fat cells is closely related to the extracellular signal-regulated kinase (ERK) 1/2 signaling pathway. However, research on the characteristics of lipid metabolism-regulating kinase ERK1/2 expression in osteoporosis patients with different traditional Chinese medicine (TCM) patterns and their correlations is currently limited. Therefore, this study aims to explore the correlation between TCM patterns and lipid metabolism-regulating kinases in elderly osteoporosis patients.

Methods • A total of 88 elderly osteoporosis patients with hip joint diseases who underwent total hip arthroplasty at our hospital from October 2017 to October 2022 were selected for this study, and all included patients met the complete inclusion criteria. The 88 patients were divided into groups according to TCM pattern differentiation, with 37 cases of liver and kidney deficiency pattern (Group A), 28 cases of spleen and kidney yang deficiency pattern (Group B), and 23 cases of qi stagnation and blood stasis pattern (Group C). On the second day of hospitalization, 5 ml of morning fasting venous blood was collected from patients in the three groups and used to detect and compare the differences in total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels. Patients underwent 7 days of in-hospital conditioning before undergoing total hip arthroplasty. After surgery, bone specimens were collected from the femoral neck bone defect and preserved for testing. Western blot was used to detect the protein expression levels of ERK1/2 and p-ERK1/2 in bone specimens of patients with osteoporosis. Subsequently, the differences in ERK1/2 and p-ERK1/2 protein expression levels among the three groups were compared. Finally, multifactorial logistics regression analysis was used to determine the correlation between different TCM patterns and blood lipid indicators and lipid metabolism-regulating kinases.

Results • There was no significant difference in TC levels among osteoporosis patients with TCM patterns in the three groups (all, $P > .05$). TG levels in Group B osteoporosis patients were significantly higher than those in Group A and Group C (all, $P < .05$). HDL-C levels in Group A osteoporosis patients were significantly higher than those in Group B and Group C (all, $P < .05$). LDL-C levels in Group C osteoporosis patients were significantly lower than those in Group A and Group B (all, $P < .05$). There was no significant difference in ERK1/GAPDH and ERK2/GAPDH levels among osteoporosis patients with TCM patterns in the three groups (all, $P > .05$). The levels of p-ERK1 and p-ERK2 in bone specimens of osteoporosis patients in Group C were significantly higher than those in Group A and Group B (all, $P < .05$). The level of p-ERK2 in bone specimens of osteoporosis patients in Group A was significantly higher than that in Group B ($P < .05$). There was a significant positive correlation between TCM pattern and TG levels in patients in Group B ($r = 0.221$, $P < .05$) and a significant negative correlation with p-ERK1/2 values ($r=-0.547$, $P < .05$).

Conclusion • Osteoporosis patients with different TCM patterns exhibit significant differences in blood lipid indicators and lipid metabolism-regulating kinase levels. Additionally, there is a close relationship between different TCM patterns of osteoporosis and lipid metabolism-regulating kinases. (*Altern Ther Health Med.* 2024;30(7):263-267).

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INTRODUCTION

Osteoporosis is a prevalent systemic endocrine bone metabolic disorder closely associated with the natural processes of aging and degeneration.[1] This multifaceted condition is characterized by reduced bone mass, resulting in thinning, fractures, and decreased bone trabecular density, accompanied by porous and thinning bone cortex. It substantially elevates the patient's bone fragility and the risk of fractures.[2] Geriatric osteoporosis, often referred to as type II primary osteoporosis, predominantly affects individuals over the age of 60, and recent years have witnessed a notable increase in its prevalence.[3] The study of osteoporosis, particularly in the elderly population, assumes paramount significance. Osteoporotic fractures not only lead to significant morbidity and mortality but also have substantial economic implications for healthcare systems. Therefore, comprehending the underlying mechanisms of osteoporosis and identifying effective treatment strategies is pivotal in addressing this burgeoning public health issue.

Traditional Chinese medicine (TCM) offers a unique viewpoint on osteoporosis, characterizing it as "bone paralysis and bone impotence".[4] Within the TCM framework, osteoporosis pathogenesis is thought to implicate multiple organs and systems within the body. TCM evidence types play a central role in guiding tailored treatment approaches that consider the specific characteristics of each patient's condition.[5] Understanding the TCM perspective on osteoporosis is essential for holistic and personalized patient care, offering valuable insights that could enhance therapeutic outcomes. Furthermore, recent research has emphasized the interplay between lipid metabolism and bone health. Elevated adipocyte expression is often associated with reduced bone mineral density and bone mass, with adipocyte differentiation regulated in part by the extracellular regulated protein kinases (ERK) 1/2 signaling pathway.[6] Disruptions in lipid metabolism have been linked to disorders of bone metabolism and the development of osteoporosis.[7] Investigating lipid metabolism and its regulation through kinases holds promise for a more comprehensive understanding of osteoporosis.

Despite prior research, a substantial gap remains in our understanding regarding the relationship between different TCM evidence types and lipid metabolism indices, as well as lipid metabolism-regulating kinases in elderly patients with osteoporosis. This gap presents a vital area for further exploration and investigation. This study seeks to address this knowledge deficit by examining the correlation between TCM evidence types and lipid metabolism-regulating kinases in elderly patients with osteoporosis.

MATERIALS AND METHODS

Participants

Referencing the "Practical Guidelines for the Prevention and Treatment of Osteoporosis",[8] 88 elderly osteoporosis patients were classified into different traditional Chinese medicine (TCM) patterns, including Spleen-Kidney Yang Deficiency, Liver-Kidney Deficiency, and Qi Stagnation with Blood Stasis. TCM pattern classification was performed by four traditional Chinese medicine practitioners at our hospital. In cases where there was a significant difference in conclusions among the four practitioners for a particular patient, that patient was excluded to ensure the accuracy of the study. After TCM pattern differentiation by the traditional Chinese medicine practitioners, a total of 88 patients were included in this study, comprising 37 patients in Group A (Liver-Kidney Deficiency), 28 patients in Group B (Spleen-Kidney Yang Deficiency), and 23 patients in Group C (Qi Stagnation with Blood Stasis). General demographic information, including gender, age, and BMI, was collected for patients in the three groups. This study complied with the requirements of the "Helsinki Declaration" and was approved by the Ethics Committee of Changzhou Traditional Chinese Medical Hospital affiliated to Nanjing University of Traditional Chinese Medicine (Approval No.: CSU-2018-0258). All study participants were informed about the study and had signed relevant informed consent forms.

TCM Pattern Classification

Spleen-Kidney Yang Deficiency: Manifestations include aversion to cold, cold limbs, pale complexion, sore and weak lower back and knees, abdominal pain with a sensation of coldness. Chronic diarrhea or prolonged dysentery, frequent bowel movements, especially diarrhea that occurs in the early morning and is clear like rice water. Difficulty urinating, edema in the extremities, and in severe cases, abdominal distension. Some patients may experience frequent urination with incomplete emptying or nocturia. The tongue is pale and swollen with tooth marks on the edges, and the tongue coating is white and slippery. The pulse is deep, fine, and weak.

Liver-Kidney Deficiency: Symptoms include chronic joint pain that does not improve, restricted joint movement, muscle wasting, sore and weak lower back and knees, aversion to cold with cold limbs, impotence, spermatorrhea, or bone-steaming and fever with restlessness and dry mouth. The tongue is pale red with thin or scanty coating, and the pulse is deep, fine, and weak or fine and rapid.

Qi Stagnation with Blood Stasis: Characteristics involve stabbing pain that is aggravated by pressure and remains fixed, dusky or dark complexion with ecchymosis or petechiae. The pulse is fine, choppy, or deep and hesitant, or it may be wiry or have pulse knots.

Inclusion and exclusion criteria

Inclusion criteria: 1) patients were aged 60-80 years; 2) patients had a T value of ≤-2.5 on dual-energy X-ray bone density test; 3) patients were diagnosed with osteoporosis by clinical test results; 4) comorbid hip diseases; 5) who received total hip replacement (THR) in our hospital; 6) good cooperation with the study.

Exclusion criteria: 1) patients with mismatched age; 2) traumatic, septic, and rheumatoid arthritis that affects THR; 3) malignant neoplastic diseases; 4) serious infectious diseases; 5) serious visceral diseases; 6) mental, cognitive, and other dysfunctional diseases; 7) poor cooperation.

Detection methods and evaluation criteria

Blood lipid indices assay: Fasting venous blood (5ml) was collected on the morning of the day of admission and centrifuged to obtain the serum. Total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were determined by the automatic biochemical analyzer in the three groups of patients.

Western blot: The osteoporotic bone specimens retrieved from the femoral neck opening after THR were retained for the determination of the expression of ERK1/2 and p-ERK1/2 protein in the specimens using Western blot. Bone specimens were rinsed twice using PBS, bone tissue was ground under liquid nitrogen cryogenic conditions and processed by adding RIPA lysis solution, followed by centrifugation to extract total protein. 5 μL of protein samples were collected and quantified using the BCA protein quantification kit. 10 μL of protein samples were added with an appropriate amount of concentrated SDS-PAGE protein loading buffer and heated in boiling water for 3-5 min. After cooling to room temperature, the protein samples were placed in SDS-PAGE gel spiked wells for electrophoresis with a constant pressure setting of 120 V and an electrophoresis time of 90 min. The prepared proteins were transferred to the PVDF membrane by wet transfer using Bio-Rad, with a transfer current at 300 mA, and blocked for 2 h in TBST containing 5% skimmed milk at room temperature. Primary antibodies [anti-ERK 1/2 antibody, anti-Phospho-ERK 1/2 antibody, anti-GAPDH antibody at a dilution of 1:1000] were added at 4°C and incubated overnight. On day 2, PVDF membranes were rinsed 5 times using PBST for 5 min each time, and secondary antibodies containing labeled horseradish peroxide was added and shaken at room temperature for 2 h. Color development was performed using the ECL luminescence reagent method. The absorbance values of each band were determined by the gel analysis software, and the absorbance values were used to represent the protein expression levels. The ratio of the expression level of the protein to be measured to the expression level of the internal reference GAPDH indicates the relative expression level of the target protein.

Statistical analysis

Data analysis involved SPSS version 26.0. Categorical data was represented as n(%), and statistical differences were assessed using the chi-square test (χ^2). Continuous data was presented as mean (± standard deviation), and the *t* test was applied to examine statistical variances. Correlation analysis utilized binary logistic regression analysis. The significance threshold was established at $P < .05$, indicating statistical significance. In the data analysis, specific comparisons were conducted in the ANOVA and post-hoc tests to assess group differences. The rationale for employing binary logistic regression analysis for correlation was based on its suitability for the study's objectives.

RESULTS

Baseline Patient Profiles (Table 1)

The baseline patient profiles are summarized in Table 1. Group A consisted of 16 males and 21 females, aged 63-80 years (mean age 74.23±3.84), with a BMI range of 19-25 kg/m² (mean BMI 21.36±1.31). Group B included 12 males and 16 females, aged 61-78 years (mean age 74.62±3.77), with a BMI range of 20-26 kg/m² (mean BMI 21.52±1.34). Group C comprised 10 males and 13 females, aged 61-79 years (mean age 74.58±3.85), with a BMI range of 20-24 kg/m² (mean BMI 21.29±1.36). The three groups demonstrated no significant differences in baseline patient profiles ($P > .05$).

Table 1 Baseline patient profiles

	Group A (n = 37)	Group B (n = 28)	Group C (n = 23)	F/χ^2	*P* value
Sex				2.174	.298
Male	16	12	10		
Female	21	16	13		
Age (year)	63-80	61-78	61-79		
Mean age (year)	74.23±3.84	74.62±3.77	74.58±3.85	0.102	.903
BMI(kg/m²)	19-25	20-26	20-24		
Mean BMI(kg/m²)	21.36±1.31	21.52±1.34	21.29±1.36	0.207	.813

Lipid Index Levels in Patients with Different TCM Evidence Types of Osteoporosis (Table 2)

Table 2 displays the lipid index levels among patients with different TCM evidence types of osteoporosis. The three groups exhibited similar serum TC levels ($P > .05$). However, patients with osteoporosis characterized by spleen and kidney yang deficiency demonstrated significantly higher serum TG concentrations compared to those with liver and kidney deficiency or qi stagnation and blood stasis ($P < .05$). Furthermore, osteoporosis characterized by liver and kidney deficiency was associated with elevated serum HDL-C concentrations compared to other TCM evidence types, while the qi stagnation and blood stasis type displayed notably lower LDL-C levels ($P < .05$).

Table 2 Lipid index levels in patients with different TCM evidence types of osteoporosis (mmol/L)

Groups	n	TC	TG	HDL-C	LDL-C
Group A	37	4.37±0.13	1.86±0.22^{a}	1.21±0.20^{a,b}	2.95±0.27^{a,b}
Group B	28	4.39±0.11	2.41±0.18	1.01±0.14$^{\#}$	2.81±0.23^{b}
Group C	23	4.41±0.10	1.95±0.21	0.89±0.10	2.39±0.19

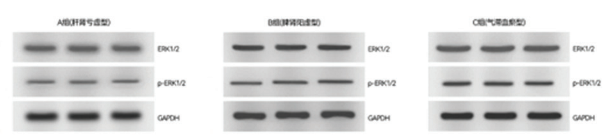
[a]indicates $P < .05$ when compared with group B
[b]indicates $P < .05$ when compared with group C

Table 3 ERK1/2 and p-ERK1/2 protein expression levels in patients with different TCM evidence types of osteoporosis

Groups	n	ERK1/GAPDH	p-ERK1	ERK2/GAPDH	p-ERK2
Group A	37	1.07±0.26	0.66±0.13^{b}	1.19±0.15	0.91±0.14^{a,b}
Group B	28	1.12±0.37	0.71±0.09^{b}	1.17±0.03	0.77±0.03^{b}
Group C	23	1.18±0.19	1.21±0.15	1.23±0.12	1.03±0.09

[a]indicates $P < .05$ when compared with group B
[b]indicates $P < .05$ when compared with group C

Figure 1 Western blotting bands of bone specimens from patients with different TCM evidence of osteoporosis



ERK1/2 and p-ERK1/2 Protein Expression Levels (Table 3 and Figure 1)

Table 3 presents the protein expression levels of ERK1/2 and p-ERK1/2 in patients with different TCM evidence types of osteoporosis. No statistically significant differences were observed in the levels of ERK1/GAPDH and ERK2/GAPDH among the three groups ($P > .05$).

However, as shown in Figure 1 and Table 3, the expression of p-ERK2 in bone specimens was highest in patients with qi stagnation and blood stasis osteoporosis, followed by liver and kidney deficiency, and then spleen and kidney yang deficiency ($P < .05$). Patients with qi stagnation and blood stasis osteoporosis also exhibited significantly higher levels of p-ERK1 compared to those with other TCM evidence types ($P < .05$).

Correlation Between Different TCM Evidence Types and Lipid Indicators and Kinases

The severity of spleen and kidney yang deficiency osteoporosis demonstrated a positive correlation with serum TG concentrations (r = 0.221, *P* < .05) and a negative correlation with p-ERK1/2 expression (r = -0.547, *P* < .05).

DISCUSSION

TCM physical signs refer to the morphological, structural, physiological, psychological and other comprehensive traits of the human body formed by innate endowment and acquired by the body.[9] According to TCM, a link exists between congenital signs and health, and changes in their signs may determine the body's susceptibility to certain disease-causing factors.[10] The pathogenesis of osteoporosis is closely associated with factors such as environment, exercise, age, and diet.[11] Osteoporosis features a slow onset and atypical symptoms in its early stages, and disease progression will increase the risk of fracture and seriously compromise the health of patients.[12] Traditional Chinese medicine holds a relatively unique theoretical system for the prevention and treatment of osteoporosis, and different schools of TCM treat the etiology and pathogenesis of osteoporosis differently.[13] To date, no unified criteria have been established in TCM regarding the typology of osteoporosis. Li et al.[14] classified osteoporosis mainly into the liver and kidney yin deficiency, spleen and kidney *yang* deficiency, and kidney deficiency and blood stasis, and Zhao et al.[15] categorized osteoporosis into four types by studying previous literature: kidney yang deficiency, liver and kidney *yin* deficiency, spleen and kidney *yang* deficiency, and blood stasis and *qi* stagnation. In the present study, the TCM classification of patients with osteoporosis was carried out with reference to the Practical Guidelines for the Prevention and Treatment of Osteoporosis, and it was found that most of the patients with osteoporosis had varying degrees of kidney deficiency, which is consistent with the TCM theory that "the kidney governs the bone marrow".[16] It was also suggested[17] that liver-yin deficiency, spleen deficiency, and blood stasis are important causes of the development of osteoporosis. The liver and kidney are both the source of essence and blood, which are related to the strength of the bones, so patients with a weak liver and kidney are prone to bone impotence. The spleen is the source of qi and blood biochemistry, and there exists a close relationship between the strength of the spleen and the energy and vitality of the blood. The deficiency of the body's vital energy will lead to poor blood flow, resulting in blood stasis and stagnation, abnormal transportation of *qi* and blood, *yin* and *yang*, and reduced bone density, which causes damage to the microstructure of bone tissue.

A related study[18] indicated that dyslipidemia could lead to impaired bone marrow microcirculation and disorders of bone metabolism, which consequently cause bone-related diseases such as osteoporosis. It was also reported[19] that dyslipidemia could significantly reduce osteocalcin expression, which could promote osteoclast differentiation and activation, thereby inhibiting the bone formation and exacerbating bone resorption in patients. In the present study, The three groups of patients exhibited similar serum TC levels (*P* > .05). Patients with osteoporosis of spleen and kidney *yang* deficiency showed higher serum TG concentrations versus those with osteoporosis of liver and kidney deficiency or *qi* stagnation and blood stasis (*P* < .05). Osteoporosis of liver and kidney deficiency was associated with higher serum HDL-C concentrations versus other TCM types, and the *qi* stagnation and blood stasis type was linked to significantly lower LDL-C levels versus other TCM types (*P* < .05). The results were consistent with previous research findings,[20] suggesting that changes in lipid levels affect human bones and that lipid levels differ between patients with different TCM evidence of osteoporosis. Prior research[21] has also identified a close association of bone metabolism indicators with lipid metabolism indicators. Adipocyte differentiation is influenced by the ERK1/2 signaling pathway. The ERK signaling pathway, as a member of the MAPK family, is mainly delivered by ERK1/2 to ERK and regulates the proliferation and differentiation of cells.[22] Lannigan et al.[23] showed that the ERK1/2 signaling pathway plays an important role in the proliferation and differentiation of osteoblasts and is closely related to the development of osteoporosis. A study[24] demonstrated that activation of the MAPK-ERK1/2 pathway could effectively increase osteogenic gene expression and thus promote osteoblast proliferation. Here, statistical significance was absent in the levels of ERK1/GAPDH and ERK2/GAPDH between the three groups (*P* > .05). The expression of p-ERK2 in the bone specimens was the highest in patients with *qi* stagnation and blood stasis osteoporosis, followed by liver and kidney deficiency, and then spleen and kidney yang deficiency (*P* < .05). Patients with *qi* stagnation and blood stasis also presented significantly higher levels of p-ERK1 than those with other TCM types (*P* < .05). This suggests a significant difference between phosphorylated ERK1/2 protein expression in patients with different TCM evidence types of osteoporosis. Some similar studies have reported an association between the ERK signaling pathway and the onset and progression of osteoporosis. For instance, He et al.[25] presented comparable findings, indicating a potential link between the activation of ERK1/2 and the qi stagnation and blood stasis pattern in osteoporosis. However, it is worth noting that other studies[26,27] have also identified variations in the ERK signaling pathway under different TCM patterns, particularly in terms of disease progression and treatment response. Taking these research results together, we postulate that various TCM patterns may exert distinct roles in the pathogenesis of osteoporosis, with the ERK signaling pathway potentially serving as a pivotal regulatory factor. Moreover, the severity of spleen and kidney yang deficiency osteoporosis was positively correlated with serum TG concentrations (r = 0.221, *P* < .05) and negatively correlated with p-ERK1/2 expression (r = -0.547, *P* < .05), which were in line with the results by Long,[28] whose found that the spleen-kidney-*yang* deficiency phenotype was positively correlated with TG levels and negatively correlated with the severity of osteoporosis. MAPK-ERK1/2 promotes osteogenesis as well as inhibits lipogenesis; thus, the diminished p-ERK1/2 expression will affect osteogenesis and the differentiation of osteoblasts in patients.[29] However, in our study, we did not find a significant association between total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and the severity of osteoporosis. These findings may suggest that, in spleen and kidney yang deficiency osteoporosis, triglycerides (TG) and p-ERK1/2 may play important roles, while the influence of TC, LDL, and HDL may be relatively minor. Nevertheless, further research is needed to gain deeper insights into the relationship between other lipid markers and different TCM pattern types of osteoporosis, in order to elucidate their potential biological mechanisms.

The study has some limitations. Firstly, all participants were individuals with hip diseases, potentially impacting the study outcomes. Additionally, the relatively small sample size restricted a comprehensive exploration of the relationship between lipid indices and lipid metabolism-regulated kinase signaling pathways in patients with each TCM evidence type. Further research is required to mitigate potential biases arising from other conditions or influencing factors. Future studies should encompass more diverse patient populations and employ larger sample sizes, longitudinal investigations, and controlled study designs. These approaches can enhance our understanding of the interplay between lipid metabolism, kinase signaling pathways, and TCM patterns in osteoporosis, ultimately leading to improved diagnostic and treatment strategies.

CONCLUSION

In summary, there are significant differences in blood lipid levels and lipid metabolism regulatory kinase activities among osteoporosis patients with different traditional Chinese medicine (TCM) patterns. Furthermore, there exists a close relationship between different TCM patterns of osteoporosis and lipid metabolism regulatory kinases. In clinical practice, understanding the TCM patterns of osteoporosis patients can provide an initial assessment of the disease's progression and severity for that particular type. These research findings can be applied to guide healthcare providers in gaining a better understanding of the characteristics associated with different TCM patterns among osteoporosis patients. They serve as a foundation for the development of personalized diagnostic and treatment plans, ultimately enhancing the effective management of osteoporosis.

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