STUDY ON THE EFFECT OF LIUZIJUE EXERCISE ON BLOOD PRESSURE AND OXIDATIVE STRESS IN PATIENTS WITH ESSENTIAL HYPERTENSION

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ABSTRACT

Introduction: *To explore the effect of Liuzijue exercise on blood pressure and oxidative stress of patients with essential hypertension.*

Materials and methods: *41 patients diagnosed with essential hypertension were recruited and randomly divided into Liuzijue exercise group and waiting treatment group, and 15 healthy people were recruited as the healthy group. On the basis of maintaining normal work and rest pattern and continuing the original drug treatment plan, Liuzijue exercise group entered into a 12-week Liuzijue exercise plan, and the waiting treatment group did not have any form of fitness exercise intervention. The two groups and healthy group had blood pressure, superoxide dismutase (SOD), glutathione (GSH), Malondialdehyde (MDA); Glutathione peroxidase (GSH-PX); γ-glutamyl peptide aminotransferase (GGT) levels in blood monitored on the first day and the end of the intervention.*

Results: *After the intervention, the SBP and Pulse Pressure in Liuzijue exercise group were significantly decreased($P<0.01$), compared with Waiting treatment group, the level of SBP, DBP and Pulse Pressure of Liuzijue group decreased($P>0.05$). Compared with the Waiting treatment group, the level of GSH-PX in Liuzijue exercise group was higher ($P<0.01$), in Liuzijue exercise group, the expression of SOD was higher after intervention. Other indicators had no statistically significant changes ($P>0.05$).*

Conclusion: *The 12-week Liuzijue exercise can enhance antioxidant capacity, improve oxidative stress, reduce systolic and diastolic blood pressure, by increasing the activity of GSH-PX in patients with essential hypertension.*

Keywords: *Essential hypertension, liuzijue exercise, oxidative stress, SOD, GSH-PX.*

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Introduction

Essential hypertension (EH) is a chronic non-communicable disease. Hypertensive patients often have vascular endothelial dysfunction, which is closely related to oxidative stress. Oxidative stress can break the balance between vasoconstrictor and diastolic factors, resulting in weakened vasodilation and enhanced systolic function, further aggravating the occurrence of hypertension and atherosclerosis(1). Studies have found that the core cause of endothelial dysfunction is the activation of vascular endothelial oxidation(2). Clinical studies have shown that compared with normal people, hypertensive patients have higher levels of oxidative markers and insufficient endogenous antioxidants(3). Oxidative stress is a cytotoxic change caused by ROS, which is key in the cardiovascular system and inflammation, hypertrophy, proliferation, apoptosis, and migration. These pathological changes are the key factors in the formation of abnormal endothelial function in hypertension, cardiac remodeling and other cardiac diseases(4). SOD and MDA are potent factors characterizing oxidative stress(5,6). In addition, studies have shown that oxidative stress indicators such as SOD are also related to the expression levels of VEGF receptor and nitric oxide synthase (eNOS) in patients with hypertension, which further proves that there is a relationship between oxidative stress and endothelial functional impairment. The combination of aerobic exercise and drugs is regarded as an emerging trend in the treatment of cardiovascular and cerebrovascular diseases. Its feasibility has been confirmed by a number of studies, aerobic exercise can effectively improve the blood pressure stability of hypertensive patients(7); Petrella(8) believed that low-intensity aerobic exercise is specially effective in treating essential hypertension. It was studied that aerobic exercises reduce the plasma endothelin level and improves the endothelial system(9).

Liuzijue exercise can effectively affect the blood pressure of the middle-aged and elderly(10). However, it hasn't been reported whether Liuzijue exercise participates in the regulation of blood pressure by improving the level of oxidative stress. Based on this, this study conducted a 12-week Liuzijue exercise in hypertensive patients to study the effect of Liuzijue exercise on blood pressure and oxidative stress levels in hypertensive patients.

Materials and methods

Subject recruitment and ethic approval

Essential hypertension patients who met the inclusion criteria were selected in the Daying Hospital of TCM of Suining city, Sichuan. The study program had been approved by the Medical Ethics Committee of Affiliated Hospital of Chengdu University of TCM. All procedures of the experiment were carried out in accordance with the institutional guidelines.

Inclusion and exclusion criteria

Inclusion criteria:

- Patients whom meet the diagnostic criteria for hypertension(11), SBP ≥140mmHg (1mmHg=0.133kPa) and (or) DBP ≥90mmHg. In order to ensure the safety of the subjects, the subjects included in this study were all patients with primary hypertension graded as 1 (mild) or 2 (moderate);
- Age 45-80 years old;
- No usual exercise and fitness habits (exercise time <30 minutes/day, less than 2 days per week);
- Single-drug antihypertensive therapy with calcium channel blockers (such as nifedipine), and patients who have not changed their medication regimen recently;
- Those who have no related diseases that affect motor function and severe motor dysfunction;
- Those who have not participated in the clinical trials of other traditional Chinese medicine exercise therapy such as Taijiquan and Baduanjin in the past six months;
- Have an informed understanding of the trials, have the ability to self-organize, voluntarily participate in this study, and be able to cooperate till the completion of the test index detection.

Exclusion criteria:

- Patients who had secondary hypertension;
- Patients with blood pressure reaches grade 3 hypertension;
- Patients with liver, kidney and heart dysfunction, diabetes and other serious complications;
- Long-term smokers and alcohol abusers, Patients who are not suitable to participate in sports;
- Those who have regular exercise and fitness habits;
- Those who are mentally ill or have poor compliance.

Research methods

Waiting treatment group: no exercise intervention in any form was given, and the previous routine drug treatment plan was maintained. After the 12-week intervention, subjects in the WLG could choose whether to practice Liuzijue according to their own wishes.

Liuzijue exercise group: continue the routine drug treatment, and the patients in the LEG will practice Liuzijue exercise for a period of 12 weeks on the basis of maintaining a standard diet and daily life. Exercise was carried out 3 times per week, at 14:00-15:00 in the afternoon for 1 hour, and the exercise includes: 2 minutes of preparation activities, 56 minutes of Liuzijue exercise (a set of movements is about 13 minutes, 4 times in total, with a 2-minute rest in between, and about 2 minutes of finishing activities.

Blood pressure measurement

The subjects were at sitting position for at least 5 minutes while the Blood Pressure was measured using Omronan electronic sphygmomanometer. The patient was seated and kept quiet when the measurement was performed. BP was measured three times with intervals longer than 1 minute. The average values of BP measured in the three times taken as the final value.

ELISA

Blood samples were collected on the day before the Liuzijue exercise began, and on the first day after the end of the experiment. All subjects were instructed to avoid strenuous activity 12 hours in advance and strictly fasted food and water. The peripheral venous blood was drawn from the subjects at 8:00 am on the test day. Then, the blood samples were set at 23 degree centigrade for 30-40 min. After the blood was completely coagulated, centrifuge at 3000 r/min for 20 min in a centrifuge. Absorb the upper serum and store it in an EP tube, and put it in a -80 °C refrigerator for 2 hours. After the samples were collected, the Elisa kit was used for detection. Monitoring indicators are, SOD; GSH; MDA; GSH-PX; GGT.

Statistical analysis

Using SPSS 25.0 to analyze the mathematical statistics. The data were described as $\bar{X}$±SD. The comparison among groups was performed by one-way ANOVA, and the intra-group comparison before and after the experiment was performed by paired-samples t-test. The test standard was set as P<0.05 for statistical significance, and P<0.01 for significant statistical significance.

Results

Baseline information

The baseline information including gender, age, Height, weight and BMI. According to statistical results, there were no statistical differences in gender, age, height, weight, and BMI between LEG and WLG (P>0.05), and the baselines were comparable. See Table 1.

Index	LEG (n=21)	WLG (n=20)	P value
Gender Male/Female	6/15	7/12	0.577
Age(year)	65.19±6.48	60.42±8.15	0.050
Height (cm)	153.86±7.94	156.84±8.74	0.265
Weight (kg)	56.60±7.52	61.47±8.53	0.062
BMI (kg/m^2)	23.88±2.44	24.94±2.56	0.189

Table 1: Comparison of baseline.
Note: BMI: body mass index.

Blood pressure

Compared with the healthy group, both LEG and WLG had higher blood pressure, all patients met the including criteria. Blood pressure level in the LEG and the WLG were measured and collected before and after the intervention. The results showed that the level of SBP in LEG decreased after intervention (P=0.001<0.01; before intervention: 154.50±12.38mmHg; after intervention: 140.95±10.69mmHg); the level of DBP in LEG was slightly decreased after intervention (P=0.164>0.05; before intervention: 87.55±10.88mmHg, after intervention: 83.45±6.95mmHg).

The Pulse Pressure was significantly decreased after 12-week Liuzijue exercise in LEG (P=0.004<0.01, before intervention: 66.95±10.81mmHg, after intervention: 57.80±8.68mmHg), Compared with the WLG, the SBP, DBP, and Pulse Pressure level was lower in LEG with no statistical difference. See Figure 1.

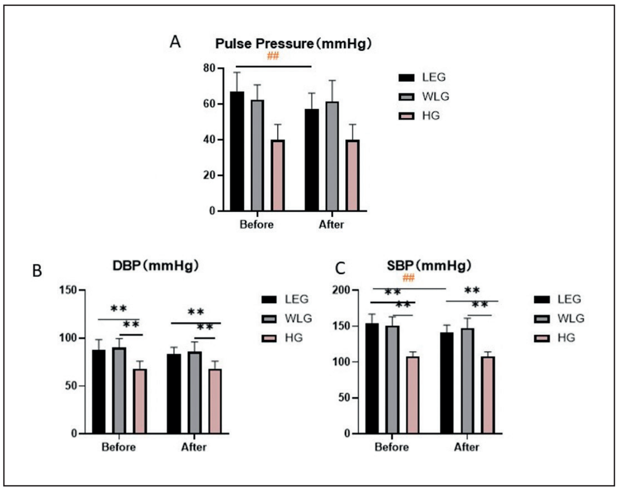

Figure 1: Change of Pulse Pressure (1A), DBP(1B), and SBP(1C) in three groups. LEG: Liuzijue exercise group; WLG: waiting treatment group; HG: healthy group. SBP: systolic blood pressure; DBP: diastolic blood pressure.
$^{**}P<0.01$ (compared with HG), $^{\#\#}P<0.01$ (Intra group comparison), ▲▲$P<0.01$ (compared with WLG).

Oxidative stress

After the 12-week Liuzijue exercise intervention, the results of the oxidative stress response in the LEG, the WLG and the healthy group were further analyzed. Compared with the healthy group, both LEG and WLG had an increased GSH-PX level (P<0.01), compared with WLG, the GSH-PX express in Liuxijue exercise group was significantly higher (P=0.003<0.01). After 12 weeks of Liuzijue exercise intervention, the level of GGT content has not changed significantly, compared with WLG, the expression of GGT in Liuxijue exercise group was increased, however with no statistical difference. After 12 weeks of Liuzijue exercise intervention, the SOD content of Liuzijue exercise group increased (P=0.001<0.01, before intervention: 9.46±3.53; after intervention: 13.80±3.85); the SOD content of WLG decreased, but there was no significant difference. After 12 weeks of Liuzijue exercise intervention, MDA content in Liuzijue intervention group decreased (before intervention: 4.40±1.48; after intervention: 4.13±1.01). See Figure 2 and Figure 3.

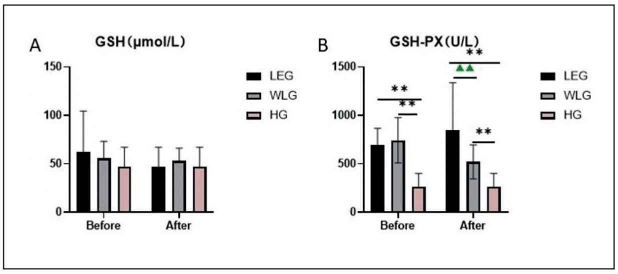

Figure 2: Change of GSH(2A) and GSH-PX(2B) in three groups. LEG: Liuzijue exercise group; WLG: waiting treatment group; HG: healthy group.
***P<0.01 (compared with HG), ##P<0.01 (Intra group comparison), ▲▲P<0.01 (compared with WLG).*

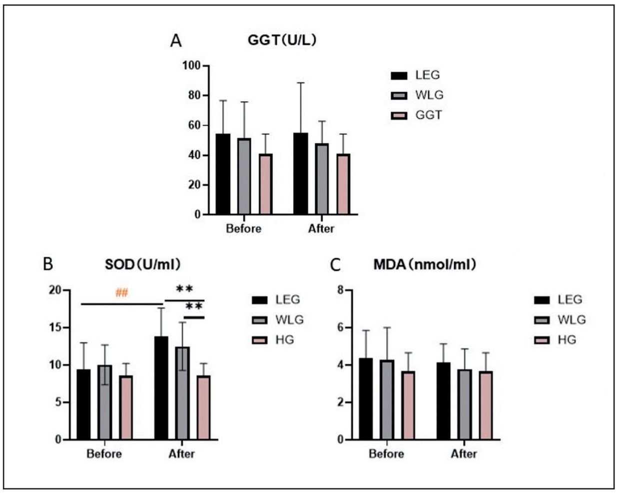

Figure 3: Change of GGT(3A), SOD(3B) and MDA(3C) in three groups. LEG: Liuzijue exercise group; WLG: waiting treatment group; HG: healthy group.
***P<0.01 (compared with HG), ##P<0.01 (Intra group comparison), ▲▲P<0.01 (compared with WLG).*

Discussion

In addition to the influence of unhealthy lifestyle and genetic factors, the pathogenesis of essential hypertension is related to the activation of humoral factors, activation of the nervous system, vascular endothelial dysfunction, impaired capillary blood flow, and inflammatory mediators[12]. SOD is an effective factor to measure oxidative stress, which belongs to one of the key antioxidant enzymes and important in the oxidative stress cascade. Under the influence of SOD, superoxide anion is converted into hydrogen peroxide and oxygen, thus reducing the amount of superoxide anion. In addition, SOD can compete and couple with NO, affecting the bioavailability of the latter, and target organ damage. Li's team[13] conducted a cross-sectional study to assess the connection of SOD3 with cardiovascular disease, all 1047 patients took standard echocardiography and had SOD3 activity measured, as a result, they found that the serum SOD3 activity of patients with cardiovascular disease decreased significantly, and multiple logistic regression analysis also found that when serum Ec-SOD activity increased 10 U/mL, the proportion of cardiovascular patients decreased by more than 16%. In addition, SOD3 can reduce the production of superoxide anion and play a role in lowering blood pressure by altering endothelium-dependent relaxation function and renal sodium transport function(14). In a hypertensive model with two-kidney-one-clip method and Ang II injection, SOD3 reduction resulted in increased arteriogenesis and impaired endothelium-dependent relaxation[14, 15].

The results of our study showed that the subjects' serum SOD was increased after Liuzijue intervention, indicating that Liuzijue exercise may improve the pathological state of hypertensive vascular lesions by restoring SOD. Some scholars have found that compared with the hypertensive patients, the plasma SOD concentration of the intervention patients was increased, and the SOD concentration gradually increased with the improvement of the hypertension course[13]. Qing[16] found that the concentration of SOD is mainly affected by the pathological conditions of the body, and SOD will not change significantly under physiological conditions. Li[13] also found that the serum SOD concentration of hypertensive patients without target organ damage was not significantly different from that of healthy people.

GSH-PX is a member of the peroxidase family. Many studies concluded that GSH-PX is related to many cardiovascular and cerebrovascular diseases closely, such as hypertension[17]. Ji Shulan[17] compared the serum of hypertensive patients and non-hypertensive subjects and showed that the levels of SOD and GPX in hypertensive patients decreased, while LPO increased, and the contents of SOD and GPX were negatively correlated with blood pressure. Qiu[18] found that the serum glutathione peroxidase and catalase activities in hypertensive subjects were weaker than those in healthy subjects. Pavlovich[19] also found that the GSH-PX levels in hypertensive patients or subjects with target organ damage were lower than those in the control group. GSH is a substrate of GSH-

PX. GSH can remove hydrogen peroxide and lipid peroxides from cells. GSH can also combine with other free radicals. In the above reaction variation, GSH is depleted in cells and possibly converted to the disulfide oxidized form, which can be converted to GSH by glutathione reductase with NADPH as a coenzyme[20]. Solveigh[21] found that GSH and GSH-PX can inhibit NF-κB-driven inflammatory response through a mechanism of peroxidative activity.

And this channel is also an important reason for the formation of hypertension, indicating that GSH is the key reason for the regulation of blood pressure[22]. Rybka[23] conducted a study on blood collected from 31 subjects and found that the peripheral blood GSH and GSH-PX-related defense and anti-oxidative stress system of hypertensive subjects became stronger, indicating that GSH is crucial in regulating blood pressure. Our study showed that after 12 weeks of intervention, the serum GSH-PX level of hypertensive patients in Liuzijue exercise group was increased. In addition, the change of each component of GSH concentration level was also consistent with the GSH-PX reaction. This study confirmed the possibility of excessive oxidative stress in patients with essential hypertension, and their anti-oxidative stress ability was improved after the exercise of Liuzijue. The possible mechanism is that Liuzijue exercise may reduce the level of inflammation by restraining the activation of nuclear transcription factor-kB (NF-kB) through the redox activity of GSH-PX. NF-kB can respond to vascular injury mediators and activate inflammatory pathways, resulting in increased secretion of inflammatory factors and disordered oxidative stress response. Liuzijue exercise may improve vascular inflammation and hypertension by inhibiting NF-kB activation through GSH-PX redox activity.

Most of GGT is stored in the liver, it is also distributed in the kidney, pancreas, spleen, intestine, brain, lung, skeletal muscle, myocardium, and vascular endothelium[24]. The function of intracellular GGT is to metabolize extracellular GSH, assimilating precursor amino acids and incorporating GSH in cells. It can be seen that serum GGT is a marker component of oxidative stress, and oxidative stress can reduce the transformation of GSH. GGT can be converted into other reactive oxygen species (ROS) in the redox changes of extracellular metal ions, which further lead to DNA damage and pro-oxidation to regulate cell proliferation, transformation, and death[25]. Meanwhile, GGT can strengthen the combination of endogenous substances and certain exogenous substances with GSH, thereby promoting the body's metabolism and excretion. GGT is a risk factor for vascular and non-vascular disease. GGT was considered to be associated with impairment of blood pressure, arterial stiffness and aortic elasticity[26]. In an observational study involving 38,806 participants, Wu[27] conducted an observational study on the relationship between serum γ-glutamyl transferase and hypertension. The results showed that in people who have no metabolic syndrome, GGT levels were positively correlated with the occurrence of hypertension, and the correlation was stronger in women. Our results showed that the GGT concentration of hypertensive patients was higher than that of healthy people. After a 12-week Liuzijue exercise intervention, GGT levels in hypertensive patients slightly decreased, indicating that Liuzijue exercise is beneficial to restore the balance of oxidative stress in hypertensive patients. The possible mechanism is Liuzijue may restore the steady state of oxidative stress by reducing the concentration of GGT and thus the production of reactive oxygen species.

MDA increases with the increase of oxidative stress response capacity, which will further enhance the damage of vascular endothelial cells[28]. SOD and GSH-PX are important antioxidant enzymes that can remove excess free radicals in the human body and protect cell membranes from free radical damage. The higher the activity of MDA, the lower the possibility of cell membrane damage[29]. When the activity of MDA increases and the activities of SOD and GSH-PX decrease, the balance of oxidation and antioxidants is broken, which further causes severe damage to the vascular endothelium, leading to the progress of hypertension[30]. Our study showed that the level of MDA decreased after Liuzijue intervention. The possible mechanism is that Liuzijue intervention improved the oxidative stress response in patients and reduced the lipid peroxidation of oxygen free radicals in the body[31].

In conclusion, there is an imbalance of oxidative stress response in hypertension patients. Oxidative stress response is one of the important factors that cause vascular endothelial lesions. Vascular endothelial lesions in turn will aggravate the imbalance of oxidative stress response, which together promote blood pressure and damage target organs. This study found that 12-week Liuzijue exercise could reduce the concentration of GGT and MDA in patients with essential hypertension, and increase the concentration of GSH-PX and

SOD to improve oxidative stress in patients with essential hypertension. Liuzijue exercise may regulate blood pressure by reducing oxidative stress. Liuzijue exercise, as a low-intensity aerobic exercise, may reduce blood pressure, inhibit vascular inflammation, restore the balance of redox reactions in hypertensive patients, and improve the overall life quality of patients[32, 33].

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