EXPLORING THE MECHANISM OF LIUZIJUE EXERCISE PROTECTING THE VASCULAR ENDOTHELIUM OF HYPERTENSION BASED ON "EMPS-GUT FLORA"

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ABSTRACT

Introduction*: By observing the effect of Liuzijue exercise on vascular endothelial microparticles and intestinal flora in patients, to explore the effect of Liuzijue exercise mechanism of exercise prevention and treatment of hypertensive vascular lesions.*

Materials and methods*: 26 patients with hypertension were randomly divided into two groups: the Liuzijue exercise intervention group and the waiting intervention group. Each group had 13 patients in the Liuzijue intervention group for consecutive 12 weeks, 3 times a week, 1 hour each time to analyze the changes of EMPs content and intestinal flora in the two groups before and after intervention.*

Results*: Liuzijue exercise could reduce plasma EMPs level in hypertensive patients; subjects Escherichia, Escherichia coli were positively correlated with EMPs (P< 0.05).*

Conclusion*: Liuzijue exercise maybe regulate the species of Escherichia coli and Escherichia coli, and change the content of endothelial particles, thereby protecting the blood vessels of patients with hypertension skin.*

Keywords: *Liuzijue exercise, hypertension, vascular endothelial function, gut microbes.*

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Introduction

Liuzijue exercise is the sports that cooperates with different mouth pronunciations after inhalation and exhalation. Liuzijue exercise could regulate the blood pressure of hypertensive patients. It can also protect vascular endothelial function in patients with essential hypertension. EMPs is a small vesicle structures generated when endothelial cells are stimulated or destroyed, which can effectively reflect the vascular endothelial injury in hypertension[1,2]). Therefore the change of EMPs content in patients with high blood pressure was selected in this study to explain the impaired vascular endothelial function of patients. At the same time, this study used 16srDNA. High-throughput sequencing method to observe the changes of intestinal flora in hypertensive patients after treatment. To explore the mechanism of Liuzijue exercise to prevent and treat hypertensive vascular disease, so as to provide new information for the prevention and treatment of hypertensive vascular disease.

Materials and methods

Subject recruitment

From March 2019 to June 2019, 26 Grade 1 and Grade 2 essential hypertension patients who met the standards were selected in the Ninth People's Hospital of Qingyang District, Chengdu.

Object grouping

The patients were randomly divided into Liuzijue exercise intervention group and the waiting list group with 13 cases each group, by random number table method.

Inclusion criteria

a) Patients who met the diagnostic criteria of grade 1 and 2 for essential hypertension and had low or medium risk of cardiovascular risk stratification.

Patients who used calcium channel blockers (such as nifedipine and amlodipine) for single-drug antihypertensive therapy before eye and had relatively stable medication regimen;

b) Age 45-80 years old, gender is not limited;

c) No habit of overeating, eating relatively regular;

d) Those who do not exercise regularly and systematically (<20 minutes/time, <2 times a week);

e) Those without severe motor dysfunction;

f) Not taking antibiotics, steroids, microbial preparations, traditional Chinese medicines for promoting blood circulation and removing blood stasis in the past month, and no diarrhea or other stomach diseases history of bowel disorders;

g) Those who have not participated in other clinical trials of exercise therapy such as Taijiquan and Baduanjin in the past six months;

h) Be aware of the trial, voluntarily participate in the trial research, sign the informed consent, and be able to cooperate with the completion of the trial index inspection test, good compliance.

Exclusion criteria

• Patients with secondary hypertension, such as: hypertension or drug-induced hypertension caused by primary aldosteronism, acromegaly, Cushing's syndrome and other diseases;

• Blood pressure reaches grade 3 of hypertension classification (systolic blood pressure≧180mmHg and/or diastolic blood pressure≧110mmHg), hypertensive heart disease vascular risk level stratification reaches high-risk and very high-risk levels;

• Patients with heart, liver and kidney dysfunction, diabetes and other serious complications;

• Patients with chronic gastrointestinal diseases;

• Those who smoke and drink heavily for a long time;

• Patients with a history of severe trauma surgery within the last six months.

Intervention methods

Before intervention, the age, sex, height, body mass index (BMI), heart rate, smoking history and drinking history of subjects in the two groups were recorded. The 24-hour ambulatory blood pressure of subjects before and after baseline intervention was measured, and the immediate blood pressure was measured before and after each intervention.

Control group

Patients with WLG receive conventional drug therapy (eg: nifedipine, amlodipine), and maintain daily living habits. At 12 after the weekly trial and the 3-month follow-up, Liuzijue intervention was performed according to the subjects' wishes.

Intervention group

LTG patients should add Liuzijue exercise on the basis of conventional drug treatment. The subjects concentrated on the LiuZijue exercise under the guidance.

1 hour each time (9:00-10:00 in the morning), three times a week for a total of 12 weeks. Subjects take blood pressure measurements centrally before exercise. After 5 minutes of warm-up activities, and then according to the standards of the General Administration of Sports of the People's Republic of China, the exercise of LiuZijue is carried out for 60 minutes, and finally Blood pressure was measured again after 5 minutes of simple relaxation exercise.

Main experimental instruments

Medical electronic sphygmomanometer; flow cytometer; PacBio sequencing platform.

Detection indicators

Blood pressure

In order to observe the short-term effect after intervention and avoid the occurrence of white overcoat hypertension, before and after each intervention, members of the research group immediately measured the blood pressure of the subjects with a qualified upper arm medical electronic sphygmomanometer. The 24-hour mean systolic blood pressure (MSBP)/diastolic blood pressure (MDBP) at night before and after 12 weeks of intervention, respectively. Maximum systolic/diastolic blood pressure.

Endothelial Microparticles (EMPs)

The plasma endothelial microparticles (EMPs) content of subjects were detected by flow cytometry.

Gut microbial structure and function

This experiment is based on the PacBio sequencing platform using 16SrDNA high-throughput sequencing technology to identify 16SrDNA (DNA sequence of prokaryotic ribosomal small subunit rRNA (16S rRNA) variable region V3-V4 region for PCR amplification and high-throughput assay To identify the composition, classification, expression abundance, population structure, diversity, etc. of microorganisms (bacteria) in human fecal samples.

Statistical methods

SPSS25.0 software was used for statistical analysis of the experimental data, and a two-tailed P <0.05 indicated a significant statistical difference. For the measurement data conforming to the normal distribution, independent samples t-test and paired t-test were used to compare the differences between groups and within groups. The Wilcoxon rank-sum test was used to analyze the differences between groups; the one-way ANOVA test was used to analyze the differences under different time windows. (After 1 month, 2 months, and 3 months) differences in blood pressure levels within the LTG group; paired t-test was used to compare the differences in different time windows (after 1 month, 2 months, 3 months later), differences in blood pressure levels before and after Liuzijue intervention. The enumeration data were analyzed using the chi-square test. Pearson correlation analysis was used to test the correlation between clinical and biochemical parameters.

Results

Baseline Comparison Results

The trial finally included 25 subjects, including 13 cases of LTG and 12 cases of WLG. There were 9 males and 4 females in LTG subjects. The average age was 65.38±4.46 years old, 3 had a history of smoking and 4 had a history of drinking; WLG subjects were 5 males and 7 females. The average age was 63.08±5.12 years old, 8 had smoking history, 8 had drinking history; gender, age, smoking history and drinking history between groups match. There was no significant difference in the average systolic blood pressure, diastolic blood pressure, heart rate and BMI between the two groups. They are comparable.

Comparison of blood pressure improvement values

After the intervention, the daytime MSBP and daytime MDBP of the LTG subjects decreased (4.65±4.04, 0.85±1.79), and the WLG daytime MSBP and daytime MDBP increased (7.17±7.80, 10.05±2.74), and there were significant differences between groups (P <0.05, P >0.01); nighttime MSB and nighttime MDBP decreased in LTG subjects after intervention (8.42±3.55, 2.25±1.78), WLG nocturnal MSBP decreased (0.67±3.85), nocturnal MDBP increased (1.67±6.64), no comparison between groups Statistical difference (P >0.05). See Table 1.

Analytical Metrics	Index	Liuzijue exercise intervent	Awaiting intervention group	Statistics	*p*
24h mean blood pressure	24hMSBP	-5.85±13.14	9.17±7.67**	t=-3.52	0.002
	24hMDBP	-1.62±6.12	6.42±6.19**	t=-3.226	0.003
Daytime mean blood pressure	Daytime MSBP	-4.65±4.04	7.17±7.80*	t=-2.556	0.019
	Daytime MDBP	-0.85±1.79	10.05±2.74**	t=-3.376	0.003
Night mean pressure	Night MSBP	-8.42±3.55	-0.67±3.85	t=-1.483	0.152
	Night MDBP	-2.25±1.78	1.67±6.64	t=-1.502	0.147

Table 1: Differences in blood pressure improvement before and after intervention in the two groups (Mean± SD, mmHg).

*Note: The improvement value is the difference between the blood pressure after treatment and the blood pressure before treatment. The value is "-", which means blood pressure drops, otherwise it means blood pressure rises; *p< 0.05 compared with before intervention, **p< 0.01 compared with before intervention.*

Changes in plasma EMPs levels

The results of the plasma EMPs levels of the two groups of subjects showed that compared with before the intervention, the plasma EMPS levels of the LTG subjects after the intervention were lower than those before the intervention. decreased (1638.54±478.50 pcs/μl and 1274.92±465.66 pcs/μl), and the levels of CD31+/CD42- increased (2.80, 3.52%), there was no statistical significance within the group (P >0.05); the plasma EMPS levels of WLG subjects increased (752.33±190.32 pcs/μl, 1465.83±533.57 pcs/μl), CD31+/CD42- levels decreased (3.52, 2.89%), the intra-group comparison was not statistically significant (P >0.05).

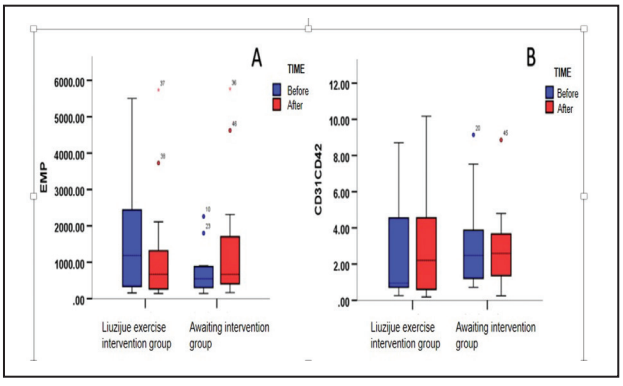

Figure 1: Differences in plasma EMPS between the two groups before and after the intervention; A is the change in the number of plasma EMPS in the two groups; B is the plasma EMPS CD31+/CD42- in the two groups (%) Change graph; *P <0.05 before intervention, there is a statistical difference.

Before intervention, LTG subjects had higher plasma EMPS levels than WLG, CD31+/CD42-lower than WLG, there was no statistical significance between groups (P >0.05); after intervention, the plasma EMPS level of LTG subjects was lower than WLG, CD31+/CD42- was higher than WLG, and there was no statistical significance between groups (P >0.05). The specific data are shown in Figure 1.

16SrDNA PCR sequencing results of intestinal flora

Valid sequenced

In this study, a total of 50 stool samples were collected from hypertensive subjects before and after intervention, and the stool microflora DNA was extracted from the collected samples. After sequencing, a total of 550,391 CCS sequences were obtained through Barcode identification, with each sample generating at least 3,345 CCS sequences and an average of 11,008 CCS sequences.

OUT Analysis

OUT number distribution

The number of OTUs in each sample was obtained by clustering. The distribution results of the number of OUTs showed that the total number of OTUs before LTG intervention was 193.

The prognosis was 202; the total number of OTUs before WLG intervention was 197, and after the intervention was 190; indicating that the intestinal flora of subjects in the six-character group.

Species diversity increased, while species diversity of gut microbiota decreased in WLG subjects. Before the intervention of the intervention group, WTGA is after the intervention of the waiting intervention group; the number on the column of each group is the number of OTUs of the corresponding sample.

OTU cluster Venn diagram

Venn diagram of OTU clustering showed that the subjects in the two groups before intervention had the same 171 OTUs, 22 OTUs unique to LTG, and 22 unique OTUs to WLG 26 OTUs; after the intervention, the subjects in the two groups had the same 180 OTUs, 22 LTG-specific OTUs, and WLG-specific OTUs 10; There were 187 identical OTUs in LTG subjects before and after the intervention, 6 unique OTUs before the intervention, 15 unique OTUs after the intervention, and the overall OTU abundance increased. WLG subjects had 177 identical OTUs before and after the intervention, 20 unique OTUs before the intervention, and 13 unique OTUs after the intervention.

The abundance of OTUs decreased; indicating that the species diversity of gut microbiota increased in subjects in the six-character group, while more species in the gut microbiota in subjects with WLG. The sample quality decreased.

Annotation of flora species

The species distribution map at each level of the tested samples indicates that the relative abundance distribution of each sample phylum, class, order, family, genus and species has changed. There were differences in flora structure and proportion of flora, but the difference was not statistically significant (P >0.05).

Door level difference

After Liuzijue intervention, Firmicutes, Proteobacteria, Verrucomicrobiota and Desulfobacterota. The contents of Bacteroidota and Actinobacteriota decreased with no statistical significance (P >0.05).

Differences in class, order and subject level

After the intervention of Liuzijue exercise, *Gammaproteobacteria*, *Bacteroides* and *Clostridia's* content decreased, in the intestine of patients with essential hypertension. *Negativicutes* and *Fusobacteri* content increased significantly; The levels of *Enterobacterale*, *Bacteroidale*s, *Oscillospirale*s and *Verrucomicrobiales* decreased. The levels of *Veillonellales-Selenomonadales* increased. *Fursona cteriales* increased significantly; *Enterobacteriaceae, Ruminococcaceae*, *Prevotellaceae* decreased, *Bacteroidaceae*, *Ruminococcacea*e increased, and the content of *Fusobacteriaceae* increased significantly. Accounting significance (P> 0.05).

Genus level difference

After Liuzijue exercise intervention, enteric *Klebsiella* and *Prevotella* in patients with essential hypertension and *Faecalibacterium* decreased, *Escherichia*, *Ruminococcus* and *Bacteroides* content increased, there was no statistical significance (P>0.05).

Level Differences

After Liuzijue exercise intervention, intestinal *Escherichia_coli* and Prevotella copri in patients with essential hypertension decreased, *Bacteroides_ vulgatus* and the content of *Subdoligranulum_* variabile increased without statistical significance (P > 0.05).

β-diversity between groups

NMDS analysis

Non-Metric Multi-Dimensional Scaling (NMDS) was used for intra-group and ranking between groups, NMDS results showed that the samples of LTG and WLG subjects were scattered before the intervention, and there was no significant difference; The distance between groups of patients

in the groups was close and clustered to a certain extent, while the distance between groups was far; indicating that there were differences in the bacterial community structure between groups, see Figure 2.

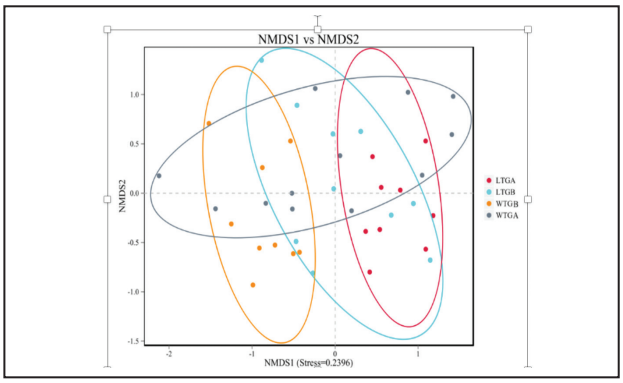

Figure 2: Differences in NMDS analysis between the Liuzijue intervention group and the waiting intervention group: the dots in the figure represent each sample, and different colors represent different In the same grouping, the distance between points indicates the degree of difference, and the samples with closer distances on the coordinate graph have higher similarity. LGB for Liu Zi Jue Intervention Before group intervention, LTGA was the Liuzijue intervention group after intervention, WTGB was the waiting intervention group before intervention, and WTGA was the waiting intervention group after intervention.

UPGMA-bar analysis

The UPGMA dendrogram showed that after intervention, the samples in the LTG and WLG groups had close distances, short branch lengths, and high similarity; the distance is far, the branch length is long, and the similarity is low, which proves that there are differences in the flora among the groups.

Heat map analysis of distance between samples

The results of distance Heatmap showed that although there was no obvious regular difference in the sample flora of LTG and WLG subjects after intervention, the intra-group distance was closer than the inter-group distance, indicating that there were differences in the flora among the groups. The results are shown in Figure 3.

Correlation analysis between changes in intestinal flora and changes in blood pressure and endothelial function in subjects

The correlation results showed that the subjects Ruminococcus, Prevotella copri was positively and negatively correlated with 24hMSBP (r=0.592, r=0.566, P <0.05, respectively), *Escherichia*, *Escherichia coli* was positively correlated with EMPs (r=0.459, r=0.415, P <0.05, respectively), F/B There was no significant correlation between the changes of the main flora at the level of other genus and blood pressure and EMPs (P >0.05). See table 2.

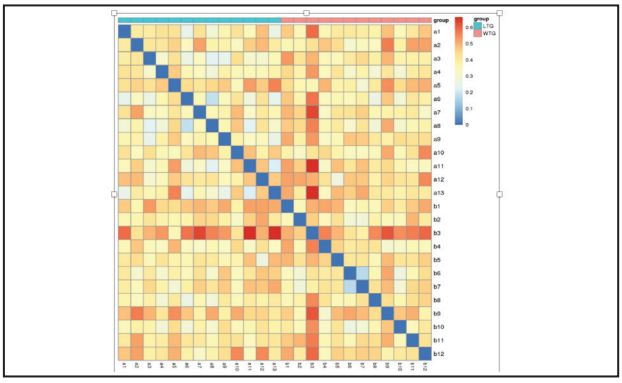

Figure 3: Heat map of distance between samples: the color gradient from blue to red indicates the distance between samples from near to far; LTG is Liuzijue intervention group, WTG is waiting for intervention Group.

Index	24h MSBP		24h MDBP		EMPs	
	r	*P*	r	*P*	r	*P*
F/B	-0.234	0.441	-0.161	0.599	0.069	0.739
Bacteroides	0.455	0.119	0.233	0.443	-0.350	0.079
Prevotella	-0.549	0.052	-0.511	0.074	0.268	0.185
Escherichia	-0.138	0.654	-0.311	0.301	0.459*	0.018
Faecalibacterium	0.135	0.660	0.144	0.638	-0.037	0.875
Klebsiella	0.026	0.934	-0.080	0.795	0.196	0.338
Ruminococcus	0.592*	0.033	-0.250	0.410	-0.158	0.440
Escherichia_coli	-0.138	0.654	-0.311	0.301	0.415*	0.035
Prevotella_copri	0.566*	0.044	-0.523	0.067	0.141	0.491
Faecalibacterium prausnitii	-0.083	0.788	0.239	0.432	-0.123	0.550

Table 2: The correlation between changes in intestinal flora and changes in blood pressure and endothelial function in subjects (*P< 0.05).

Discussion

Blood pressure refers to the lateral pressure value on blood vessels when blood flows, and is used as a direct indicator for the diagnosis of hypertension. Blood pressure can classify the risk of hypertension and judge the prognosis, recent studies have shown that the blood pressure value of hypertensive patients is closely related to cardiovascular and cerebrovascular diseases[3-5]. The blood pressure value has a strong research value. By observing the experimental data, the intervention group subjects' 24hMSBP and 24hMDBP decreased (5.85±13.14, 1.62±6.12, respectively), daytime MSBP, daytime MDBP decreased (4.65±4.04, 0.85±1.79, respectively), nighttime MSBP and nighttime MDBP decreased (8.42±3.55,2.25±1.78), indicating that Liuzijue exercise has a direct regulatory effect on the blood pressure value of hypertensive patients, and can also improve blood pressure. Abnormal fluctuations can help patients with hypertension avoid violent fluctuations in blood pressure under external stress, which is conducive to the stability of blood

pressure. It can maintain and protect target organs, thereby delaying the development of hypertension and improving the survival time and quality of patients. Vascular endothelial microparticles are small vesicle material structures produced when endothelial cells are stimulated or apoptotic, which can effectively reflect vascular lesions, inflammatory diseases and endothelial cell status, and participates in various reactions in the body, which is the link between apoptosis and neogenesis product of homeostasis[6]. The experimental data showed that after 12 weeks of intervention treatment, the plasma EMPs value decreased, and the level of CD31+/ CD42- has improved, indicating that the Liuzijue exercise has an improving effect on hypertension and vascular endothelium. Meanwhile the value of EMPs in the waiting group increased significantly, indicating that in the case of the development of the disease and the aggravation of endothelial damage in the control group EH patients, Liuzijue exercise can effectively slow down the trend of endothelial damage, and reverse the damage to the improvement effect, which has the function of protecting blood vessels. Endothelial cells improve vascular damage and reduce the production of EMPs.

The intestinal flora is a complex micro-ecosystem in the intestinal tract, which is involved in various aspects such as human immunity, metabolism, and neuromodulation. It plays an indelible role in maintaining the homeostasis of the human body. In recent years, the intestinal flora has been found to be related to a variety of diseases[7,8]. Disease treatment provides new ideas. Due to the extensive role of intestinal flora, the intestine is known as the second brain, which has a great impact on human health. The improvement of physical function plays a huge role. Through the analysis of the experimental data, it can be seen that at the genus level, the intervention of Liuzijue can reduce the relative abundance of Klebsiella prevotella and Faecalis, and increase the relative abundance of Escherichia ruminococcus and Bacteroidetes in the intestines of patients with low EH. At the species level, intervention with Liuzijue could reduce the relative abundance of E. coli prevotella copri and increase the relative abundance of Bacteroides and Subdoligranulum-variable in EH patients These results suggest that the Liuzijue can reduce the increase of pathogenic Klebsiella prevotella and Faecalis in EH patients, and make the intestinal flora of EH patients develop into beneficial flora, which may be the key bacteria of Liuzijue affecting blood pressure.

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