ORIGINAL RESEARCH

The Analysis of Serum Klotho Protein Level and Related Gene Polymorphism in Osteoporotic Fracture of Elderly Patients with Osteoporosis

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ABSTRACT

Objective • Klotho protein level are reported to play important roles in the osteoporosis. To investigate the correlation between serum Klotho protein level and related gene (Klotho G395-A gene) polymorphism and osteoporotic fracture in elderly patients with osteoporosis.

Methods • A total of 62 elderly patients with osteoporosis admitted to the Department of Orthopedics of our hospital from January 2021 to June 2022 were included in the study group. Another 62 elderly patients without osteoporosis who underwent a physical examination at the same time were selected as the control group. Patients in the study group were divided into group A (n = 23, osteoporotic fracture) and group B (n = 39, osteoporotic fracture) according to the occurrence of osteoporotic fracture. Serum Klotho protein level was detected in all patients, and its related gene (Klotho G395-A gene) polymorphism was analyzed. After fasting in the morning (fasting for more than 8 hours), 3-5 ml venous blood was collected and immediately placed in a centrifuge tube. Serum was separated and serum Klotho protein level was measured by enzyme-linked immunosorbent assay kit. Polymorphism typing was performed by Taqman allele-specific hybridization analysis. At the same time, general information (gender, age, body mass index, systolic blood pressure, diastolic blood pressure, glycated glucose protein, low-density lipoprotein cholesterol, bone mineral density) was collected. The differences in general data, serum Klotho protein level and Klotho G395-A gene polymorphism between the study group and the control group were analyzed. Spearman analysis was used to analyze the correlation between general data, serum Klotho protein level and Klotho G395-A gene and osteoporotic fracture. Logistic analysis was used to analyze the independent risk factors of osteoporotic fracture.

Results • There was no significant difference of the sex, systolic blood pressure (SBP), diastolic blood pressure(DBP), Klotho G395-A genotype GG and alleles A and G between the study group and the control group. There was significant difference of body mass index (BMI), glycated glucose protein, low-density lipoprotein cholesterol(LDL-C), bone mineral density, serum Klotho protein level and Klotho G395-A genotype AA and AG were between the study group and the control group. Gender, age, glycated glucose protein and Klotho G395-A genotype AA were positively correlated with osteoporotic fracture ($P < .05$), while bone mineral density was negatively correlated with osteoporotic fracture ($P < .05$). There was no correlationship between the serum Klotho protein level and the incidence of osteoporotic fracture ($P > .05$). Logistic analysis showed that age, bone mineral density and Klotho G395-A genotype AA were independent risk factors for osteoporotic fracture.

Conclusion • The level of serum Klotho protein and related gene polymorphisms are both related to osteoporotic fracture in elderly patients with osteoporosis. It is significant to reduce the incidence of osteoporotic fractures. In future, more experiments are needed to explore the underlying mechanism. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Osteoporosis is a systemic disorder of bone metabolism, characterized by decreased bone mass per unit volume, bone thinning, increased bone fragility, weakened bone strength, and damage to bone microstructure.[1-2] Osteoporosis has become the focus of many problems and one of the disease types of global concern.[3-4] Statistics show that the number of osteoporosis patients in the world is about 200 million in 2020.[5] According to the Centers for Disease Control and

Prevention (CDC), from 2017 to 2018, 8.4% of adults aged 50-64 and 17.7% of adults aged 65 and older had osteoporosis. During these years, 39.3% of adults aged 50-64 and 47.5% of adults aged 65 and older had low bone mass prior to osteoporosis. Osteoporosis is four times more common in women than in men. Osteoporotic fracture is a common fracture type in orthopedics. It is caused by osteoporosis and often occurs when walking or falling, or after a strong cough or heavy lifting.[6] The main symptom of this disease is obvious pain at the lesion site.

With the further development of the disease, the body's activity function will be significantly affected, and even the spinal nerves will be involved, which will lead to the limitation of living ability and the deterioration of quality of life.[7] Serum Klotho protein level is a unidirectional transmembrane protein, which plays a significant role in inhibiting oxidative stress and regulating calcium and phosphorus metabolism, and its expression on osteocytes also plays a role in regulating mineral and bone metabolism.[8] klotho gene is an anti-aging gene, which is mainly expressed in the kidney and brain. Its secreted protein is an anti-aging hormone, and its low expression level will accelerate skin aging and cause muscle atrophy, which is also significantly correlated with osteoporosis and other aging processes.[9] However, whether serum Klotho protein level and related gene polymorphisms are necessarily associated with osteoporotic fractures in elderly patients with osteoporosis needs to be verified. There are little studies that demonstrate the relationship between Klotho and osteoporotic fractures, which needs futher studies.

Based on this, this study analyzed the medical records of 62 elderly patients with osteoporosis and 62 elderly patients without osteoporosis in the Department of Orthopedics of Huai 'an Fifth People's Hospital. The aim of this study was to investigate the correlation between serum Klotho protein level and related gene (Klotho G395-A gene) polymorphism and osteoporotic fracture in elderly patients with osteoporosis.

DATA AND METHODS

General Information

62 elderly patients with osteoporosis admitted to the Department of Orthopedics of our hospital from January 2021 to June 2022 were selected and included in the study group. Another 62 elderly patients without osteoporosis who underwent physical examination at the same time were selected as the control group. Patients in the study group were divided into group A (23 patients with osteoporotic fracture) and group B (39 patients without osteoporotic fracture) according to whether they had osteoporotic fracture or not. The study required ethical approval from the ethics committee of the Huai 'an Fifth People's Hospital.

Inclusion Conditions and exclusions

Inclusion conditions: (1) Complete medical records; (2) Age ≥60 years; (3) All patients with osteoporosis were confirmed by bone mineral density examination; (4) All patients with osteoporotic fracture were confirmed by medical history, physical examination and imaging examination, and all of them were single vertebral osteoporotic compression fracture. (5) Patients or their family members were aware of the study, voluntarily joined, and signed informed consent.

Exclusion rules: (1) Secondary osteoporosis; (2) Serious diseases of major organs; (3) Taking drugs that affect bone metabolism in the past 6 months; (4) Severely abnormal coagulation function; (5) Infectious diseases; (6) Mental illness; (7) Malignant tumor; (8) poorly controlled diabetes mellitus (HbA_{1C} > 7%); (9) Hyperthyroidism.

Methods

Detection of serum Klotho protein level. After fasting in the morning (fasting for more than 8 hours), 3-5 ml venous blood was collected and immediately placed in a centrifuge tube. Serum was separated and serum Klotho protein level was measured by enzyme-linked immunosorbent assay kit (CUSABIO Biotech, Newark, DE). The serum Klotho protein level was measured by enzyme-linked immunosorbent assay.

Polymorphism analysis of Klotho G395-A gene. Polymorphism typing was performed by Taqman allele-specific hybridization analysis. 3ml peripheral venous blood was collected and anticoagulated with 3.2% sodium citrate. Plasma and leukocytes were separated. The leukocyte genome was extracted by classical phenol-chloroform method and stored in a refrigerator at -70°C. Primers were set and synthesized according to the whole DNA sequence of KLOTHO gene in GENEBANK. It is estimated that the fragment length of G-395A amplification product is 389bp. The upper and downstream primers are TAGGGCCCGGCAGGAT and CCTGGAG-CGGCTTCGTC, FAM detector A is CCCCAAGTCGGGAAAAGTTGGTC, and HEX probe G is: CCCCCAAGTCGGGGAAAGTTGGTC. The total volume of the polymerase chain reaction system of G-395A was 20 μL, and the concentrations of the upper and downstream primers were 0.8μmol/L. The final concentrations of FAM labeled probe A and HEX labeled probe G were 0.075 μmol/L and 0.25nμol/L, respectively. The polymerase chain reaction conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 1 minute, 65°C for 30 seconds and 72°C for 48 seconds, followed by final extension at 72°C for 5 minutes and cooling at 4°C for 5 minutes. Amplification was carried out on fluorescence quantitative polymerase chain reaction instrument, and electrophoresis was used to observe whether the bands of amplification products were clear and single. Polyphred 5.04 system was used to detect and analyze the polymorphism of Klotho G395-A gene. All polymorphisms were manually calibrated, including genotypes AA, AG, GG and alleles A and G.

Analysis of general data. General information was collected, including gender, age, body mass index, systolic blood pressure, diastolic blood pressure, glycated glucose protein, low-density lipoprotein cholesterol, and bone mineral density.

Body mass index: weight and height were measured, and body mass index was calculated using the formula: body mass index = weight (kg)/height (m^2). Systolic blood pressure and diastolic blood pressure: Omron HEM-7052 upper arm automatic electronic sphygonometer was used to measure the blood pressure of the brachial artery of the right upper extremity of the patient. The blood pressure was measured twice (10 minutes apart) in strict accordance with the method recommended by the Chinese Blood Pressure Measurement Guidelines, and the average of the two measurements was taken. If the difference between the two measurements was more than 10mmHg, the third measurement was performed, and the average of the three measurements was taken. The patient was asked not to drink coffee or alcohol 30 minutes before blood pressure measurement, not to do strenuous exercise, keep calm mood, sit quietly and rest for 10 minutes.

Glycosylated glucose protein: Before eating in the morning (fasting for more than 8 hours), 3-5 ml blood was collected from the elbow vein, centrifuged at low temperature (3500 RPM) for 15 minutes, and stored in the refrigerator at -20°C to be tested. The high pressure liquid phase method was used for detection. The instrument and kit were all from Bole Company.

Low-density lipoprotein cholesterol: Before eating in the morning (fasting for more than 8 hours), 3-5 ml blood was collected from the elbow vein, centrifuged at low temperature (3500 RPM) for 15 minutes, and stored in the refrigerator at -20°C to be tested. Siemens 2400 automatic biochemical analyzer was used for detection by direct method (selective inhibition method). The kit was purchased from Beijing Jiuqiang Company.

Bone mineral density value: Quantitative computed tomography (QCT) is a form of medical imaging that uses data from a series of X-ray images to create a two-dimensional or three-dimensional model of a part of the body. In general, computed tomography (CT) refers to the practice of using this type of X-ray image to create a more complete image. Adopt QCT method, using QCT image automatic analysis software and specific body model of 16 row helical CT machine (American GE company), before the determination requirements take off all metal objects of the patients, take supine position, put in patients with lumbar back under the special body model, adjust position, ensure the below the midline of the lumbar cone, make the waist close to body model, try to reduce the gap. Parameters are set as follows: current 250mA, voltage 120kV, bed height 154cm, spiral 0.938, scan for 5 seconds. During the QTC scan, the patient was asked to remain in position for axial images of the L1-L5 phase scanned through the central layer of the L2, L3, and L4 vertebral bodies. The level vertebra and the lift model were measured on the workstation processing software, and the Z-Score value, BMD value, T-Score value, and L2-4 BMD value were obtained.

Blood pressure: Normal blood pressure value is the concept of "high normal blood pressure" first proposed by medical scientists in 1984.When the systolic blood pressure is 17.3 ~ 18.6kPa (130 ~ 139mmHg), the systolic blood pressure is 17.3 ~ 18.6kPa (130 ~ 139mmHg). Diastolic blood pressure of 11.3 ~ 11.9kPa (85 ~ 89mmHg), or as long as one of the two reaches this level, is "high normal blood pressure", that is, blood pressure "high normal value".

Observation Indicators

(1) The differences of general data, serum Klotho protein level and Klotho G395-A gene polymorphism between the study group and the control group were analyzed. (2) The differences of general data, serum Klotho protein level and Klotho G395-A gene polymorphism between group A and group B were analyzed. (3) Spearman analysis was used to analyze the correlation between general data, serum Klotho protein level and Klotho G395-A gene and osteoporotic fracture. (4) logistic analysis was used to analyze the independent risk factors of osteoporotic fracture.

Statistical methods

SPSS22.0 software was used. Measurement data were represented as ($\bar{x} \pm s$), *t* test was performed, count data were represented as %, χ^2 test was performed. Spearman analysis was used for correlation analysis, and logistic analysis was used for independent risk factor analysis.

RESULTS

Comparison of general data, serum Klotho protein level and Klotho G395-A gene polymorphism between the study group and the control group

Sex, systolic blood pressure, diastolic blood pressure, Klotho G395-A genotype GG and alleles A and G were compared between the study group and the control group, all $P > .05$; Age, body mass index, glycated glucose protein, low density lipoprotein cholesterol, bone mineral density, serum Klotho protein level and Klotho G395-A genotype AA and AG were compared between the study group and the control group, all $P < .05$. See table 1.

Table 1. Comparison of general data, serum Klotho protein levels and Klotho G395-A gene polymorphisms between the study group and the control group

Project	Study group (n = 62)	Control group (n = 62)	χ^2/t	*P* value
Gender (male/female, n)	30/32	33/29	0.290	.590
Age ($\bar{x} \pm s$, years)	79.32±7.11	76.34±6.24	2.480	.007
Body mass index ($\bar{x} \pm s$, kg/m²)	23.45±2.67	21.46±2.74	4.150	.000
Systolic blood pressure ($\bar{x} \pm s$, mmHg)	135.20±17.52	132.25±18.45	1.310	.096
Diastolic pressure ($\bar{x} \pm s$, mmHg)	78.34±12.48	76.40±12.41	0.868	.194
Glycosylated glycemic protein ($\bar{x} \pm s$, %)	6.82±0.63	5.62±0.84	8.999	.000
Low-density lipoprotein cholesterol ($\bar{x} \pm s$, mmol/L)	2.93±1.33	2.46±1.13	2.121	.018
Bone density values ($\bar{x} \pm s$, *t* values)	-2.87±0.36	0.36±0.43	45.351	.000
Serum Klotho protein level ($\bar{x} \pm s$, pg/ml)	883.67±40.24	673.47±41.40	28.668	.000
Klotho G395-A gene polymorphisms[n(%)]				
Genotype AA	13(20.97)	5(8.06)	4.159	.041
Genotype AG	43(22.58)	16(25.81)	23.571	.000
Genotype GG	36(58.06)	41(66.13)	0.857	.355
Allele A	19(30.65)	13(20.97)	1.516	.218
Allele G	43(69.35)	49(79.03)	1.516	.218

Table 2. General information of groups A and B, serum Klotho protein levels and Klotho G395-A gene polymorphisms Comparison of differences

Project	A (n = 23)	B (n = 39)	χ^2/t	*P* value
Gender (male/female, n)	7/16	23/16	4.719	.030
Age ($\bar{x} \pm s$, years)	81.670±7.210	78.040±6.830	1.980	.026
Body mass index ($\bar{x} \pm s$, kg/m²)	24.530±2.800	23.620±2.710	1.262	.106
Systolic blood pressure ($\bar{x} \pm s$, mmHg)	136.030±17.610	134.960±17.280	0.234	.408
Diastolic blood pressure($\bar{x} \pm s$, mmHg)	78.790±12.510	77.830±12.450	0.293	.385
Glycosylated glycemic protein ($\bar{x} \pm s$, %)	7.150±0.690	6.34±0.56	5.043	.000
Low-density lipoprotein cholesterol ($\bar{x} \pm s$, mmol/L)	2.99±1.40	2.87±1.25	0.349	.364
Bone density values ($\bar{x} \pm s$, *t* values).	-3.15±0.44	-2.64±0.40	4.673	.000
Serum Klotho protein level ($\bar{x} \pm s$, pg/ml)	893.15±40.33	875.35±40.07	1.686	.049
Klotho G395-A gene polymorphisms[n(%)]				
Genotype AA	8(34.78)	5(12.82)	4.211	.041
Genotype AG	17(73.91)	25(64.10)	0.637	.425
Genotype GG	16(69.57)	20(51.28)	1.986	.159
Allele A	9(39.13)	10(25.64)	1.239	.266
Allele G	18(78.26)	25(64.10)	1.365	.243

Table 3. Correlation analysis of general data, serum Klotho protein level and Klotho G395-A gene and osteoporotic fracture

Index	R value	*P* value
gender	0.100	<.05
age	0.159	<.05
Glycosylated glycemic proteins	0.247	<.05
Bone density value	-0.210	<.05
Serum Klotho protein level	0.610	>.05
Klotho G395-A genotype AA	0.389	<.05

Table 4. Logistic analysis of inguinal hernia recurrence

Variable name	B	SE	Wald value	*P* value	OR	95%*CI*
Gender	1. 098	0.632	1.257	.104	0.502	0.143~0. 648
Age	0.867	0.833	9.365	.018	1.418	1. 109~3.457
Glycosylated glycemic proteins	1. 053	0.257	1.834	.192	0.745	0.214~0. 831
Bone density value	-0. 654	0.285	8.732	.011	1.454	1. 195~7.935
Klotho G395-A genotype AA	0. 599	0.521	4.821	.036	1.168	1. 089~10.815

Comparison of general data, serum Klotho protein level and Klotho G395-A gene polymorphism between group A and group B

The body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), low density lipoprotein cholesterol (LDL-C), Klotho G395-A genotypes AG, GG and alleles A and G were compared between group A and group B, all $P > .05$; The gender, age, glycated glucose protein, bone mineral density, serum Klotho protein level and Klotho G395-A genotype AA of group A and group B were compared, all $P < .05$. Are shown in table 2.

Correlation analysis of general data, serum Klotho protein level and Klotho G395-A gene with osteoporotic fractures

Gender, age, glycated glucose protein and Klotho G395-A genotype AA were positively correlated with osteoporotic fracture , with the R value 0.100 , 0.159 and 0.247, respectively ($P < .05$). The bone mineral density was negatively correlated with osteoporotic fracture, with the R value -0.210 ($P < .05$). Serum Klotho protein level was not significantly correlated with osteoporotic fracture ($P > .05$). See table 3.

Analysis of independent risk factors for osteoporotic fracture

OR value is mainly used in retrospective case-control studies, by obtaining the data of the case group and the control group, to establish the association between the event outcome and a certain factor, so as to study the correlation between the two. Logistic analysis showed that age, bone mineral density and Klotho G395-A genotype AA were independent risk factors for osteoporotic fracture. See table 4.

DISCUSSION

Osteoporosis is a polygenic disorder, generally determined by the combined effects of several genes and environmental factors. The main pathogenesis of osteoporosis is that bone resorption by osteoclasts is greater than bone formation by osteoblasts, resulting in a negative balance of bone reconstruction.[10] The development process of the disease has no obvious symptoms, usually appear osteoporosis fracture after being noticed, but once the osteoporotic fracture, the patient often arise bone pain, low back pain, the symptom such as scoliosis deformity, compression fracture, the typical symptoms will make the activity ability and life quality of life of patients with affected by large, serious when even will cause the residual dead.[11] The research has shown that Siglec-15 gene-deficient mice exhibit mild osteoporosis and Siglec-15 gene is involved in osteoclast differentiation induced by estrogen deficiency.[12]

Serum Klotho protein level is a single transmembrane protein encoded by the Klotho gene with a molecular weight of 135kDa. Many existing studies have confirmed that Klotho is closely related to aging, and the decrease of its level will lead to a syndrome similar to human aging, and then cause vascular calcification, atherosclerosis, skin atrophy and osteoporosis.[13] It is associated with atherosclerosis, diabetes, kidney injury, osteoporosis, etc. Previous study found that klotho G395-A gene polymorphism is associated with osteoporosis in elderly men, possibly AA genotype. An animal experimental study found that regulating the level of Klotho in aged mice or alleviating the deficiency of Klotho can restore the muscle regeneration in aged mice after damage, suggesting that Klotho protein may be a related factor causing aging.[14] Klotho is generally expressed as a soluble protein in the blood, which is the main functional form of Klotho in the body circulation and plays a role in regulating the balance of calcium and phosphorus and vitamin D metabolism.[15] Human bone mineral density is the result of the interaction between genes and environment. Klotho gene polymorphism may be related to osteoporosis, and Klotho gene products may affect osteoblast activity.[16] The results of this study showed that the serum Klotho protein levels and the AA and AG genotypes of Klotho G395-A were significantly different between the study group and the control group, suggesting that the serum Klotho protein levels and related gene polymorphisms may be correlated with the occurrence of osteoporosis. The comparative analysis of patients with osteoporosis and osteoporotic fracture showed that there were significant differences in serum Klotho protein levels and Klotho G395-A genotype AA between the two groups, suggesting that serum Klotho protein levels and related gene polymorphisms were associated with the occurrence of osteoporotic fracture.

In this study, the influencing factors of osteoporotic fracture were analyzed. The results showed that gender, age, glycated glucose protein, Klotho G395-A genotype AA were correlated with the occurrence of osteoporotic fracture. Osteoporosis in the elderly is often primary osteoporosis. Due to a variety of reasons, bone density and bone quality decline, the microstructure of bone is destroyed, resulting in increased bone fragility, which is prone to fracture. A woman's risk of osteoporotic fracture is higher than male, with the proportion about 4:1, of which the highest prevalence of perimenopausal women, analysis because may lie in: perimenopausal women estrogen secretion significantly lower in the body, resulting in a decrease of bone cells express bone element, inhibition of bone resorption, which in turn leads to less bone formation, the change of bone and bone resorption increased. Women are more prone to osteoporosis aftermenopause, because of the lack of calcium in the bones, calcium deficiency. Too little calcium intake, bad bone absorption, made postmenopausal women suffer from osteoporosis . osteoporosis is a skeletal state, but also the result of bone calcium loss. The body's bone mass loss rate and environment factors related to factors such as sports, muscle strength can improve bone mass and bone mass loss prevention, while men exercise muscle strength is opposite bigger, stronger, and therefore less bone mass loss, make the corresponding lower the incidence of osteoporosis fracture. There is a significant correlation between the occurrence of osteoporosis and age, and the elderly are more susceptible to the disease. The analysis of the reason is directly related to the reduction of bone mineral density, bone structure destruction, calcium metabolism disorder, bone synthesis and other factors in the elderly. Related studies have also pointed out that the calcium in bone tissue will slowly lose with age, and the loss of calcium will cause a series of physiological changes, such as decreased bone mineral density, bone trabecular sparsity, cortical lamellar structure disorder, porous, and so on, which will lead to the increase of osteoporosis and osteoporotic fracture incidence.

Glycated glycaemic protein is an important reference for assessing the blood glucose level of patients. The increase of its level indicates a high blood glucose level, which will affect the metabolic function of the body, cause calcium metabolism disorder, and then increase the risk of osteoporotic fracture. Sub-standard glycated hemoglobin, hypoglycemia, and complications of diabetes will further increase the risk of osteoporosis forming habitual fractures. To stay away from fractures caused by osteoporosis, the first step should be to smoothly reduce blood sugar and avoid hypoglycemia, which is the key to the prevention and treatment of diabetic osteoporosis. Bone densitometry is an important method for the diagnosis of osteoporosis. At present, QCT is the only method to measure the bone mineral density of cancellous bone and cortical bone respectively, which can provide a new way for early screening, diagnosis, etiology analysis and monitoring of osteoporosis to a large extent. The main function of QCT is to measure the bone density of the human bone and measure the volume bone density (vBMD) of the human bone. QCT uses the X-ray attenuation principle of clinical CT machine and QCT professional quality control body model to accurately convert the CT value of the scan image into the equivalent density of hydroxyapatite.

The results of this study also confirm that BMD is an independent risk factor for osteoporotic fracture. Analysis of the relationship between serum Klotho protein level and related gene polymorphisms and osteoporotic fractures showed that Klotho G395-A genotype AA was positively correlated with osteoporotic fractures, while serum Klotho protein level was not significantly correlated with osteoporotic fractures. It is suggested that AA genotype at G395-A of Klotho gene or the susceptibility gene of osteoporotic fracture should be monitored, which is basically consistent with the report of Liu Ju et al.[16]

CONCLUSION

To sum up, the level of serum Klotho protein and related gene polymorphisms are both related to osteoporotic fracture in elderly patients with osteoporosis. It is significant to reduce the incidence of osteoporotic fractures. In future, more experiments are needed to explore the underlying mechanism.

DATA AVAILABILITY

The data could be obtained by contacting corresponding author.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING

This study did not receive any funding in any form.

ACKNOWLEDGEMENTS

All authors contributed to the study and agreed to be listed as authors.

REFERENCES

1. Yin X, He Z. Research progress on the anti-renal fibrosis mechanism of Klotho protein and the intervention of traditional Chinese medicine in Klotho protein [J]. *Liaoning Zhongyiyao Daxue Xuebao*. 2020;22(4):216-220.
2. Jin H, Wang H. Zeng Z. Association between elderly osteoporotic fractures and polymorphisms at rs900798 of CYP11A1 gene [J]. *Zhongguo Laonianxue Zazhi*. 2019;39(12):2961-2964.
3. Zhi LI, Haihui WU. Correlation between 8-site polymorphisms of CYP11A1 gene rsrs90079 and bone mineral density and bone metabolism markers in senile osteoporotic fracture [J]. *Journal of Clinical and Experimental Medicine*. 2018;17(16):1744-1747.
4. Xu M, Xu G, Yang X, et al Expression and significance of serum bone metabolism indexes and Klotho protein in serum of osteoporosis patients[J]. *Journal of North Sichuan Medical College*,2020,35(4):691-693,709
5. Wang A, Liang L. Influencing factors related to fractures in elderly women with primary osteoporosis [J]. *China Maternal and Child Health*. 2021;36(11):2621-2623.
6. Qu X, Jiang T, Cao Z, et al. Comparative study of bone turnover markers and bone density in predicting osteoporotic fracture in elderly women [J]. *Zhongguo Guzhi Shusong Zazhi*. 2020;26(1):31-36.
7. Zhang P. Cai H. Expression of plasma Klotho protein, sST2 and Gal-3 levels and correlation with left ventricular hypertrophy in elderly hypertensive patients [J]. *Chinese Journal of Evidence-Based Cardiovascular Medicine*. 2021;13(8):950-953.
8. Lin W, Li X, Wang Y, et al. Analysis of changes in blood Klotho protein and hormone levels before and after osteoporosis patients with osteoporosis [J]. *Guangdong Yaoxueyuan Xuebao*. 2020;36(6):880-885.
9. Song Y, Tong W, Zhang H, et al. Correlation between systemic inflammatory indicators and fracture occurrence in elderly osteoporosis patients [J]. *Lab Med*. 2022;37(3):235-239.
10. Liu K, Zhang X, Ding T. Study on the relationship between spine-related parameters and clinical features of senile osteoporosis and the risk of osteoporotic fracture in the elderly[J]. *Clinical Research in Medicine*,2021,38(6):834-837,842
11. Liu C. Clinical effect of zoledronic acid combined with salmon calcitonin in the treatment of elderly patients with osteoporotic fractures [J]. *Journal of Clinical Rational Medication*. 2021;14(1):81-82.
12. Wang X, Diao L, Sun D, et al. OsteoporosAtlas: a human osteoporosis-related gene database. *PeerJ*. 2019;7:e6778. doi:10.7717/peerj.6778
13. Zhao X, Zhang Z. Risk of osteoporotic fracture in middle-aged and elderly people in Shanghai based on fracture risk assessment tool (FRAX®)[J]. *Chinese Journal of General Practice*,2020,23(19):2429-2434
14. Li B, Zhong M, Jiang Y, et al. Correlation between elderly osteoporosis and plasma levels of Klotho protein and FGF23 protein [J]. *Zhongguo Laonianxue Zazhi*. 2018;38(1):180-182.
15. Dong Y, Sun Y, Ji Z, et al. Correlation between the detection of bone mineral density and the expression of Klotho protein in patients with renal bone disease [J]. *China Modern Drug Application*. 2018;12(24):56-57.
16. Zhang X, Pei F, Wang X. Characteristics and clinical significance of KIothor level changes in osteoporosis patients[J]. *Journal of Laboratory and Laboratory Medicine*,2019,37(3):389-391,394
17. Liu J, Lei Y, Chen H, et al. Correlation between serum Klotho protein levels and KlothoG395-A gene polymorphisms and osteoporotic fractures in elderly osteoporosis patients [J]. *Zhongguo Laonianxue Zazhi*. 2021;41(17):3737-3739.