Clinical Study of Radiofrequency Ozone and Injection of Anti-inflammatory Analgesic Solution into the Internal Orifice of Nerve Root Combined with Traditional Chinese Medicine Hook Operation in the Treatment of Lumbar Disc Herniation

WanHai Zhang (wanhaizhangdr@163.com)
Xianghe Country People's Hospital

ZhiGang Wang
Xianghe Country People's Hospital

JianWei Yin
Xianghe Country People's Hospital

YuanYuan Bai
Xianghe Country People's Hospital

FengChao Qiu
Xianghe Country People's Hospital

HaiDong Zhang
Xianghe Country People's Hospital

HuiYi Zhang
Xianghe Country People's Hospital

Bao Wang
Xianghe Country People's Hospital

LiHua Wang
Xianghe Country People's Hospital

FengNing Zhang
Xianghe Country People's Hospital

ShuJuan Ji
Xianghe Country People's Hospital

MingXing Yuan
Xianghe Country People's Hospital

HongWei Zhao
Xianghe Country People's Hospital

He Yang
Xianghe County People's Hospital
Keywords: radiofrequency, ozone, internal orifice of nerve root, traditional Chinese medicine hook operation, lumbar disc herniation

DOI: https://doi.org/10.21203/rs.3.rs-138941/v1

License: ☕️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

**Background:** This study aims to explore the clinical efficacy of radiofrequency ozone and injection of anti-inflammatory analgesic solution into the internal orifice of nerve root combined with traditional Chinese medicine hook operation in the treatment of lumbar disc herniation.

**Methods:** The patients with lumbar disc herniation in our hospital from December 20, 2017 to June 19, 2019 were selected as the main research subjects, and the patients were numbered according to the order of their first visits, and the included patients were divided into treatment group and control group using random number table method. Patients in the treatment group were treated with radiofrequency ozone and injection of anti-inflammatory analgesic solution into the internal orifice of nerve root combined with traditional Chinese medicine hook operation. Patients in the control group were treated with traditional lumbar intervertebral disc radiofrequency treatment. The clinical treatment effects of the two groups were observed.

**Results:** A total of 113 patients were included in this study, of which 73 were in the treatment group and 40 were in the control group. The results of the study showed that the NRS scores of patients in the treatment group before treatment were 5±1.68 points, and the NRS scores were 2±0.78 points, 1±0.54 points, and 1±0.77 points 1 month, 3 months, and 1 year after treatment, respectively. The NRS scores of patients in the control group were 3±0.48 points, 2±0.63 points, and 2±0.85 points 1 month, 3 months, and 1 year after treatment. Compared with before treatment and the control group, there were significant differences (P<0.01).

**Conclusion:** Compared with single lumbar intervertebral disc radiofrequency treatment, radiofrequency ozone and injection of anti-inflammatory analgesic solution into the internal orifice of nerve root combined with hook operation can obtain good short-term and medium-term effects in the treatment of lumbar disc herniation. It is a safe and effective minimally invasive treatment method.

**Background**

Lumbar disc herniation is caused by lumbar disc degeneration, annulus fibrosus rupture, nucleus pulposus protrusion, stimulation and (or) compression of the spinal nerve or spinal cord, resulting in low back and leg pain with root sciatica as the main symptom. It is a common clinical disease and frequently-occurring disease,[1–2] traditional conservative treatment methods are generally not satisfactory and often require surgical fenestration treatment. No matter single segment or multi segment fenestration, the spinal biomechanical damage is inevitable, and long-term complications often occur.[3–4]

Minimally invasive interventional treatment avoids damage to the spine and preserves the mechanical stability of the spine and the balance of the biological environment.[5–7] For example, intervertebral disc radiofrequency thermocoagulation and traditional Chinese medicine hook operation. In radiofrequency
thermocoagulation treatment, under the precise positioning of imaging and precise guidance of the navigation system, the electrode needle is introduced through the needle path, and the radiofrequency technology is used to output energy to act on the nucleus pulposus tissue, and directly denature, coagulate, contract and reduce the volume of nucleus pulposus in the diseased part of the herniated nucleus pulposus, and relieve the compression on nerve roots, and rarely damage the normal nucleus pulposus tissue, and at the same time repair the rupture of the annulus fibrosus, inactivated the newly-developed hypersensitive nerve terminals in the disc, and directly block the release of glycoprotein and β protein in the nucleus pulposus fluid. The warming effect has a good therapeutic effect on the injured annulus fibrosus, edema of nerve root and inflammatory reaction in spinal canal [8–9]. Traditional Chinese medicine hook operation and small curved needle is a kind of painless and minimally invasive closed treatment method of traditional Chinese medicine bone injury, which is between operation, non-operation and intervention, with large strength, small difficulty, small damage to patients, low cost, good effect and instant effect. In our department, the traditional Chinese medicine small curved needle is used for the hook operation, which can achieve the purpose of local decompression, tension reduction, release and dredging, so as to rejuvenate the local area, relax the tendons and activate the blood circulation, be active and flexible, and move freely.

At present, the clinical efficacy of minimally invasive interventional treatment of lumbar disc herniation is not clear. Therefore, this study focuses on patients with lumbar disc herniation as the main research object. It aims to explore radiofrequency ozone and injection of anti-inflammatory analgesic solution into the internal orifice of nerve root combined with Chinese medicine hook operation for treatment of lumbar disc herniation.

**Materials And Methods**

1.1 **Research object**

The patients with lumbar disc herniation in our hospital from December 20, 2017 to June 19, 2019 were selected as the main research subjects, and the patients were numbered according to the order of their first visits, and the included patients were divided into treatment group and control group using random number table method. The patients in the treatment group were treated with radiofrequency ozone and injection of anti-inflammatory analgesic solution into the internal orifice of nerve root combined with traditional Chinese medicine hook operation. The patients in the control group were treated with traditional lumbar intervertebral disc radiofrequency treatment. The clinical treatment effects of the two groups were observed. This study complies with the "Declaration of Helsinki of the World Medical Association" and has been approved by the ethics committee of our hospital. All patients signed an informed consent form.

1.2 **Diagnostic criteria**
In this study, the diagnostic criteria of lumbar disc herniation refer to Hu Yougu's "Lumbar Disc Herniation" [10]. Based on the clinical history, physical signs and imaging examination, the diagnosis basis of lumbar disc herniation is as follows: 1. Leg pain is heavier than low back pain, and leg pain is typical pain in the sciatic nerve distribution area. 2. The skin in the nerve distribution area feels numb when being pressed. 3. The straight leg raising is reduced by 50% compared with the normal, and there may be positive results in good leg raising test. The bowstring test is performed by pressing the tibial nerve in the popliteal area to cause radiating pain at both ends of the limb. 4. Two of the four neurological signs appear (muscle atrophy, motor weakness, hypoesthesia and weakened reflexes). 5. Imaging findings are consistent with clinical examinations, which include spinal canal imaging, CT or MRI, etc.

1.3 Inclusion criteria

The inclusion criteria of this study include: 1. The degree of low back pain is greater than that of back pain; 2. The imaging examination shows that it is of protruding type; 3. Conservative treatment for more than 6 weeks without improvement; 4. Straight leg raising and strengthening test is positive; 5. The height of the involved disc was greater than 75% of the adjacent disc height; 6. Age over 18 years old; 7. Patients who have signed an informed consent.

1.4 Exclusion criteria

The exclusion criteria of this study include: 1. The height of the involved intervertebral disc is less than 25% of the adjacent disc height; 2. Complete destruction of the nucleus pulposus; 3. The imaging examination shows that it is of free prolapse type; 4. Spinal fracture, tumor, infection, coagulation disorders; 5. Spondylolisthesis above 5°, spinal stenosis; 6. Patients with incomplete case data.

1.5 Treatment methods

In this study, patients in the control group were treated with traditional lumbar intervertebral disc radiofrequency treatment, and patients in the treatment group were treated with radiofrequency ozone and injection of anti-inflammatory analgesic solution into the internal orifice of nerve root combined with traditional Chinese medicine hook operation. The radiofrequency treatment operation method is as follows: the patient is in the prone position, the responsible disc intervertebral space is positioned under the C-arm, and after routine disinfection and draping, local anesthesia is performed in the responsible disc intervertebral space or 7-8cm next to the intervertebral space, using radiofrequency puncture needle to puncture at this point. Confirm the position is correct after C-arm irradiation, the needle tip is located at the protrusion of the intervertebral disc, and a radiofrequency instrument is connected to give sensory stimulation and motor stimulation tests. The electrophysiological results confirm that the puncture needle is at the protrusion and far away from the nerve root. Radiofrequency ablation treatment is given three time: at 70°C for 60 seconds, 80°C for 60 seconds, and 90°C for 60 seconds. When heating, the patient feels soreness in the lumbosacral area and the affected limb. Intravertebral disc ozone therapy is given again, the injection concentration is 60mg/L ozone 10ml, the lumbosacral and affected limbs will also experience soreness during the ozone injection process, and the soreness disappears after the injection.
Withdraw the needle slowly and when the gas injection resistance of 5ml syringe disappears, the needle tip is confirmed to be located at the internal orifice of nerve root after C-arm irradiation. Inject 10ml of anti-inflammatory analgesic solution. The patient has no symptoms of discomfort. Pull out the puncture needle, and then perform paravertebral 2.5-3.0cm local anesthesia for the responsible disc and inject anti-inflammatory analgesic solution at the paravertebral, around the facet joints, and the external orifice of intervertebral foramen. The crochet needle expands the puncture needle eye and then pierces the hook to a depth of about 3.5cm-5.0cm outward to the outer edge of the small joint capsule of the responsible disc and the posterior branch of the spinal nerve root for loosening, and then insert the curved side of the small curved needle between the two intervertebral spaces, and press the protrusion of the intervertebral disc to the ventral and medial side, and release the outlet spinal nerve root at the posterior lower edge of the upper vertebral body toward the cephalic side. Finally, turn the bend of the needle outward and enter the lateral recess from the medial edge of the spinal facet joint capsule, the curved needle is drawn from the intervertebral foramen to loosen the adhesion of the spinal cord and the nerve root, and paste the dressing at the puncture site after finishing.

1.6 Statistical analysis

In this study, SPSS 20.0 statistical software is used for data processing, and measurement data are expressed as mean ± standard deviation (x̄±s). Count data is expressed in percentage (%). The comparison between the two groups that obey the normal distribution uses the t test; the comparison between the groups that does not obey the normal distribution uses the non-parametric test. Chi-square test is used for count data. P<0.05 indicates that the difference is statistically significant.

The clinical efficacy comparison (NRS score) of the two groups of patients and comprehensive clinical efficacy statistics showed that the effective rate of the treatment group was significantly higher than that of the control group, with statistical difference (P<0.05).

Results

2.1 General information

A total of 113 patients were enrolled in this study, including 73 in the treatment group, 22 males and 51 females; age 41–65 years old, average (53.32 ± 11.46) years old; the shortest course of disease was 2 months, the longest 5.4 years, average (6.41 ± 3.53) months. The control group consisted of 40 cases, 14 males and 26 females; age 44–68 years old, average (54.97 ± 14.03) years old; the shortest course of disease was 4 months, the longest 5 years, average (6.74 ± 3.37) months. There was no statistically significant difference in general conditions between the two groups of patients (P > 0.05), and they were comparable.

2.2 Clinical manifestations of lumbar disc herniation in the two groups
Among the 113 patients enrolled in this study, there are 21 cases of pure L4/5 intervertebral disc herniation, 16 cases of pure L5/S1 intervertebral disc herniation, 28 cases of two-segments (L3/4 and L4/5) protruding, 40 cases of two-segment (L4/5, L5/S1) protruding and 8 cases of three-segments (L3/4, L4/5, L5/S1) protruding. The symptoms and signs of the patients were consistent with the impact data, and they all had typical clinical manifestations of lumbar disc herniation, with varying degrees of low back pain and symptoms and signs of unilateral or bilateral lower extremity nerve damage.

2.3 Comparison of clinical efficacy between the two groups of patients

The follow-up period was 1–12 months after treatment, with an average of 7.5 months. The NRS scores of the patients in the treatment group before treatment were 5 ± 1.68 points, and the NRS scores were 2 ± 0.78 points, 1 ± 0.54 points, and 1 ± 0.77 points 1 month, 3 months, and 1 year after treatment, respectively. The NRS scores of the control group were 3 ± 0.48 points, 2 ± 0.63 points, and 2 ± 0.85 points 1 month, 3 months, and 1 year after treatment. Compared with before treatment and the control group, there were significant differences (P < 0.01).

Discussion

Medical ozone is an allotrope of oxygen composed of three oxygen atoms. It is the most active form of oxygen. Ozone can decompose into oxygen and single oxygen atoms in about 20 minutes under normal temperature and pressure. A single oxygen has a strong activity, and has a strong oxidizing effect on bacteria, viruses and other microorganisms, as well as proteoglycans, lipids, and inflammatory mediators. Oxidized proteoglycan: It oxidizes nucleus pulposus proteoglycan, destroys nucleus pulposus cells, makes the osmotic pressure of the nucleus pulposus matrix unable to be maintained, and loses water, thereby reducing the pressure in the intervertebral disc, retracting the disc herniation, and eliminating the compression on the nerve root. Anti-inflammatory: Ozone can antagonize the immune factors and inflammatory mediators released in the inflammatory response, reduce nerve root edema and adhesion, and achieve the purpose of anti-inflammatory. Analgesia: The strong oxidation of ozone can quickly inactivate inflammatory mediators and eliminate pain. Loosen: Loosen the adhesion of nerve roots and intervertebral discs and surrounding tissues.

The anti-inflammatory analgesic solution is composed of 0.25% lidocaine needle, 4 mg triamcinolone acetonide injection, 0.9% sodium chloride solution. Lidocaine is a local anesthetic of the amide type. It has obvious excitatory and inhibitory bidirectional effects on the central nervous system, and there can be no pioneer excitement. When the blood concentration is low, analgesia and expansion of blood vessels can improve local blood circulation. Triamcinolone acetonide injection is an adrenal cortex hormone drug, which has anti-inflammatory and anti-allergic effects and suppresses immune response. This study found that injecting anti-inflammatory analgesic solution into the internal orifice of the nerve root can reduce nerve root edema and achieve the purpose of anti-inflammatory and analgesic.
Treatment of lumbar disc herniation should be more humane. In the treatment of lumbar disc herniation, more doctors prefer open surgery \([12-13]\). Although our department does not deny that open surgery is intuitive and thorough in the treatment of lumbar disc herniation, we do not approve of it as a routine treatment, because this method is severely destructive in the normal biomechanics of the human body \([14-16]\), we have compressed the original indications for open surgery and recommend more patients to use minimally invasive treatment to maintain biomechanical stability. Practice has proved that minimally invasive treatment is effective in treating lumbar disc herniation and does not affect any subsequent treatment.

Indications and contraindications for minimally invasive treatment of lumbar intervertebral disc herniation \([17-18]\): Absolute indications: a. Slow onset of the disease for no more than 1 year; b. Incomplete nerve injury; c. Patients accept minimally invasive treatment. Relative indications: a. Symptoms appear in slow-onset cases between 1 and 3 years with intermittent symptoms; b. It is accompanied by cardiovascular and cerebrovascular diseases at the same time; c. Patients have doubts about minimally invasive treatment. Contraindications: a. The disease persists for more than 3 years; b. Complete nerve damage; c. The prominent nucleus pulposus is completely free; d. Patient have severe cardiovascular and cerebrovascular diseases and mental diseases.

The key points of minimally invasive treatment of lumbar disc herniation: first of all, the diagnosis must be accurate. This requires clinicians engaged in minimally invasive treatment to have a solid theoretical foundation and sufficient clinical experience, especially the differential diagnosis should be accurate \([19-20]\). Secondly, the positioning must be accurate. This requires doctors not only not to be sloppy in the diagnosis of nerve positioning, but also to be particularly accurate in positioning during the treatment process, otherwise the treatment effect will plummet \([21]\).

In addition, it is very important to provide psychological counseling for patients with lumbar disc herniation. Patients with lumbar disc herniation have a long onset period. Most patients will experience depression and even commit suicide. In the treatment of lumbar disc herniation, patients must be encouraged and helped to build up the self-confidence to overcome the disease.

With the advancement of science and technology and the continuous improvement of material and cultural living standards, our work and learning environment and living habits have changed, and the incidence of a series of spinal diseases such as cervical spondylosis and lumbar disc herniation has increased year by year. And the trend is that there are more young patients. Lumbar disc herniation is mainly manifested by pain in the lower back and legs, or not accompanied by pain and numbness in the lower limbs. Most patients have limited mobility and motor dysfunction, which brings great pain and inconvenience to the work and life of patients. Rapid pain relief and improvement of motor dysfunction have become the most urgent requirements of patients. We are constantly exploring clinically, consulting medical-related literature, going out to learn, summing up experience, and actively exploring new treatment methods. To sum up, through clinical application, we found that "clinical study on the treatment of lumbar disc herniation with radiofrequency ozone and injection of anti-inflammatory
analgesic solution into the internal orifice of nerve root combined with hook operation of traditional Chinese medicine" has significant clinical effect compared with single lumbar disc radiofrequency treatment for lumbar disc herniation, which can quickly relieve pain symptoms, improve motor function, and directly treat the target of the lesion, that is the protrusion. It has been recognized and praised by the majority of patients, and has good social and economic benefits. In qualified hospitals, efforts should be made to relieve the pain of patients with lumbar disc herniation and resume normal life as soon as possible.

This research still has the following shortcomings. First of all, although this study is a randomized controlled experiment, it is not blinded. Secondly, this study is a single-center clinical study, and a multi-center clinical study is still needed for further discussion. Finally, the sample size included in this study is relatively small, and it is still necessary to increase the sample size for further research.

**Conclusion**

Compared with single lumbar intervertebral disc radiofrequency treatment, radiofrequency ozone and injection of anti-inflammatory analgesic solution into the internal orifice of nerve root combined with hook operation can obtain good short-term and medium-term effects in the treatment of lumbar disc herniation. It is a safe and effective minimally invasive treatment method.

**Declarations**

**Ethics approval and consent to participate**

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Xianghe County People's Hospital. Written informed consent was obtained from all participants.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

Not applicable.
Authors' contributions

Zhang WH and Wang ZG have made substantial contributions to conception and design, Yin JW, Bai YY, Qiu FC, Zhang HD and Zhang HY acquisition of data, analysis and interpretation of data; Wang B, Wang LH, Zhang FN and Ji SJ have been involved in drafting the manuscript and revising it critically for important intellectual content; Yuan MX, Zhao HW and Yang H have given final approval of the version to be published.

Acknowledgements

Not applicable.

References

1. Ozcan S, Muz A, Altun A Y, Onal S. Intradiscal ozone therapy for lumbar disc herniation[J]. Cellular and molecular biology (Noisy-le-Grand, France), 2018, 64(5):52.
2. Tang S, Mo Z, Zhang R. Acupuncture for lumbar disc herniation: a systematic review and meta-analysis[J]. Acupuncture in Medicine, 2018: acupmed-2016-011332. doi:10.1136/acupmed-2016-011332.
3. Fjeld O R, Grøvle, L, Helgeland J, Småstuen M C, Solberg T K, Zwart J A, et al. Complications, reoperations, readmissions, and length of hospital stay in 34 639 surgical cases of lumbar disc herniation[J]. The Bone & Joint Journal, 2019, 101-B(4): 470-477. doi:10.1302/0301-620X.101B4.BJJ-2018-1184.R1.
4. Liu Y, Zhao J X, Tian Y X. Efficacy and safety of electroacupuncture in treatment of lumbar disc herniation: a protocol for a cohort study[J]. Journal of Traditional Chinese Medicine, 2019, 39(01):131-136.
5. Hao K N, Tang S Y, Xie H N, Li X M, He X F. Application of ozone therapy in interventional medicine[J]. Journal of Interventional Medicine, 2019, 2(1):8-11. https://doi.org/10.1016/j.jimed.2019.05.003.
6. Chen W Y, Wang K, Yuan W A, Zhan H S. [Relationship between lumbosacral multifidus muscle and lumbar disc herniation][J]. Zhongguo Gu Shang, 2016, 29(6):581-584.
7. Stagni S, Santis F D, Cirillo L, Dall’olio M, Princiotta C, Simonetti L, et al. A minimally invasive treatment for lumbar disc herniation: DiscoGel chemonucleolysis in patients unresponsive to chemonucleolysis with oxygen-ozone[J]. Interventional Neuroradiology, 2012, 18(1):97-104. doi:10.1177/159101991201800113.
8. Zeng Z, Yan M, Dai Y, Qiu W D, Deng S, Gu X Z. Percutaneous bipolar radiofrequency thermocoagulation for the treatment of lumbar disc herniation[J]. Journal of Clinical Neuroscience, 2016:39-43. doi:10.1016/j.jocn.2015.10.050.
9. Ankur Jain V R S G, Singh G. Comparative study of oxygen ozone therapy, percutaneous radiofrequency thermocoagulation and their combined effects for the treatment of lumbar disc herniation[J]. Spine Journal, 2014, 10(9): S70-S71. doi:10.1016/j.spinee.2010.07.191.
10. Hu YG. Lumbar disc herniation, 2nd Ed. Beijing: People's Medical Publishing House, 1995.2.21.

11. Wei C J , Li Y H , Chen Y , Wang J Y,Zeng Q L,Zhao J B ,et al. [Percutaneous intradiscal oxygen-ozone injection for lumbar disc herniation: no need of perioperative antibiotic prophylaxis].[J]. Nan Fang Yi Ke Da Xue Xue Bao, 2007, 27(3):384-386.

12. Paulsen R T , Carreon L Y , Andersen M . Patient-reported Outcomes After Surgery for Lumbar Disc Herniation, a Randomized Controlled Trial Comparing the Effects of Referral to Municipal Physical Rehabilitation Versus No Referral[J]. Spine, 2020, 45.doi:10.1097/BRS.0000000000003221.

13. Ricarda L, David P, Krismer M, Haid C, Obwegeser A, Thaler M. Braking reaction time before and after surgery for patients with recurrent lumbar disc herniation.[J]. Journal of neurosurgery. Spine, 2019:1-5.doi:10.3171/2019.1.SPINE18859.

14. Andersen S B , Birkelund R , Andersen M , Carreon L Y, Coulter A , Steffensen K D. Factors Affecting Patient Decision-making on Surgery for Lumbar Disc Herniation[J]. Spine, 2018, 44(2):1.doi:10.1097/BRS.0000000000002763.

15. Kim C H . Surgical Timing in Lumbar Disc Herniation Surgery[J]. Neuro spine, 2020, 17(1):213-214.doi:10.14245/ns.2040068.034.

16. Postacchini F.Lumbar disc herniation: a new equilibrium is needed between nonoperative and operative treatment[J]. Spine, 2001, 26(6):601. doi:10.1097/00007632-200103150-00008.

17. Li S H , Zhou M W , Li Y J , Liang X Y. [Update of research on minimally invasive treatment of lumbar disc herniation].[J]. China Journal of Orthopaedics & Traumatology, 2012, 25(4):348-352.

18. Wu Z Q , Wei L X , Li J , Wang Y Q, Ni D H,Yang Pet al. Percutaneous treatment of non-contained lumbar disc herniation by injection of oxygen–ozone combined with collagenase[J]. European Journal of Radiology, 2009, 72(3):499-504. doi:10.1016/j.ejrad.2008.07.029.

19. Alvi M A , Kerezoudis P , Goncalves S , Goyal A , Bydon Operative Approaches for Lumbar Disc Herniation: A Systematic Review and Multiple Treatment Meta-Analysis of Conventional and Minimally Invasive Surgeries[J]. World Neurosurgery, 2018, 17(10):S266.doi:10.1016/j.wneu.2018.02.156.

20. Salame K , Lidar Z . Minimally invasive approach to far lateral lumbar disc herniation: technique and clinical results[J]. Acta Neurochirurgica, 2010, 152(4):663-668.doi:10.1007/s00701-009-0519-7.

21. Yeung A T , Yeung C A . Minimally invasive techniques for the management of lumbar disc herniation.[J]. Orthopedic Clinics of North America, 2007, 38(3):363-372.doi: 10.1016/j.ocl.2007.04.005.

Table

Table 1. Clinical efficacy comparison (NRS score) of the two groups of patients
| Group/NRS score | Before treatment | 1 month after treatment | 3 months after treatment | 1 year after treatment |
|----------------|------------------|-------------------------|--------------------------|-----------------------|
| Treatment Group | 5±1.68           | 2±0.78                  | 1±0.54                   | 1±0.77                |
| Control Group   | 5±1.68           | 3±0.48                  | 2±0.63                   | 2±0.85                |

Note: Compared with the control group, P<0.05