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Anti-inflammatory Effect of Heat-sensitive Moxibustion via the NF-κB Signaling Pathway on Cerebral Ischemia/Reperfusion Injury in Rats

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Abstract: Ischemic stroke is universally acknowledged as a common cause of long-term disability or even death. Suspended moxibustion, an indirect form of moxibustion, is when moxibustion is placed superficially over the skin without being in contact with it. Some researchers have used this method to treat stroke patients, but strong evidence of its therapeutic effectiveness is lacking. However, the effect of traditional suspended moxibustion has recently been improved with the development of heat-sensitive suspended moxibustion. Our previous studies showed that moxibustion for 35 min provided a more effective treatment strategy than moxibustion for 15 min, and moxibustion by 35 min with tail temperature increase had a better outcome than that without, however, the mechanism underlying the effect is not clear. In this study, we treated the stroke rats with moxibustion by 35min and divided them into non-heat sensitive moxibustion(NHSM) group and heat sensitive moxibustion (HSM) group according to difference in the tail temperature increase, then we compared the effect and investigated the mechanisms between NHSM and HSM. We found that HSM significantly decreased tail-flick latency, increased neurological function score, decreased infarct volume, reduced inflammatory cells, decreased the expression of inflammatory factor ICAM-1 and reduced the expression of NF-κB p65 and p-IKKα/β in rats with focal cerebral ischemia/reperfusion injury. Our experimental findings revealed that HSM exerted its anti-inflammatory and neuroprotective effects from MCAO-induced injury by decreasing the expression of the NF-κB signaling pathway.

Keywords: Auspended Moxibustion; Heat-sensitive moxibustion; Middle cerebral artery occlusion; Cerebral ischemia/reperfusion injury; Tail temperature; Tail-flick latency; Infarct volume; Inflammatory cells; CD11b; ICAM-1; NF-κB p65; p-IKKα/β; Traditional Chinese Medicine

Research Highlights: (1) Application of moxibustion at acupoint Dazhui (DU14) with tail temperature increase (HSM) significantly reduced focal cerebral ischemia/reperfusion injury.(2) Heat-sensitive moxibustion significantly decreased tail-flick latency, increased neurological function score, decreased infarct volume, reduced inflammatory cells (microglia) infiltration and decreased inflammatory factor ICAM-1 expression in rats with focal cerebral ischemia/reperfusion injury.(3) Heat-sensitive moxibustion significantly reduced the expression of NF-κB p65,p-IKKα/β in rats with focal cerebral ischemia/reperfusion injury.

1. Introduction

Suspended moxibustion, an indirect form of moxibustion, is when moxibustion is placed superficially over the skin without being in contact with it. Similar to Chinese herbal medicine,[1] moxibustion is widely used in China and has been adopted by many for the treatment of various chronic diseases or symptoms as "deficient conditions" (weakness), including stroke patients, however, the results seems elusive.[2],[3] Recent developed "heat-sensitive" suspended moxibustion has been shown to be neuroprotective in stroke patients, even more so than traditional suspended moxibustion[4]. Traditionally, the duration of suspended moxibustion is 15 minutes, and an extended period of moxibustion (30–60 minutes) may provide an effective treatment strategy, which is one of the reasons for heat-sensitive moxibustion high efficiency.[4],[5] Our previous research showed that stroke rats treated with moxibustion for 35min had a better outcome than those for 15min.[6] Our lab further study found that some stroke rats with moxibustion for 40 min exhibited a definite tail temperature increase, while moxibustion for 15 min did not result in tail temperature increase; [7] and 40 min moxibustion with tail temperature increase was more efficient than that without[7]. These studies suggest that moxibustion's efficacy increases when distant heat is appeared. But the detail mechanism has not been explored.

Many complex factors are involved in the pathophysiology of stroke, including inflammation, disruption of cellular metabolism in the brain, excitatory amino acid toxicity, intracellular calcium overload, oxidative stress injury and nerve cell apoptosis.[8] It is well known that inflammation is an important pathological mechanism of cerebral infarction.[9],[10],[11],[12],[13],[14] The inflammatory response involves inflammatory cells and inflammatory mediators.[15] Elango et al[10] demonstrated that occlusion followed by reperfusion increased inflammatory cell activation and infiltration, pro-inflammatory cytokine(IL-1β, TNF-α, IL-6) and inflammation-related gene(intercellular adhesion molecule-1,ICAM-1) expression in the ipsilateral brain, which could result in the development of cerebral ischemia/reperfusion injury. ICAM-1 produced by endothelial cells regulates the leukocyte recruitment and participates in inflammatory events. [10],[14],[16] Myeloperoxidase abundantly presents in inflammatory cells (such as neutrophils and macrophages). [10],[16],[17] The main mediators of neuroinflammation are glial cells, and neuroinflammatory reaction is significantly influenced by the activation of microglia.[18] NF-κB is an important transcription factor involved in inflammatory responses and detected in almost all the cells including neuronal and microglial cells following ischemic injury or other inflammation-related diseases, [19],[20] when it is activated, it can promote the transcription of a variety of certain genes, releasing a series of pro-inflammatory cytokines, arachidonic acid derivatives, glutamate, and free radicals which contribute to inflammation and then lead to neuronal damage. Our previous experiment showed that there was a significant increase in myeloperoxidase activity, microglia infiltration and NF-κB protein level in cerebral cortex of rats following IR insult, [6],[21],[22] and 35-min moxibustion reduced myeloperoxidase activity, microglia infiltration and NF-κB protein level in cerebral cortex of rats following IR insult. [6],[21],[22]

However, it remains elusive whether suspended moxibustion for 35 min with tail temperature increase really has greater efficacy than that without, and does it exert its neuroprotective effect through anti-inflammation involving the NF-κB signaling pathway? Therefore, in the present study, we treated the stroke rat with moxibustion for 35min and divided them into non-heat-sensitive moxibustion group and heat-sensitive moxibustion group according to difference in the tail temperature increase. Then we examined the neuroprotective effects of suspended moxibustion, compared their efficacy between non-heat-sensitive moxibustion group and heat-sensitive moxibustion and investigated whether these anti-inflammatory effects were related to NF-κB signaling pathway in order to gain a clearer understanding of the molecular biological mechanisms.

2. Materials and Methods
2.1 Animals

Male Sprague-Dawley rats, weighing 220–280 g, were purchased from Hunan SJA Laboratory Animal Co., Ltd., Hunan, China. Animals were housed under controlled conditions (07:00–19:00 lighting, five rats/cage before MCAO and one rat/cage after MCAO, 50–60% humidity, and 22°C-25°C) with free access to water and food. The study was carried out in strict accordance with recommendations in the Guide for the Care and Use of Laboratory Animals formulated by the Ministry of Science and technology of China (2006). All animal experiments were performed at the Experimental Animal Center of Jiangxi University of Traditional Chinese Medicine, Jiangxi, China.

A total of 80 rats were randomly divided into three groups: a sham-surgery group (n=11), a stroke model group (n=23), a moxibustion-treated group (n=46). Rats in a moxibustion-treated group were put a lit cigar made of mugwort above Dazhui acupoint for 35min and divided into NHSM group(the tail temperature after moxibustion treatment rises no more than 1℃ , or more than 1℃ but less than 2 times) and HSM group(the tail temperature rises more than 1℃ , and more than 2 times) according to difference in the tail temperature before and after moxibustion at 2h,24h, 48h and 72h(data not shown). 5 rats from the stroke only group, 9 rats from the moxibustion-treated group died of cerebral hemorrhage and subarachnoid hemorrhage after surgery, and 2 rats with 0 score, 2 rats with four scores from the stroke only group, and 5 rats with 0 scores, 4 rats with four scores from the moxibustion-treated group were excluded from the study. Therefore, a total of 53 rats were included in the final analysis. The experimental protocol is shown in Figure 1.
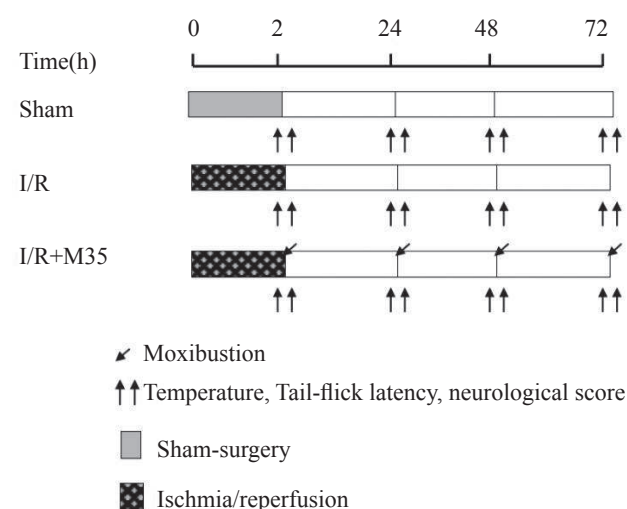

Figure 1. Experimental Design and Timelines

Sham: control animals underwent general anesthesia and sham surgical procedure without ischemia and reperfusion. I/R: ischemic-reperfusion animals underwent cerebral ischemia- reperfusion induced for 2 hours by transient middle cerebral artery occlusion (MCAO), followed by 3 days of reperfusion. I/R+M35: I/R animals with suspended moxibustion for 35 min. Slanted arrows: indicate the time of moxibustion administration. Double arrows indicate the time that tail temperature, rectal temperature, tail-flick latency and neurological function scores were conducted at 2h, 24h, 48h and 72h after surgery. At 3 days after surgery, TTC staining, HE staining and immunohistochemical staining were performed.

2.2 Focal Cerebral Ischemia/Reperfusion Injury

The focal cerebral ischemia/reperfusion injury model was induced for 2 hours by MCAO followed by three days of reperfusion in rats. All animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (3% (w/v)) at a dose of 40 mg/kg. Body temperature was maintained at $37.0 \pm 0.5°C$ using a heat lamp and heating pad. MCAO was achieved using the intraluminal filament method, as described previously[6],[7],[21],[22] with minor modifications. Briefly, a midline neck incision was made, and the left common carotid artery, internal carotid artery and external carotid artery were isolated. The external carotid artery was ligated with 4-0 silk suture distal from the carotid bifurcation and the common carotid artery was ligated with 4-0 silk suture at the proximal end. Another 4-0 silk suture was tied loosely around the common carotid artery close to the carotid bifurcation. A piece of fishing line (Simago Fishing Tackle Company, Hangzhou, Zhejiang, China) 0.237 mm in diameter and 5 cm in length with a rounded tip was introduced into a small incision in the common carotid artery and gently advanced to the origin of the middle cerebral artery (20–22 mm from carotid bifurcation). The silk suture around the common carotid artery stump was tied tightly to prevent bleeding and to secure the fishing line. After 2 hours of occlusion, the fishing line was withdrawn to allow for reperfusion. Sham-surgery group rats were manipulated in the same way, but the middle cerebral artery was not occluded.

The following exclusion criteria are applied:

(1) Death within 72 hours after focal cerebral ischemia/reperfusion;

(2) Neurological severity score = 0 or ≥4 (0 hour after focal cerebral ischemia/reperfusion). 2.3 Suspended Moxibustion Treatment

Dazhui points (region in C7–T1), considered to be important for brain function,[23] were heated by suspended moxibustion using a moxibustion-cigar produced from mugwort (custom-made for use with animals, length 12 cm, diameter 0.6 cm, made in the Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, Jiangxi, China.) at a height of approximately 3 cm over a hairless area of skin once a day for three days. Stimulation of moxibustion was performed at 2h after surgery and a duration of 35 minutes was employed. According to the difference of tail temperature before moxibustion and at 2h, 24h, 48d, 72d after moxibustion, moxibustion-treated rats were divided into the non-heat-sensitive moxibustion group and heat-sensitive moxibustion group.

2.4 Tail Temperature and Rectal Temperature

At 2h, 24h, 48h and 72h after surgery, tail temperature was measured before and after moxibustion at the point between the proximal third and the middle third of the whole tail in rats with focal ischemia-reperfusion injury using a digital thermometer (ST-1, Shanghai medical instrument factory, Shanghai, China.). In the meantime, rectal temperature was also measured using an electronic thermometer (T103, Bioland Medical equipment Co., Ltd., Shenzhen, China).

2.5 Tail-flick Latency Test

Before surgery and 2h, 24h, 48h and 72h after surgery, the latency of tail flick was measured to observe the effect of suspended moxibustion on pain threshold with rat/mice-tail light and thermalgesia algometer(YLS-12A, Jinan yiyan science technology development co., ltd., Jinan, China.).The basic principle of the tail-flick latency is that a light beam irradiates onto the tail, the tail temperature rises and produces pain. When pain intensifies to some extent that animals can't tolerate, the animals will move their tails away. For the tail flick test, the intensity of the power was set at 36W, rat was placed in a cylinder and the cylinder was put onto the fixed seat of the instrument. Then adjusted the cylinder position so that the tail between the middle third and the distal third of the tail was placed in the middle of the photoelectric pore, pressed the start button, the timer started, when the tail swings away, photoelectric switch turned off automatically, the timer ended. That was tail-flick latency. Each rat was twice measured.

2.6 Neurological Function Scores

At 2h, 24h, 48h and 72h after surgery, neurological assessment was performed according to Garcia[24] by an investigator who was blinded to the experimental groups, using a 18-point scale. The score given to each rat at the completion of the evaluation was the summation of all six individual test scores including spontaneous activity:

symmetry in the movement of four limbs, forepaw out-stretching, climbing, body proprioception, response to vibrissae touch. The minimum neurological score is 3 and the maximum is 18.

2.7 Infarct Volumes of the Brain

3 days after surgery, rats were anesthetized with an intra-peritoneal injection of 3% (w/v) sodium pentobarbital at a dose of 40 mg/kg, and their brains in sham, I/R and mox-ibution-treated group were removed. Shortly the brains were sectioned coronally into 2-mm slices and incubated in 1% (w/v) 2, 3, 5-triphenyltetrazolium chloride (TTC) in PBS solution at 37°C for 30 minutes, then the stained slic-es were photographed using a Nikon COOLPIX L1 cam-era (Nikon, Tokyo, Japan) and the areas of ipsilateral and contralateral hemispheres were measured using INFINI-TY ANALYZE software (Lumenera Corporation, Ottawa, ON, Canada). After correcting for edema, the infarct vol-umes were calculated as described previously measured for the ischemic lesion.[25] The ischemic lesion percentage of each slice was calculated as follows: corrected infarct volume (%) = [contralateral hemisphere volume − (ipsilat-eral hemisphere volume-infarct volume)]/the whole brain section volume ×100%.

2.8 Brain Histological Structures

After TTC staining and lesion area analysis, the slices were fixed in 10% (v/v) formalin, then embedded with paraffin(Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China), and the third TTC staining slice of each brain was used for hematoxylin-eosin(HE) stain-ing. A series of adjacent 5-μm thick slices were sectioned in the coronal plane. Standard HE staining was performed, and histopathological changes were photographed with the use of image analysis system (Leica 2000; Leica Mi-crosystems GmbH, Wetzlar, Germany).

2.9 Immunofluorescent Staining

3 days after surgery, the rats from each group were anes-thetized with an intraperitoneal injection of 3% (w/v) so-dium pentobarbital at a dose of 40 mg/kg and fixed with a PBS-buffered 4% paraformaldehyde solution(Sinopharm Chemical Reagent Co., Ltd., Beijing, China) by transcar-dial perfusion. Subsequently, the intact brains were re-moved, the cerebral cortices were separated and immersed in fixative 4 % paraformaldehyde for 2h, then they were transferred to 10%, 20%, 30 % sucrose in PBS solution respectively at 4°C until it sank.

Then the cerebral cortices were washed with PBS solution and sectioned at a thickness of 35 μm on a Leica CM1950 cryostat (Leica Biosystems Nussloch GmbH, Nussloch,Germany), all the sections were preserved in a

-20˚C refrigerator. In order to quantify the number of pos-itive cells, cortical sections from each brain were rinsed with PBS solution for three times, then the sections were incubated with 4 % goat serum for 2h and detected with rabbit antibody against CD11b (ab75476, diluted 1:1000, abcam, Cambridge, UK.) and rabbit antibody against p-IKKα/β(#2697, diluted 1:400, cell signaling technology Inc, Danvers, Massachusetts,USA.) at 4 °C overnight. After rinsing with PBS solution threetimes for 10 min, the tissue was incubated in a Alexa Fluor®568 goat anti-rab-bit IgG (H+L) secondary antibody（A11011, diluted 1:500, Invitrogen, Carlsbad, CA, USA）at room temperature for 2 h. After the slices were washed in PBS solution 3 times for 10 min, they were mounted using ProLong Gold an-tifade reagent (P36931, Invitrogen, Carlsbad, CA, USA). The sections were then imaged with Leica microscope in conjunction with LAS V3.7 microscope software (Leica 2000; Leica Microsystems GmbH, Wetzlar, Germany). The expression of CD11b or p-IKKα/β protein was stained red, and the nucleus was stained blue. Three-five coronal brain sections from each brain and 3 brains from each group were used in order to quantify the percentage of positive cells. The percentage of positive cells was calcu-lated as the number of CD11b- or p-IKKα/β-positive cells /the whole cells ×100% in per × 20 field separately.

2.10 Immunohistochemical Staining

3 days after surgery, the rats from each group were anes-thetized with an intraperitoneal injection of 3% (w/v) so-dium pentobarbital at a dose of 40 mg/kg and fixed with a PBS-buffered 4% paraformaldehyde solution(Sinopharm Chemical Reagent Co., Ltd., Beijing, China) by transcar-dial perfusion. Subsequently, the intact brains were re-moved; the cerebral cortices were separated and immersed in 10 % (v/v) formalin.

Then cerebral cortices were washed three times with PBS and sectioned at a thickness of 5 μm. The antigen retrieval of paraffin sections was performed with the high pressure after dewaxing and dehydration. Brain sections were firstly perforated in 3% triton solutions for 30 min at room temperature, and then washed three times with PBS solution for 10 min. The tissue was immersed in 1% H2O2 for 30 min to quench the endogenous peroxi-dase. After rinsing with PBS solution for three times, the sections were incubated with 5% goat serum for 30 min. Following incubation in serum, they were incubated with mouse monoclonal antibody against ICAM-1 （SC-8439, diluted 1:15, Santa Cruz Biotechnology Inc, Dallas, Texas, USA）and rabbit polyclonal antibody against NF-κB p65 (sc-30080, diluted 1:50, Santa Cruz Biotechnology Inc, Dallas, Texas, USA) at 4°C overnight. After incubation,

the slice was rinsed in PBS solution 3 times for 5 min and then incubated in a peroxidase-conjugated goat anti-mouse secondary antibody (ZB-2305, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China.) and peroxidase-conjugated goat anti-rabbit secondary antibody (ZB-2301, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China.) indoor for 1h. Following another series of washing in PBS solution, the tissue was placed in a solution of 0.5 mg/ml diamino-benzidine (DAB) for 5-10 min until the desired staining intensity was achieved. Ultimately, the tissue was washed and mounted onto super frost glass slides and left to dry. The tissue was photographed with Leica microscope in conjunction with LAS V3.7 microscope software (Leica 2000; Leica Microsystems GmbH, Wetzlar, Germany). The ICAM-1 and NF-κB p65-positive cells were stained brown. Three-five coronal brain sections from each brain and 3 brains from each group were used in order to measure the average integrated optical density (IOD), and the average IOD of ICAM-1 and NF-κB p65-positive cells of the whole field(scale bar=50μm) was analyzed using the Image-ProPlus 5.0 software (Media Cybernetics, USA) separately.

2.11 Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 13.0 software. Quantitative data were expressed as mean ± standard error of the mean, SEM). Multiple group analysis was performed using one-way analysis of variance (ANOVA) followed by Fisher-LSD (least significant difference) post hoc or Dunnett's test for multiple pairwise comparisons. P values of less than 0.05 ($p < 0.05$) were considered statistically significant. All experiments and analysis were performed in a blinded manner, as the experimenters were not aware of the treatment conditions.

3. Results

3.1 Heat-sensitive Moxibustion Improved Sensory Function and Increased Neurological Function Scores

Neurological behavioral evaluation was used to investigate whether HSM could reduce brain injury in rats, we determined sensory function and neurological function recoveries by assessing tail-flick latency and neurological function scores. All the tests were carried out in rats of sham, I/R and moxibustion-treated groups at various time points following IR insult.

Tail-flick latency was used as one of the key standards in identification of potential therapeutics. In this study, we measured tail-flick latency before surgery, 2h, 24h, 48h

and 72h after surgery in rats. Results showed that tail-flick latency didn't exhibit a significant difference among four groups before surgery (sham:3.885±0.140; I/R: 3.897±0.126; NHSM: 3.918±0.104; HSM:4.030±0.089), then after surgery tail-flick latencies in rat with focal cerebral ischemia/reperfusion injury(2h: 13.24±0.56; 24h: 12.88±0.40; 48h:12.41±0.30; 72h:12.32±0.35. $p < 0.05$) were longer than those of sham-surgical rats(2h: 3.901±0.175; 24h: 3.87±0.15; 48h:3.87±0.15; 72h: 3.88±0.13) at 2h, 24h, 48h and 72h. While suspended moxibustion could reduce tail-flick latency. At 24h, heat-sensitive moxibustion (24h: 11.52±0.23. $p < 0.05$) significantly reduced tail-flick latency compared to I/R group (24h: 12.88±0.40). With time, both non-heat-sensitive moxibustion (48h: 11:00±0.36; 72h: 9.25±0.35. $p < 0.05$)and heat-sensitive moxibustion(48h: 10:20±0.18; 72h:8.66±0.13. $p < 0.05$) significantly reduced tail-flick latency compared to I/R group(48h: 12:41±0.30; 72h:12.32±0.35) at 48h and 72h after surgery, and heat-sensitive moxibustion(48h: 10:20±0.18. $p < 0.05$) significantly reduced tail-flick latency compared to non-heat-sensitive moxibustion (48h: 11:00±0.36.) at 48h after surgery. These results indicate that HSM significantly improves sensory function after cerebral ischemia/reperfusion injury. (Figure 2a)

Similarly, neurological function scores were used to evaluate whether heat-sensitive moxibustion would improve neurological function. In this study, we assessed neurological function scores at 2h, 24h, 48h, and 72h of MCAO followed by reperfusion in rats according to Garcia et al (24). Results showed that the neurological function scores after surgery were as follows (2h: 7.36±3.50; 24h: 7.64±3.39; 48h: 8.00±3.72; 72h: 8.43±3.84. $p < 0.05$) in I/R group in comparison to 18 scores at all the above time points in sham group, which suggested that the rat model of focal cerebral ischemia/reperfusion injury was successful. Suspended moxibustion increased neurological function scores, rats treated with non-heat-sensitive moxibustion (24h: 10.69±3.12; 48h: 13.08±2.25; 72h: 15.54±1.27. $p < 0.05$) and heat-sensitive moxibustion (24h: 12.47±2.90; 48h: 14.40±1.96; 72h: 16.87±1.25. $p < 0.05$) at 24h, 48h, 72h exhibited a significant increase in neurological function scores compared to I/R group(24h: 7.64±3.39; 48h: 8.00±3.72; 72h: 8.43±3.84), but rats in non-heat-sensitive moxibustion group (2h: 7.69±3.12) and heat-sensitive moxibustion group(2h: 7.53±3.14) at 2h exhibited no significant in neurological function scores compared to I/R group. Heat-sensitive moxibustion(24h: 12.47±2.90; 48h: 14.40±1.96; 72h: 16.87±1.25.) increased more neurological function scores than non-heat-sensitive

moxibustion (24h: 10.69±3.12; 48h: 13.08±2.25; 72h: 15.54±1.27..) at 24h,48h and 72h after surgery, although there is no significant differrence.These results indicate that HSM improves neurological function after cerebral ischemia/reperfusion injury. (Figure 2b)
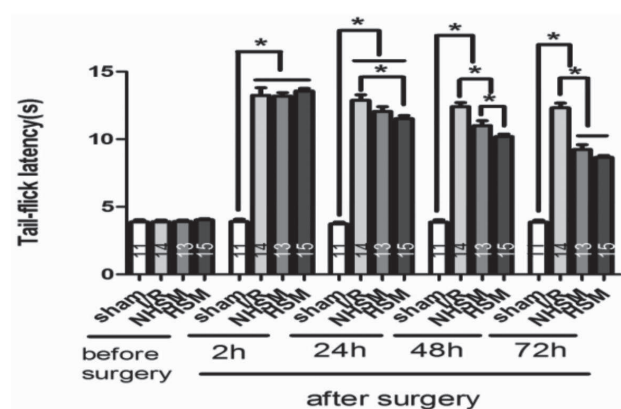
(a)
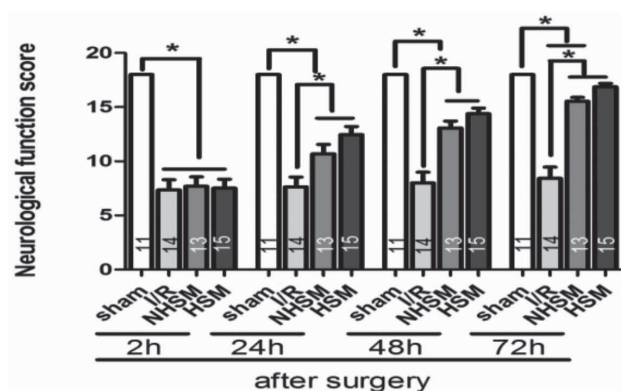
(b)

Figure 2. Effect of Heat-sensitive Moxibustion on Tail-flick Latency and Neurological Function Scores in Focal Ischemia-reperfusion Injury

(a) Summary of tail-flick latency of the sham, I/R and moxibustion-treated group. Tail-flick latencies of all focal cerebral ischemia/reperfusion injury group were rosen at 2h after surgery compared with that of sham-surgical rats, while tail-flick latency in rats treated with suspended moxibuxtion exhibited a significant decrease at 48h and 72h, and tail-flick latency in rats treated with heat-sensitive moxibuxtion even exhibited a significant decrease at 24h compared to that in I/R group. Heat-sensitive moxibustion also exerted a significant decrease at 48h compared to non-heat-sensitive moxibustion .These results indicate that suspended moxibustion, especially heat-sensitive moxibustion improves sensory function following IR insult.

(b) Summary of neurological function scores of the sham, I/R and moxibustion-treated group. The neurological function scores of sham-surgery rats were 18 at all timepoints, and neurological function scores of all focal cerebral ischemia/reperfusion injury rats were reduced at 2h after surgery. However neurological function score in rats treated with suspended moxibuxtion exhibited a significant increase at 24h, 48h, 72h compared to that in I/R group. These results indicate that heat-sensitive moxibustion also improves neurological function following IR insult.

Notes: "*": P < 0.05. Sham: Sham-surgery group; I/R: cerebral ischemia/reperfusion injury group; NHSM: cerebral ischemia/reperfusion injury with non-heat-sensitive moxibustion group; HSM: cerebral ischemia/ reperfusion injury with heat-sensitive moxibustion.

3.2 Heat-sensitive Moxibustion Reduced Brain Infarct Volume

Cerebral infarct size was also used to evaluate whether heat-sensitive moxibustion could reduce brain injury in rats following IR insult. Infarct volume was measured from the sham or I/R group with or without moxibustion. Brain slices obtained from all groups were stained with TTC 3 days after IR to investigate the efficacy of heat-sensitive moxibustion. Results revealed that suspended moxibustion significantly reduced infarct size (NHSM group: 19.75%±0.59%; HSM group: 13.06%±1.08%. P < 0.05) at 3 days after I/R when compared to I/R group (26.09%±1.33%. P<0.05), and heat-sensitive moxibustion (13.06%±1.08%. P < 0.05) significantly reduced the infarct size when compared to non-heat-sensitive moxibustion group (19.75%±0.59%). These results demonstrate that heat-sensitive moxibustion significantly reduced brain damage following IR insult in rats. (Figure 3)

(a)

(b)
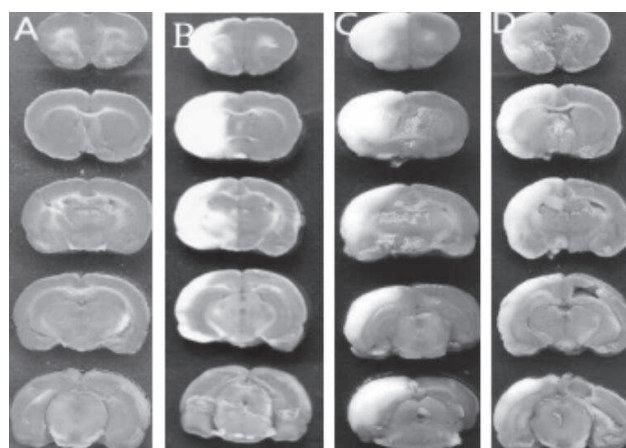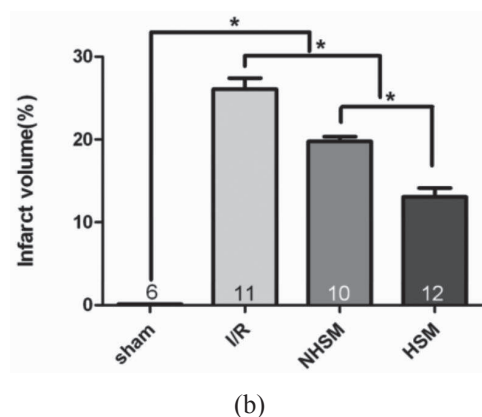
Figure 3. Effects of Heat-sensitive Moxibustion on Infarct Volume in Focal Cerebral Ischemia/Reperfusion Injury Rats

(a) Representative brain coronal slices stained with TTC. The ischemic area remained white, while the intact areastained red.

(b) Analysis of brain infarct volumes of the sham, I/R or moxibustion-treated group. Heat-sensitive moxibustion decreased brain infarct volumes of stroke rats in comparison with that of I/R group and non-heat-sensitive moxibustion group. So heat-sensitive moxibustion significantly protected the brain from cerebral ischemia/reperfusion injury.

Notes: "*": P < 0.05.

Sham(A): Sham-surgery group; I/R(B): cerebral ischemia/reperfusion injury group; NHSM（C）: cerebral ischemia/reperfusion injury with non-heat-sensitive moxibustion group; HSM（D）: cerebral ischemia/ reperfusion injury with heat-sensitive moxibustion.

3.3 Heat-sensitive Moxibustion Reduced the Presence of Inflammatory Cells in the Brain

Brain (ipsilateral) histological structure was used to evaluate whether heat-sensitive moxibustion could reduce brain injury and inhibit inflammation in rats following IR insult. Cerebral cortical slices from the sham or I/R group with or without moxibustion treatment were stained with HE (Figure 4a). Results revealed that there were damaged histological structure showing missing regions and many inflammatory cells in the cerebral cortices (ipsilateral) 3 days after surgery in the I/R group when compared to sham-surgery group. However, suspended moxibustion significantly protected the brain from cerebral ischemia/ reperfusion injury and reduced the presence of inflammatory cells; In contrast, outcomes with non-heat-sensitive moxibustion were not as good as outcomes with heat-sensitive moxibustion (Figure 4b). Further studies showed that cortical slices stained with immunofluo-

rescence (IF) had higher percentage of CD11b-positive cells (I/R group: 41.72% ± 5.78%. p < 0.05) in the cerebral cortex (ipsilateral) when compared to sham-surgery group(1.16%±0.19%). However suspended moxibustion significantly reduced the percentage of CD11b-positive cells compared to I/R group, while the percentage of CD11b-positive cells in the heat-sensitive moxibustion group(14.38%±0.74%) were less than that in the non-heat-sensitive moxibustion group(29.00%±1.00%) (Figure 4c, 4d). These results indicate that heat-sensitive moxibustion significantly attenuated brain damage resulting from inflammatory reaction following IR insult in rats.

(a)

(b)

(c)

(d)
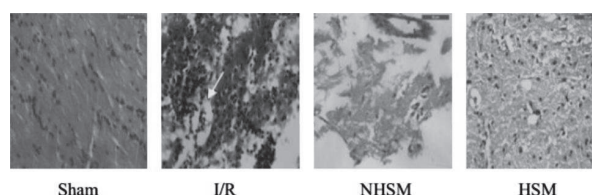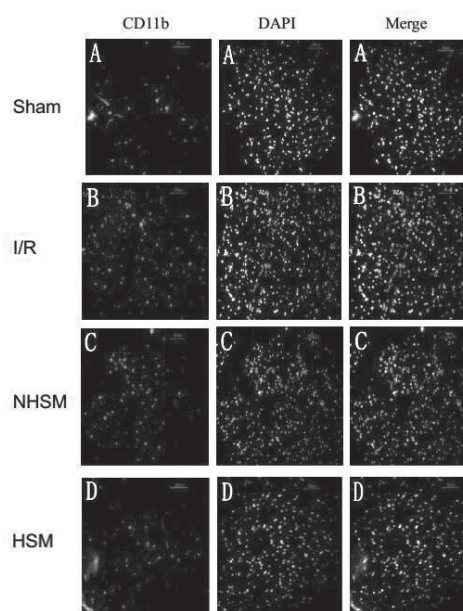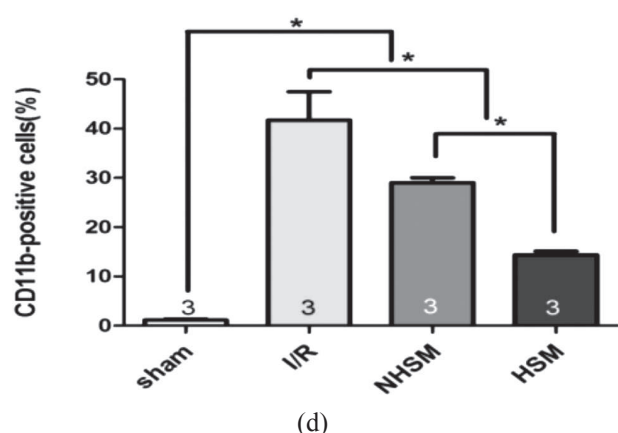
Figure 4. Effects of Heat-sensitive Moxibustion on Inflammatory Cells Infiltration in Focal Cerebral Ischemia/Reperfusion Injury Rats

(a) Rat brain coronal slice. Black box: the area for HE and immunohistochemical staining.

(b) Representative brain coronal sections stained with HE. Lots of inflammatory cells were in the cerebral cortices of I/R group in comparison to sham-surgery group. However, suspended moxibustion attenuated cerebral ischemia/reperfusion injury and significantly reduced the presence of inflammatory cells following IR insult. In contrast, outcomes in non-heat-sensitive moxibustion group were not as good as outcomes in heat-sensitive moxibustion group. Scale bars corresponds to 50μm. Green arrows indicates the inflammatory cells.

(c) Double-immunofluorescent staining of CD11b and nucleus in cerebral cortex. CD11b-positive cells stained red, while nuclei stained blue. Scale bars correspond to 50μm.

(d) Summary of CD11b-positive cells of sham, I/R and moxibustion-treated cerebral cortex. Heat-sensitive moxibustion decreased the percentage of CD11b-positive cells 3 day following IR insult in comparison to that of I/R group and non-heat-sensitive moxibustion group.
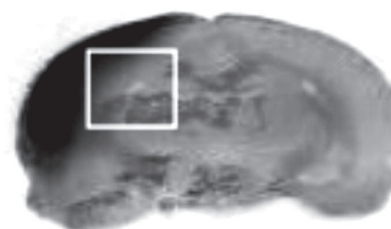
Notes: "*": $P < 0.05$. Sham(A): Sham-surgery group; I/R(B): cerebral ischemia/reperfusion injury group; NHSM(C): cerebral ischemia/reperfusion injury with non-heat-sensitive moxibustion group; HSM(D): cerebral ischemia/reperfusion injury with heat-sensitive moxibustion.

3.4 Heat-sensitive Moxibustion Reduced the Expression of ICAM-1 in the Cerebral Cortex

Intercellular adhesion molecule-1 (ICAM-1), an inflammation-related factor, produced by endothelial cells regulates the leukocyte recruitment and participates in inflammatory events. In this study, we compared the levels of the inflammation-associated proteins from ipsilateral cerebral cortices among sham, I/R and moxibustion-treated groups. As shown in Figure 5a, 5b, the average integrated optical density (IOD) of ICAM-1-positive cells in cerebral cortices was high in I/R group (3.7340±0.2094. $P < 0.05$) 3 days after surgery in comparison to that in sham-surgery rats (0.0002±0.0000). However, suspended moxibustion for 35 min significantly decreased the average integrated optical density (IOD) of ICAM-1-positive cells ($P<0.05$) in comparison to that in I/R group, and heat-sensitive moxibustion (0.6585±0.0353. $P<0.05$) decreased even more so than non-heat-sensitive moxibustion (1.7750±0.0598). These results indicated that heat-sensitive moxibustion significantly decreased inflammatory factor after the IR procedure, partly accounting for reduction of inflammatory cells in the cortical cortex.
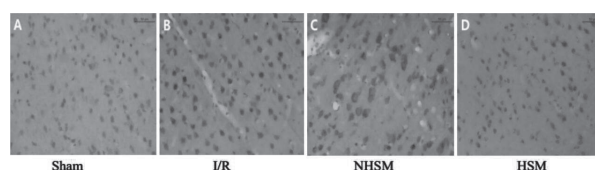
(a)
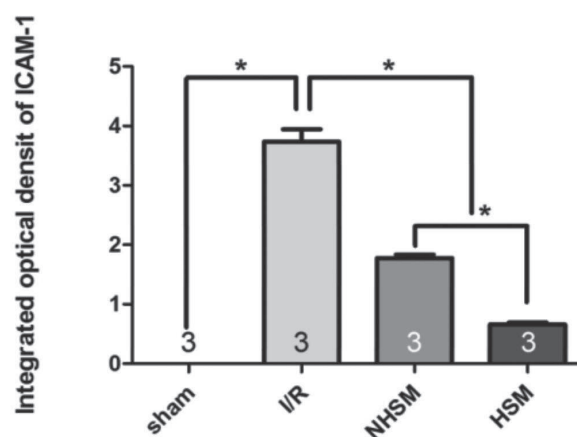
(b)

Figure 5. Effects of Heat-sensitive Moxibustion on the Expression of ICAM-1 in Focal Cerebral Ischemia/Reperfusion Injury Rats

(a) Representative immunohistochemical staining of ICAM-1 protein. ICAM-1 was stained brown. Scale bars correspondsto 50μm.

(b) Summary of the averageIOD analysis of ICAM-1 proteinin cerebral cortex of sham, I/R and moxibustion-treated group. The average (IOD of ICAM-1protein

was significantly high in the cerebral cortex 3 days following IR insult in comparison to that of sham-surgery group. However, suspended moxibustion significantly reduced the average IOD of ICAM-1 protein. In contrast, the average IOD in heat-sensitive moxibustion group were much lower than that in non-heat-sensitive moxibustion group. So heat-sensitive moxibustion significantly decreased the expression of ICAM-1 proteins following IR insult.

Notes: "*":$P < 0.05$. .Sham(A): Sham-surgery group; I/R(B): cerebral ischemia/reperfusion injury group; NHSM (C): cerebral ischemia/reperfusion injury with non-heat-sensitive moxibustion group; HSM（D）: cerebral ischemia/ reperfusion injury with heat-sensitive moxibustion

3.5 Heat-sensitive Moxibustion Reduced the Expression of NF-κ B and p-IKKα/β in the Cerebral Cortex

NF-κB is a pleiotropic transcription factor participating in inflammation. To understand whether neuroprotective effects of heat-sensitive moxibustion are mediated through suppressing NF-κB signaling pathway, we compared the levels of NF-κB signaling pathway proteins in cerebral cortex 3 days following IR insult among sham, I/R and moxibustion-treated groups. As shown in Figure 6a, 6b, the average IOD of NF-κB was high in cerebral cortices in I/R group (0.1334 ± 0.00287.$P < 0.05$) in comparison to that in sham-surgery group (0.0012 ± 0.0001). However, suspended moxibustion for 35 min significantly decreased the average IOD of NF-κB-positive cells ($P < 0.05$), and heat-sensitive moxibustion(0.0466 ± 0.0025.$P<0.05$) decreased even more so than non-heat-sensitive moxibustion (0.1023 ± 0.0016). Similarly, as shown in Figure 6c, 6d, p-IKKα/β protein level significantly increased in cerebral cortices in I/R group ($53.71\% \pm 3.54\%$, $p < 0.05$) in comparison to that in the sham group ($2.19\%\pm0.28\%$). However, suspended moxibustion for 35 min significantly reduced p-IKKα/β protein level ($p < 0.05$) in comparison to that in the I/R group, and heat-sensitive moxibustion ($15.76\%\pm0.66\%$, $p < 0.05$) reduced more p-IKKα/β protein level than non-heat-sensitive moxibustion ($32.83\%\pm0.74\%$), possibly as a result of the reduced presence of inflammatory cells in the cerebral cortex.

(b)

(c)
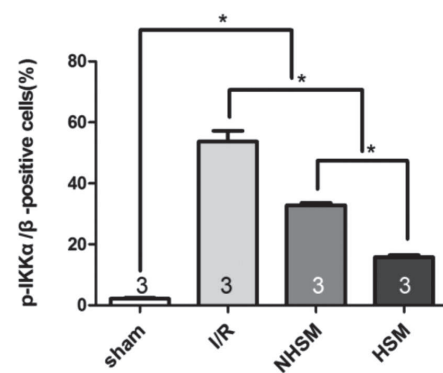
(d)

Figure 6. Effects of Heat-sensitive Moxibustion on the Expression of NF-κB and p-IKKα/β in Focal Cerebral Ischemia/Reperfusion Injury Rats

(a) Representative immunohistochemical staining of NF-κB protein 3 day following IR insult. NF-κB was stained brown. Scale bars correspond to 50μm.
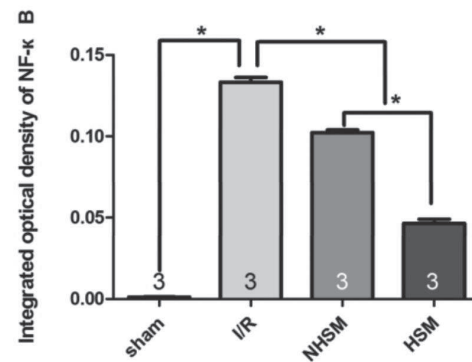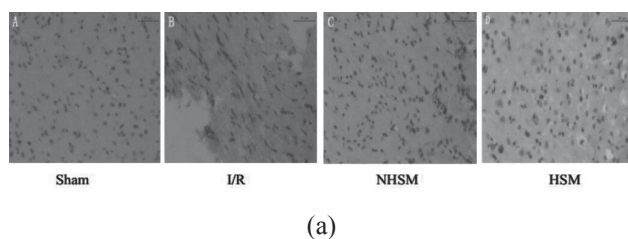
(a)

(b) Summary of the average IOD analysis of NF-κB protein in cerebral cortex of sham, I/R and moxibustion-treated group. The average IOD of NF-κB proten was significantly high in the cerebral cortex 3 days following IR insult in comparison to that of sham-surgery group. However, suspended moxibustion significantly reduced the verage IOD of NF-κB protein. In contrast, the average IOD in heat-sensitive moxibustion group were much lower than that in non-heat-sensitive moxibustion group. So heat-sensitive moxibustion significantly decreased the expression of NF-κB proteins following IR insult.

(c) Double-immunofluorescent staining of p-IKKα/β and nucleus in cerebral cortex. P-IKKα/β-positive cells stained red, while nucleus stained blue. ×200

(d) Summary of p-IKKα/β-positive cells of sham, I/R model and moxibustion-treated cerebral cortex. Heat-sensitive moxibustion decreased the expression of p-IKKα/β proteins 3 day following IR insult in comparison to that in I/R group and non-heat-sensitive moxibustion group.
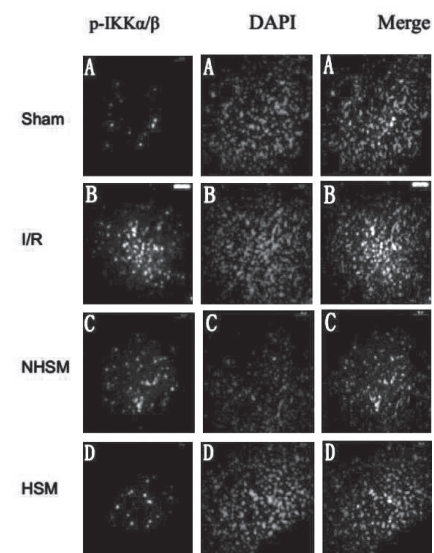
Notes: "*": $P < 0.05$. Sham(A): Sham-surgery group; I/R(B): cerebral ischemia/reperfusion injury group; NHSM (C): cerebral ischemia/reperfusion injury with non-heat-sensitive moxibustion group; HSM (D): cerebral ischemia/ reperfusion injury with heat-sensitive moxibustion.

4. Discussion

In this study, we reported that suspended moxibustion for 35 min with tail temperature increase (i.e HSM):

(1) Significantly improved sensory function and increased neurological function scores after cerebral ischemia-reperfusion injury in rats;

(2) Significantly protected the brain from cerebral ischemia/reperfusion injury;

(3) Significantly reduced the presence of inflammatory cells and CD11b-positive cells in the cerebral cortices;

(4) Significantly decreased the expressions of inflammation-related factor ICAM-1 in the cerebral cortices;

(5) Reduced the protein level of p-IKKα/β /NF-κB signaling pathway in the cerebral cortices. Our findings suggest that HSM protects brain from cerebral ischemia-reperfusion injury, which is largely mediated by anti-inflammation involved the decreased expression of p-IKKα/β /NF-κB signaling pathway during cerebral ischemia-reperfusion injury.

Suspended moxibustion is an important treatment method in Traditional Chinese Medicine. The previous

controlled trials have demonstrated the enhancement of recovery in stroke patients by 15-minute traditional suspended moxibustion on the fixed acupoints,[26],[27] however, other studies had different results.[2],[3] The newly developed application of "heat-sensitive" suspended moxibustion substantially improved upon the effect of traditional suspended moxibustion.[28] The theoretical basis for heat-sensitive moxibustion is that the acupoints which are activated from the resting state to the sensitized state by a pathological process become heat sensitive. Clinic practices demonstrated that moxibustion placed above a heat-sensitive acupoint can achieve effect namely "less stimulation, greater effect", so the efficacy of moxibustion is significantly improved.[29] Acupoint, according to the core viewpoint and theory of ancient Chinese medicine, represents the certain area of the body surface which can respond to disease. Usually, an acupoint has two states, the "resting" state and the "sensitized" state; selecting the sensitized acupoints is the key to improving efficacy.[29] There are a variety of types of sensitized acupoints; the heat-sensitive type is a new one among these.

Is an acupoint heat-sensitive or not? According to the results of clinical studies for more than 20 years by professor Chen Rixin et al[28], moxibustion above heat-sensitive acupoint can show 6 kinds of moxibustion sensation. If one of six sensations is produced, the acupoint is thought to be heat-sensitive. Among these six sensations, one is heat transduction, it means the heat of moxibustion point could transduct along a line to the distance. As the locations of acupoints are arranged along with meridians, moxibustion applied above the heat-sensitive acupoint penetrates deeply into the body and can transduct the heat to distant regions along the meridians. When such distant heat occurs, the acupoint is called heat-sensitive one, and the efficacy of moxibustion increases.[30] So in this experiment we divided moxibustion-treated mice into NHSM and HSM group according to the difference of tail temperature before moxibustion and after moxibustion.

Additionally, the amount of moxibustion is a key technology when applying heat-sensitive suspended moxibustion. When other factors are constant, duration of suspended moxibustion is a critical factor for improving the efficacy of moxibustion.[5],[7],[28] Generally, distant heat first appears on the patient at about 15 minutes into suspended moxibustion treatment, after the 15 minute time point, a rapid increase in distant heat is exhibited in the patients and maintained until the end of the treatment, usually 30 min to one hour.[31] Long-term clinical practice prove that the duration of heat-sensitive suspended moxibustion ranges from 30 minutes to 1 hour, which is usually longer

than conventional moxibustion (usually around 15 minutes), and find that prolonged application of moxibustion above certain acupoints can often induce not only internal heat-sensation but also physically detectable elevated temperature at distant locations away from the suspended moxibustion acupoint. One study showed that a 40-minute suspended moxibustion caused a measurable tail temperature increase in some MCAO rats while 15 minute treatments didn't have this effect,[7] and the occurrence rate of tail temperature increase was 54.1%.[7] Our previous study showed that suspended moxibustion for 35 minutes, but not 15 minutes, for 3 days markedly alleviated MCAO-induced infarct volume and neuronal loss, as determined using TTC, HE staining when compared with the stroke only group.[6] Among rats with suspended moxibustion for 35 minutes, those with tail temperature increase had a better outcome than those without[21]. Consistent with the previous report, our result showed that the occurrence rate of tail temperature increase was 53.6% in 35-min moxibustion group. These evidences collectively showed that prolonged moxibustion is capable of stimulating the heat-sensitive acupoints and gradually transfers the heat to a distant area, and moxibustion with tail temperature increase had a better efficacy than that without. This study showed that suspended moxibustion for 35 minutes with tail temperature increase for 3 days markedly improved sensorimotor function, reduced infarct volume and alleviated neuronal loss, as determined using neurobehavioral evaluation, TTC, HE staining when compared with the stroke only group and suspended moxibustion for 35 minutes without tail temperature increase. These results indicates that moxibustion placing above the heat-sensitive acupoint achieve a better neuroprotective effect than moxibustion placing above non- heat-sensitive acupoint.

Inflammation plays a pivotal role in the pathologic and physiological processes in cerebral ischemia-reperfusion injury, accumulation of data has revealed that cerebral ischemia-reperfusion injury is associated with a marked inflammatory reaction that contributes to the evolution of the tissue injury.[10],[11],[12],[13],[14] During brain ischemia/reperfusion insult, inflammatory responses are initiated involving infiltration into the brain of inflammatory cells and the production of inflammatory cytokines, i.e., inflammatory cells and inflammatory mediators play an important role in the brain inflammatory response to I/R, which has been observed during ischemic pathology in experimental animal models of stroke.[10],[11],[12],[13],[14] Myeloperoxidase, abundantly present in inflammatory cells (such as neutrophils and macrophages), produces hypochlorous acid from hydrogen peroxide and chloride ions

during the respiratory burst of neutrophils (abundance of reactive oxygen species). Hypochlorous acid is highly cytotoxic and has been demonstrated to damage CNS tissue during inflammation.[32] Further, myeloperoxidase activity assay has been successfully used to confirm inflammatory cell activation and recruitment in brain after MCAO.[10],[16],[17] Microglia is resident immunocompetent and phagocytic cells in the CNS, which play a critical role in the event of infection, hypoxemia, ischemia, and neurodegeneration in the central nervous system (CNS).[18] CD11b is the marker of microglia/macrophages, our previous experiment showed that there was a significant increase in CD11b-positive cells in cerebral cortex of IR model rats in comparison to those in sham-surgery group.[21] Microglial cells activation exacerbates ischemic injury in the brain.[33],[34],[35] Intercellular adhesion molecule-1 (ICAM-1) produced by endothelial cells regulates the leukocyte recruitment and participates in inflammatory events.[10],[14],[16] It is well known that an excessive inflammatory reaction can damage target cells and tissue. Therefore, inhibition of inflammatory responses provided an attractive therapeutic strategy.[10],[11],[12],[13],[14] Our previous study also showed that 35 min moxibustion reduced myeloperoxidase activity in comparison with 15 min moxibustion,[6] and 35 min moxibustion with tail temperature increase significantly decreased the CD11b-positive cells in comparison with 35 min moxibustion without tail temperature increase,[21] which suggested that prolonged moxibustion with distal heat had a more potent effect. In this study, we compare the anti-inflammatory effects between moxibustion for 35 min with tail temperature increase and that without. Our results demonstrated that 35 min suspended moxibustion treatment with tail temperature increase reduced inflammatory cells infiltration and the percentage of CD11b-positive cells, decreased the average IOD of inflammation-related factor ICAM-1-positive cells. The results indicated that 35 min moxibustion with tail temperature increase markedly reduced inflammatory reaction, which partly contributed to the neuroprotective effect of heat-sensitive moxibustion.

It is well known that NF-κB is a master regulator of inflammatory signaling pathways and involved in cerebral ischemia-reperfusion injury. It has been known that NF-κB exists in almost all the cells.[19] In the central nervous system (CNS), NF-κB activity is detected in both neuronal and glial cells following ischemic injury.[20] Then inflammatory cells activation triggers rapid transcription-factor NF-κB signal transduction cascades, mediating expression of inflammatory mediators during pathophysiological changes after brain ischemia injury.[12] The most common

form of NF-κB, a dimeric transcription factor, is a heterodimer composed of Rel A (p65), namely NF-κB (p65). Normally NF-κB p65 protein is quiet in cells, residing in the cytoplasm as a complex with inhibitory IκB that mask their nuclear localization signal. Upon brain ischemia, ischemia/reperfusion injury et al, IκB is phosphorylated by p- IKKα/β and proteolytically degraded, resulting in the translocation of NF-κB p65 to the nucleus and production of inflammatory factors. Following I/R injury NF-κB p65 expression was upregulated,[13],[14],[22],[23] so inhibition of NF-κB activation exerted anti-inflammatory and neuroprotective effects.[13],[14],[22],[23] Thus, we investigated the potential effects of heat-sensitive moxibustion on the expression of NF-κB signaling pathway. We observed that the protein level of p- IKKα/β and NF-κB p65 were high in the cerebral cortices following IR injury, while heat-sensitive moxibustion significantly decreased the expression of p- IKKα/β and NF-κB p65, consistant with the previous report.[33] We concluded that decreasing expression of the NF-κB signaling pathway partly accounted for the anti-inflammatory effect of heat-sensitive moxibustion due to reducing the production of inflammatory factors and decreasing infiltration of inflammatory cells.

5. Conclusion

In summary, our study demonstrated that heat-sensitive moxibustion significantly provided beneficial effects on cerebral ischemia/reperfusion injury by decreasing the inflammatory cells such as microglia, reducing inflammatory factors like ICAM-1and lowering the expression of NF-κB signal transduction pathway. Although there is currently no clear physiological correlate to this theory; in addition, it remains unknown what the relevant properties of the moxibustion heat stimulus are responsible for the therapeutic effects. However, the clinical evidences support the notion that moxibustion may improve physiological conditions through regulation of the homeostasis of the body. TCM claims that moxibustion functions by restoring the balance and flow of "vital energy" through sensitized acupoints, thus we suppose that under pathological conditions, the sensitized acupoints serve as the switch that controls the complicate internal regulatory system towards to dysfunction. Moxibustion applied upon sensitized acupoints can fully activate the regulatory system and enhance the internal ability to correct the imbalance of the internal regulation by inducing rapid, precise and optimal responses to harmful stimuli, and then to enhance treatment efficacy.

To sum uponclusion, the present study showed that heat-sensitive moxibustion exerts its neuroprotective effects against inflammation with the involvement of decreasing expression of NF-κB signaling pathway. These findings will further encourage heat-sensitive moxibustion to be applied in the clinic stroke field.

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Association of Genetic Polymorphism of GSTM1 and GSTT1 with the Susceptibility to Antituberculosis Drug-induced Hepatotoxicity in Chinese Population

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Abstract: Objective To investigate the relationship between the polymorphism of glutathione S transferase M1, T1(GSTM1, GSTT1) gene and the susceptibility to antituberculosis drug induced hepatotoxicity (ATDH) in patients with tuberculosis. Methods GSTM1 and GSTT1 gene polymorphisms in patients with or without liver toxicity after antituberculous treatment were analyzed using multiple PCR method. Results In ATDH group and control group, the proportion of GSTM1 gene deletion was 58.0% and 50.7% respectively, and the difference was not statistically significant (OR=1.322, 95%CI=0.921~1.878), the frequencies of GSTT1 deletion were 46.3% and 49.3%, respectively, and there was no significant difference between them. There was no significant difference in frequency of GSTM1 and GSTT1 variation between case group and control group (P> 0.05), and no synergistic effect of those two gene polymorphism were detected in the occurrence of antituberculosis drug-induced hepatotoxicity. Conclusion The polymorphisms of GSTM1 and GSTT1 genes may not be associated with the risk of ATDH.

Keywords: GSTM1; GSTT1; Tuberculosis; Hepatotoxicity; Genetic Polymorphism

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1. Introduction

Tuberculosis (TB) remains a global major infectious disease, especially in developing countries. World Health Organization (WHO) has declared TB as a public health emergency since 1993[1].

At present, Standard short-course chemotherapy based on isoniazid, rifampicin, pyrazinamide, and ethambutol has been accepted by many countries and is the basic scheme for the treatment of tuberculosis [2, 3]. A large number of clinical studies have shown that isoniazid, rifampicin and azininazinamid are to some extent susceptible to the occurrence of hepatotoxicity [3-8], especially when the above drugs were adminstored together, the incidence and severity of hepatotoxicity increased significantly. Antituberculosis drug-induced hepatotoxicity causes substantial morbidity and mortality with an incidence ranged from 2.5% to 34.9% [6,9]. The pathogenesis of antituberculosis drug-induced hepatotoxicity is unclear.

Currently, it is generally considered that the process of drug-induced hepatotoxicity is composed of multiple steps, including the entry of drugs into the liver tissue, the formation of enzyme catalyzed metabolic products, the metabolites as immunogens are then combined with en-dogenous proteins to induce immune damage or directly cause hepatocyte intoxication. In these steps, drug metabolism enzymes play a significant role in drug detoxification and activation, which exert important effect on drug efficacy and sensitivity to toxicity[10]. GSTM1 and GSTT1 are members of the GST family, and they both have detoxification functions for exogenous chemicals. Deletion homozygote of those two genes could cause the loss of enzyme activity [11]. There are reports that [12-18], GSTM1 and GSTT1 gene polymorphisms are related to the occurrence of anti tuberculosis drug hepatotoxicity, but the conclusions are not consistent. In this study, a case-control study was conducted to explore the association between GSTM1 and GSTT1 gene polymorphisms and susceptibility to antituberculosis drug-induced hepatotoxicity in Chinese population.

2. Study Objects

2.1 Study Object

Between May 2010 and March 2016, 600 patients with newly diagnosed tuberculosis who met the selection criteria were included. The case group consisted of 300 patients with hepatotoxicity after receiving first-line anti-tuberculosis treatment (2HRZE/4HR). Hepatic toxicity of anti-tu-

berculosis drugs is defined as asymptomatic or symptoms of hepatitis, such as loss of appetite, nausea, and vomiting after taking anti-TB drugs, together with at least one of the following conditions, (1) Serum AST and/or ALT is 3 times (or > 120 U/L) above the upper limit of normal, (2) In two consecutive blood samples, ALP test values is two times greater than the upper limit of normal , (3) Any increase in ALT, AST, ALP accompanied by progressively elevated bilirubin (>2.5 mg/dl). 300 patients with tuberculosis were selected as controls. These patients took the same anti-tuberculosis drugs but no hepatotoxicity appeared. All enrolled subjects must meet the following conditions: At the beginning of chemotherapy, liver function is normal and there are no other factors that may cause liver damage, such as malnutrition, HIV infection, alcohol abuse, viral hepatitis, liver disease, cardiac insufficiency, and the use other drugs, etc. In the course of treatment, changes in liver function were closely monitored in all subjects.

2.2 Methods

2.2.1 Human Peripheral Blood DNA Extraction

The whole blood sample of the tuberculosis patients mentioned above was collected, and a genome-wide extraction kit (Tiangen Biotech, Beijing, China) was used to extract DNA in blood according to the instructions, and the extract is immediately stored in a -20°C refrigerator for subsequent use.

2.2.2 Determination of GSTM1 and GSTT1 Genes

According to the report [13], the GSTM1 and GSTT1 genes were simultaneously examined by multiplex PCR and β-globin was used as an internal control. The GSTM1 ((Upstream: 5'GAACTCCCTGAAAAGCTAAAGC3', Downstream: 5'CTTGGGCTCAAATATACGGT-GG3', 219bp), GSTT1 (Downstream: 5' CTTGGGCT-CAAATATACGGTGG 3', Downstream: 5' TCAC-CGGATCATGGCCAGCA 3', 459bp), and β-globin (Upstream: 5' CAACTTCATCCACGTTCACC 3', Downstream: 5' GAAGAGCCAAGGACAGGTAC 3', 68bp) gene amplification primers were constructed successively.

The PCR reaction is a 25 ul amplification system, the final concentrations of each substances were as follows: 0.2mmol/L of each of the 4 dNTPs, 0.3mmol/L primer, 10 to 100ng DNA template, and 1U Taq DNA polymerase. The conditions for PCR were pre-denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 40 seconds, and a final extension at 72°C for 10 minutes. The PCR products were separated by 2.0% agarose gel electrophoresis, and the results were observed on a gel imaging and analysis system.

2.3 Genotyping

β-globin was used as an internal control with an amplification product showed at 268 bp, no β-globin fragment means inefficient amplification. After amplification of the DNA sample by PCR, 219 bp fragments and 459 bp fragments were generated, which were defined as GSTM1(+) and GSTT1(+), respectively. No corresponding amplification products were considered to be homozygous deletions, namely GSTM1(-) and GSTT1(-).

2.4 Statistical Analysis

Spss 13.0 software, t-test, x2 test and other methods were used to analyze the basic parameters of the two groups, and x2 test, factorial analysis and other methods were used to analyze the electrophoresis results of PCR products. The test level is α=0.05.

3. Results

3.1 Analysis of Basic Parameters

There was no significant difference in gender and age between the patient group and the control group. The values of ALT, AST, DBIL, TBIL, GGT and ALP before treatment were within the normal range. The comparison of serum ALT, AST, DBIL, TBIL, GGT and ALP between the two groups before and after treatment are shown in Table 1.

Table 1. Comparison of Patients' Parameters in Case Group and Control Group

	case group	control group	P
Gender(M/F)	174/126	160/140	0.287
Age(years)	37.19±18.193	36.41±18.661	0.167
Before			
ALT(U/L)	18.64±10.887	14.21± 6.546	
AST(U/L)	23.16±9.017	19.43±6.326	
DBIL(μmol/L)	5.628±7.012	4.325±3.246	
TBIL(μmol/L)	12.756±7.687	10.294±7.426	
GGT(U/L)	24.25±5.147	26.12±4.923	
ALP(U/L)	64.52±20.156	58.43±18.915	
After			
ALT(U/L)	196.946±208.431	18.488±9.058	
AST(U/L)	180.047±268.417	20.475±6.721	
DBIL(μmol/L)	11.231±20.754	4.513±2.013	
TBIL(μmol/L)	23.742±30.457	11.126±5.621	
GGT(U/L)	46.41±12.156	27.13±9.867	
ALP(U/L)	100.45±38.426	64.87±20.124	

3.2 PCR Amplification

If GSTM1 and GSTT1 genes are both positive (labeled as GSTM1(+)/GSTT1(+)), the DNA samples will produce 219bp, 459bp and 268bp fragments after PCR amplification. Accordingly, GSTM1(+)/GSTT1(-) genotype showed two fragments of 219bp and 268bp, GSTM1(+)/GSTT1(-) genotype has two fragments of 459bp and 268bp, while GSTM1(-)/GSTT1(-) genotype only showed 268bp fragments, as showed in Figure 1.

3.3 Relationship between GSTM1 and GSTT1 Genotypes and Susceptibility to Antituberculosis Drug-induced Hepatotoxicity

In the case group and the control group, the GSTM1 deletion genotypes accounted for 58.0% and 50.7%, respectively, the difference was not statistically significant (P>0.05), but the OR value of drug-induced hepatic impairment in GSTM1 deletion genotypes subjects is 1.32 (95% CI: 0.921 to 1.878, P>0.05), and was higher than normal controls. The GSTT1 deletion genotype accounted for 46.3% and 49.3% in these two groups, respectively, and the OR value was similar between the two groups (P>0.05). The results are shown in Table 2.

3.4 Joint analysis of GSTM1 and GSTT1 genotypes

The phenotypes of GSTM1 and GSTT1 genes were combined to analyze their relationship with ADTH. GSTM1(+)/GSTT1(+) subjects were considered as reference group. The results showed that there was no significant difference in the frequency of GSTM1(-)/GSTT1(-) genotype between the case group and the control group (p>0.05). Compared with patients with a GSTM1-deleted genotype (OR: 1.212, 95% CI: 0.762 to 1.967, P>0.05) or GSTT1-deficient genotype(OR: 0.736, 95% CI: 0.462 to 1.243, P>0.05), there was no significant increase in the risk factor for anti-tuberculous drug-induced hepatic impairment in the GSTM1(-)/GSTT1(-) genotype (OR: 1.153, 95% CI: 0.722 to 1.872, P>0.05). The results are shown in Table 3.
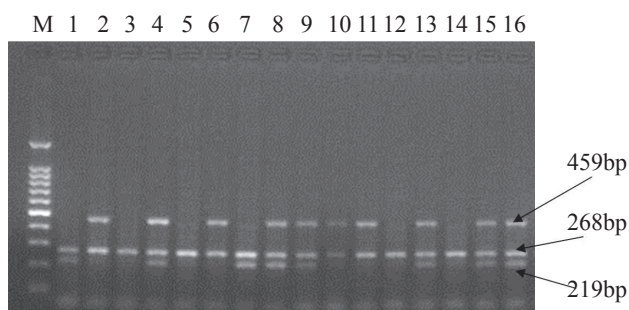
Figure 1. Agarose Gel Electrophoresis after PCR Amplification of GSTM1 and GSTT1

Table 2. Relationship between GSTM1 and GSTT1 Gene and ADTH in 600 Tuberculosis Patients

	GSTM1		GSTM1	
	+	-	+	-
case group	126	174	161	139
control group	148	152	152	148
OR(95%CI)	0.724 (0.508~1.028)	1.322 (0.921~1.878)	1.157 (0.799~1.560)	0.866 (0.618~1.217)
p	0.071		0.462	
χ2	3.251		0.541	

Table 3. Joint Analysis of GSTM1 and GSTT1 Genotypes with ATDH in 600 Tuberculosis Patients

genotype		case group	control group	OR(95%CI)	p	χ2
GSTM1	GSTT1					
+	+	72	74	1		
+	-	53	74	0.736(0.462~1.243)	0.210	1.573
-	+	92	78	1.212(0.762~1.967)	0.394	0.726
-	-	83	74	1.153(0.722~1.872)	0.537	0.382

4. Discussion

Glutathione S-transferase (GST) is an important phase II detoxification enzyme, it catalyzes the binding of the intermediate metabolites of the foreign compounds with reduced glutathione, and the resulting reduced glutathione conjugate is less toxic and easily excreted. Therefore, they have an important role in protecting cells from chemical attacks [19]. GSTM1 and GSTT1 are members of the GST family, both of which have detoxifying functions for exogenous chemicals, and its deletion homozygote could cause the loss of their corresponding enzyme activity [11].

A number of studies have been conducted to explore the relationship between GSTM1 and GSTT1 gene polymorphisms and anti-tuberculous drug-induced hepatotoxicity. In an Indian study of genetic polymorphisms of GSTM1 and GSTT1 in 33 patients with liver toxicity associated with antituberculosis drug and 33 treated TB patients without hepatotoxicity, Bidyut Roy et al[12] found that the risk for anti-tuberculosis drug-induced hepatic impairment in the GSTM1 deletion genotype was 2.13, so it might predicted the occurrence of hepatotoxicity against TB drugs. However, the difference in GSTT1 gene mutation and hepatotoxicity was not statistically significant between cases and controls. The above conclusion was supported by other researches, such as Yi-Shin Huang et al[13], but other studies have different conclusions. Virginia Leiro et al[14] conducted a retrospective study of 1,200 patients with active pulmonary tuberculosis in Spain from 1998 to 2006, they found that the GSTT1 homozygote deletion genotype was a risk factor for the development of hepatotoxicity of antituberculosis drugs (odds ratio 2.60), whereas GSTM1 was not significantly associated with hepatotoxicity. Furthermore, patients with both GSTM1 and GSTT1 deletion also had a lower risk factor for anti-tuberculosis drug-induced hepatic impairment than those with a single GSTT1 deletion. However, Studies by Sang-Heon Kim et al[15] in a Korean population showed that the GSTT1 and GSTM1 deletion variant genotypes were not associated with

the occurrence of anti-tuberculosis drug-induced hepato-toxicity, this conclusion were supported by Chatterjee S et al [20].

The results of this study show that the GSTM1 and GSTT1 gene polymorphisms are not related to the susceptibility of anti-TB drugs associated liver toxicity. The reason for the inconsistency between the findings of this study and various reports may be that there are large differences in genes from different regions and ethnicities, coupled with the small sample size of predecessors and differences in diagnostic criteria for hepatotoxicity. Moreover, anti-tuberculous drug hepatotoxicity is a multi-factor, multi-gene-related disease. When selecting cases and controls, the influence of various factors can easily be offset by each other, resulting in false negatives. Therefore, prospective randomized trials with large sample size and multivariate analysis are still needed to confirm the relationship between GSTM1 and GSTT1 and hepatotoxicity.

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Investigation and Analysis on Constitution and Syndrome Types of High-Normal Blood Pressure People in Hebei Area in the Year of 2017

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Abstract: Purpose: To investigate the distribution of population and syndrome patterns in high-normal blood pressure people in Hebei area. Methods: A total of 453 people who met the inclusion criteria were investigated using a high-normal blood pressure human mass survey questionnaire. Results: of the 453 constitution types of high-normal blood pressure patients in Hebei area, 184 were the constitution of yin-yang harmony, accounting for 40.62% of the total; 73 were the constitution of yang asthenia, accounting for 16.11% of the total; 59 were the constitution of damp-heat, accounting for 13.02% of the total; 52 were the constitution of qi asthenia, accounting for 11.48% of the total; 8 were the allergic constitution, accounting for 1.77% of the total. In addition, of their syndrome types, the syndrome of liver-fire hyperactivity were accounting for 30.24%; the syndrome of excessive phlegm-dampness were accounting for 26.71%; the syndrome of yin-deficiency and yang-predominance were accounting for 23.18%; the syndrome of deficiency of both yin and yang were accounting for 19.87%. Conclusion: Among the high-normal blood pressure people in Hebei area, the proportion of the constitution of yin-yang harmony is the highest, followed by the constitution of yang asthenia, the constitution of damp-heat, and the constitution of qi asthenia, with the lowest percentage being the allergic constitution. In addition, when it comes to the syndrome of them, the proportion of the syndrome of liver-fire hyperactivity is the highest, followed by the syndrome of yin-deficiency and yang-predominance, and the lowest is the syndrome of deficiency of both yin and yang.

1. Introduction

Normal high blood pressure is the critical state between normal blood pressure and high blood pressure, which belongs to the category of not existing diseases of TCM (traditional Chinese medicine). High-normal blood pressure has the characteristics of high detection rate and low awareness rate. However, high-normal blood pressure people have suffered different degrees of damage to the vascular endothelial function and early damage to heart, brain, kidney and other target organs. Based on the above considerations, from the perspective of "treating disease", Traditional Chinese Medicine Development Center of Hebei Province, Cangzhou Hospital of Integrated TCM-WM·Hebei, Traditional Chinese Medicine Hospital of Hebei Province and other units (hereinafter referred to as the cooperative units) unite the high-normal blood pressure population in Hebei area. The epidemiological investigation of the TCM constitution and syndrome distribution was carried out, and the correlation between high-normal people's group quality, syndrome type, risk factors, and sleep status was further analyzed, and experimental data were provided for clinical intervention. This

article examines the physical fitness of 453 eligible individuals included in the collaborative unit from March 1, 2017 to November 1, 2017. The report is now as follows:

2. Objects and Methods

2.1 Inclusion and Exclusion Criteria

Inclusion criteria: (1) The blood pressure in the consulting room meets the diagnostic criteria for high blood pressure; (2) 18≤age≤89; (3) Drugs that affect blood pressure and its rhythm cannot be taken after inclusion; (4) voluntarily accept this study and sign it Informed consent;

Exclusion criteria: (1) People with essential hypertension or secondary hypertension; (2) Severe primary diseases such as heart, brain, liver, kidney, and hematopoietic system, mental patient groups, and sleep apnea syndrome; (3)) Persons aged <18 years or older> 89; (4) Night workers; (5) Pregnant women; (6) Other clinical trial participants who are participating in the evaluation of the results of this research.

2.2 Survey Methods

The training of blood pressure measurements in the clinic is conducted to ensure the accuracy of the measured blood

pressure in the consultation room. The training on the normal high-normal blood pressure population mass survey questionnaire was conducted to ensure the consistency of the clinical questionnaire data.

2.3 Survey Objects

Based on the above-mentioned inclusion criteria, a total of 453 people aged between 18 and 89 were included in the survey from March 1, 2017 to November 1, 2017. There were 236 men and 217 women.

2.4 Survey Contents

Unified high-value blood pressure people's mass survey questionnaire was issued. The main contents of the questionnaire were related to general conditions, identification of Chinese medicine physique, syndrome type, and risk factors (Pittsburgh Sleep Quality Index).

2.5 Statistical Analysis Methods

All statistical calculations were analyzed with SPSS 21.0 statistical software. $P<0.05$ was considered statistically significant as the difference. $P>0.05$ was not statistically significant.

3. Results

3.1 General Situation

3.1.1 Distribution of Gender and Age in High-Normal Blood Pressure Population

Table 1. Distribution of Gender and Age in High-Normal Blood Pressure Population

	Male		Female	
	n	%	n	%
(18-44) Youth Age	45	9.93%	35	7.73%
(45-59) Middle Age	113	24.94%	96	21.19%
(60-89) Old Age	78	17.22%	86	18.98%

From Table 1, we can see 453 cases of this investigation, 236 men and 217 women, male to female ratio of 1.087:1. Among all age groups, the highest incidence rate was 46.13% in the middle-aged group, followed by 36.20% in the elderly group, and the youngest group had the lowest incidence rate, 17.66%. The survey data of this group suggested that the high-normal blood pressure people are most concentrated in the middle-aged age group of 45-59 years old.

3.1.2 Smoking and Drinking Population Distribution in High-Normal Blood Pressure Population

Table 2. Smoking and Drinking Population Distribution in High-Normal Blood Pressure Population

	Smoking	Drinking
n	139	116
%	30.68%	25.61%

From Table 2, we can see that of the 453 people with normal high blood pressure, 139 had smoking habits, accounting for 30.68% of the total; 116 drinking habits accounted for 25.61% of the total.

3.1.3 Distribution of Past Diseases in People with High-Normal Blood Pressure

Table 3. Distribution of Past Diseases in People with High-Normal Blood Pressure

	Diabetes Mellitus	Hyperlipidemia	Insomnia	Obesity
n	58	181	67	120
%	12.80%	39.96%	14.79%	26.49%

Table 3 shows that of the 453 patients with normal high blood pressure, in the previous history of past diseases, 181 were hyperlipidemia, accounting for 39.96% of the total; 120 were obese, accounting for 26.49% of the total; the number of people with Insomnia was 67, accounting for 14.79% of the total; and those with diabetes were 58 or 12.80% of the total.

3.2 The Distribution of Constitution and Syndrome Types of in High-Normal Blood Pressure People

3.2.1 The Distribution of Constitution Types of in High-Normal Blood Pressure People

Table 4. The Distribution of Constitution Types of in High-Normal Blood Pressure People

Constitution Types	n	%
Constitution of Yin-Yang Harmony	184	40.62%
Constitution of Qi Asthenia	52	11.48%
Constitution of Qi Stagnation	22	4.86%
Constitution of Yang Asthenia	73	16.11%
Constitution of Damp-Heat	59	13.02%
Allergic Constitution	8	1.77%
Constitution of Yin Asthenia	31	6.84%
Constitution of Phlegm-Dampness	10	2.21%
Constitution of Blood Stasis	14	3.09%

As can be seen from Table 4, among 453 patients with high-normal blood pressure, 184 were the constitution of yin-yang harmony, accounting for 40.62% of the total; 73 were the constitution of yang asthenia, accounting for 16.11% of the total; 59 were the constitution of damp-heat, accounting for 13.02% of the total; 52 were the constitution of qi asthenia, accounting for 11.48% of the total; 31 were the constitution of yin asthenia, accounting for 6.84% of the total; 22 were the constitution of qi stagnation, accounting for 4.86% of the total; 14 were the constitution of blood stasis, accounting for 3.09% of the total; 10 were the constitution of phlegm-dampness accounting for 2.21% of the total; 8 were the allergic constitution,

accounting for 1.77% of the total. The proportion of the constitution of yin-yang harmony is the highest, followed by the constitution of yang asthenia, the constitution of damp-heat, and the constitution of qi asthenia, with the lowest percentage being the allergic constitution.

3.2.2 Analysis on the Syndrome Types of High-Normal Blood Pressure People

Table 5. Analysis on the Syndrome Types of High-Normal Blood Pressure People

Distribution of Syndrome Types	n	%
Liver-fire Hyperactivity	137	30.24%
Yin-Deficiency and Yang-Predominance	105	23.18%
Excessive Phlegm-Dampness	121	26.71%
Deficiency of Both Yin and Yang	90	19.87%

As can be seen from Table 5, among 453 patients with high-normal blood pressure, 137 were the syndrome of liver-fire hyperactivity, accounting for 30.24%; 121 were the syndrome of excessive phlegm-dampness, accounting for 26.71%; 105 were the syndrome of yin-deficiency and yang-predominance, accounting for 23.18%; 90 were the syndrome of deficiency of both yin and yang, accounting for 19.87%. The proportion of the syndrome of liver-fire hyperactivity is the highest, followed by the syndrome of yin-deficiency and yang-predominance, and the lowest is the syndrome of deficiency of both yin and yang.

3.2.3 The Correlation between Constitution and Syndrome Types of High-Normal Blood Pressure People

Table 6. The Correlation between Constitution and Syndrome Types of High-Normal Blood Pressure People

	a	b	c	d	e	f	g	h	i
A	42	16	6	25	20	6	11	6	5
B	48	15	4	14	5	3	9	2	5
C	52	11	6	18	19	1	5	4	5
D	40	7	6	12	13	1	5	2	4

Notes: a, b, c, d, e, f, g, h, and i respectively represent the constitution types: Constitution of Yin-Yang Harmony, Constitution of Qi Asthenia, Constitution of Qi Stagnation, Constitution of Wang Asthenia, Constitution of Damp-Heat, Allergic Constitution, Constitution of Yin Asthenia, Constitution of Phlegm-Dampness, and Constitution of Blood Stasis;

A, B, C, and D respectively represent the syndrome types: Liver-fire Hyperactivity, Yin-Deficiency and Yang-Predominance, Excessive Phlegm-Dampness and Deficiency of Both Yin and Yang.

The syndrome types: Liver-fire Hyperactivity, Yin-Deficiency and Yang-Predominance, Excessive Phlegm-Damp-

ness and Deficiency of Both Yin and Yang: P <0.05, the differences were statistically significant, suggesting that there is a correlation between syndrome type and physical fitness.

It can be seen from Table 6 that in the syndrome of liver-fire hyperactivity, the proportion of the constitution of yin-yang harmony is the highest, accounting for 30.67%, followed by the constitution of yang asthenia, accounting for 18.25%; in the syndrome of yin-deficiency and yang-predominance, the constitution of yin-yang harmony is the highest, accounting for 45.71% , followed by the constitution of qi asthenia, accounting for 14.29%; in the syndrome of excessive phlegm-dampness, the constitution of yin-yang harmony is the highest, accounting for 42.97%, followed by the constitution of damp-heat, accounting for 15.70%; in the syndrome of deficiency of both yin and yang, T the constitution of yin-yang harmony is the highest, accounting for 44.44%, followed by the constitution of damp-heat, accounting for 14.44%.

3.3 Analysis on the Correlation between Sleep Status and Constitution and Syndrome Types of High-Normal Blood Pressure People

3.3.1 The Distribution of Sleep Status of in High-Normal Blood Pressure People

Table 7. The Distribution of Sleep Status of in High-Normal Blood Pressure People

	Basically Regular	Irregular
n	281	172
%	62.03%	37.97%

As can be seen from Table 7, among the 453 patients with high-normal blood pressure, the number of patients who have basically regular sleep status was 281, accounting for 62.03%; the number of the number of patients who have irregular sleep status was 172, accounting for 37.97%.

3.3.2 Analysis on the Correlation between Sleep Status and Constitution Types

Table 8. Analysis on the Correlation between Sleep Status and Constitution Types

	a	b	c	d	e	f	g	h	i
Irregular Sleep	57	21	10	24	33	3	8	3	13
Regular Sleep	124	30	11	48	24	4	23	7	10

Notes: a, b, c, d, e, f, g, h, and i respectively represent the constitution types: Constitution of Yin-Yang Harmony, Constitution of Qi Asthenia, Constitution of Qi Stagnation, Constitution of Wang Asthenia, Constitution of Damp-Heat, Allergic Constitution, Constitution of Yin Asthenia, Constitution of Phlegm-Dampness, and Constitution of Blood Stasis.

P <0.05, the differences were statistically significant, suggesting that there was a correlation between sleep irregularity and constitution types; P <0.05, the differences were statistically significant, suggesting that there was a correlation between sleep regularity and constitution types.

From Table 8, it can be seen that, of the people who have basically regular sleep, the proportion of the constitution of yin-yang harmony is the highest, accounting for 33.14%, followed by the constitution of damp-heat, accounting for 19.19%, while the lowest is the allergic constitution and the constitution of phlegm-dampness, accounting for 1.4%; in addition, of the people who have irregular Sleep, the proportion of the constitution of yin-yang harmony is the highest, accounting for 44.13%, followed by the constitution of yang asthenia, accounting for 17.08%, while the lowest is the allergic constitution, accounting for 1.7%.

3.3.3 Analysis on the Correlation between Sleep Status and Syndrome Types

Table 9. Analysis on the Correlation between Sleep Status and Syndrome Types

	A	B	C	D
Irregular Sleep	60	41	39	32
Regular Sleep	77	63	82	59

Notes: A, B, C, and D respectively represent the syndrome types: Liver-fire Hyperactivity, Yin-Deficiency and Yang-Predominance, Excessive Phlegm-Dampness and Deficiency of Both Yin and Yang.

P <0.05, the differences were statistically significant, suggesting that sleep irregularities are related to the syndrome types; P <0.05, the differences were statistically significant, suggesting that there is a correlation between sleep patterns and syndrome types.

From Table 9, it can be seen that, of the people who have irregular Sleep, the proportion of the syndrome of liver-fire hyperactivity is the highest, accounting for 34.88%, followed by the syndrome of yin-deficiency and yang-predominance, accounting for 23.84%, while the lowest is the syndrome of deficiency of both yin and yang, accounting for18.60%; in addition, of the people who have basically regular sleep, the proportion of the syndrome of excessive phlegm-dampness is the highest accounting for 29.18%, followed by the syndrome of liver-fire hyperactivity, accounting for27.40%, while the lowest is the syndrome of deficiency of both yin and yang, accounting for 21.00%.

4. Discussion

High-normal blood pressure is the transitional stage from normal blood pressure to hypertension, and the disease is still in its embryonic state. It belongs to the category of "occult syndromes", "Pro-disease" and "coming disease"[1]. The constitution of TCM believes that differences in constitution determine the susceptibility of individuals to diseases, and that different constitutions have certain susceptibility to the occurrence of diseases. Constitution factors are related to whether the body is affected, the tendency to disease development and the development, changes and prognosis after the disease[2]. High-normal blood pressure is a necessary stage of high blood pressure. Epidemiological investigation of the distribution of the syndromes of high-normal blood pressure people will help to understand the nature of high-normal blood pressure.

Clinical studies in the past five years have shown that there is a close relationship between high-normal blood pressure people and the type of TCM constitution. Liqun Tang et al.[3] found that the normal high blood pressure was mainly distributed in the calm-type, Qi-deficiency, and yin deficiency-type populations. There is a close relationship with qi deficiency type and yin deficiency type. And that the normal high blood pressure Chinese medicine type has its own unique performance. Hongbo Li et al.[4] investigated the prehypertension TCM constitution of community residents in Nanning and found that the prehypertensive population in Nanning had a tendency of qi deficiency and yin deficiency, and there was a rising risk of cardiovascular disease. Junhong Kong et al.[5] observed the characteristics of traditional Chinese medicine in pre-hypertension population in Changzhou City and found that the pre-hypertension risk factors were yin deficiency, blood stasis, moist heat, qi stagnation, and dampness. The crowd took corresponding physical adjustment measures. Lianhua Yin et al.[6] studied the correlation between traditional Chinese medicine constitution and arteriosclerosis in 300 prehypertensive patients and found that the prepulse patients had significantly higher bi-vessel pulse wave velocity, early changes in arterial stiffness, and hypertension. The degree of arteriosclerosis in pre-experimental patients was related to yin deficiency, phlegm and sputum blood quality. Jianxia Gu et al.[7] found that the TCM syndromes of the high-normal blood pressure group are mostly liver stagnation and spleen deficiency type, liver stagnation and fire type, and hot heat disturbance type. Chuanhua Yang et al.[8] found that the normal high blood pressure before the age of 50 was mostly irritated by liver and blood, and after the age of 60, there were more yin deficiency and impotence and yin and yang deficiency. Xi-

aoyuan Wang et al.[9] showed that the patients with normal high blood pressure were mainly in the liver, spleen, and kidney; the syndromes of traditional Chinese medicine were mostly liver and kidney yin deficiency, phlegm and blood stasis, and liver qi stagnation; Deficiency syndrome is mostly caused by liver and kidney yin deficiency.

The survey of constitution and syndrome types of 453 high-normal blood pressure patients in Hebei area revealed that: of the 453 constitution types of high-normal blood pressure patients in Hebei area, the proportion of the constitution of yin-yang harmony is the highest, followed by three biased constitution types in turn: the constitution of yang asthenia, accounting for 16.11%; the constitution of damp-heat, accounting for 13.02%; the constitution of qi asthenia, accounting for 13.02%.When it comes to their syndrome types, the proportion of the syndrome of liver-fire hyperactivity is the highest, accounting for 30.24%, followed by the syndrome of excessive phlegm-dampness, accounting for 26.71%; the syndrome of yin-deficiency and yang-predominance, accounting for 23.18%; the syndrome of deficiency of both yin and yang, accounting for 19.87%. This survey has defined the distribution law of constitution and syndrome types in the high-normal blood pressure patients in Hebei area. It will provide an important basis for future clinical intervention programs, and will also provide powerful clinical data for further popular science lectures and monographs.

With the regard to the prevention and treatment of hypertension, people are currently paying more attention to how to effectively reduce the overall risk of cardiovascular disease in patients. Therefore, the impact of various risk factors on hypertension patients has received increasing attention in recent years. China Hypertension Prevention Guidelines pointed out that high sodium, low potassium diet, overweight and obesity, drinking, mental stress and other factors are risk factors for the development of hypertension[10], Xiaohong Tang et al.[11] analyzed the normal high blood pressure of civil servants in Changsha City Investigations revealed that age, smoking, alcohol consumption, family history, blood lipids, blood glucose, and body mass index were closely related to the occurrence of high-normal blood pressure and high blood pressure. More than 10% of normal high-normal blood pressure and high blood pressure civil servants combined 3 and more than 3 risk factors. Huanhuan Zhao et al.[12] surveyed the normal high blood pressure residents in the Tianjin community and found that the body mass index, waist circumference, triglyceride, male ratio, and alcohol consumption ratio of the normal high blood pressure group in different age groups were higher than those in the normal blood pressure group. 70-year-old high-normal blood pressure residents have the phenomenon of known high blood pressure risk factors. In recent years, studies have found that insomnia is closely related to hypertension among many risk factors. Because the normal high blood pressure non-dipper population has more serious autonomic dysfunction and diurnal rhythm of melatonin secretion, resulting in non-dipper blood pressure affect brain function, so the normal high blood pressure non-dipper population than the skeleton type population Severe sleep disorders, sleep disorders become independent risk factors for non-dipper blood pressure. Jiexia Chen et al.[13] found that the resting activity and circadian rhythm of sleep arousal were changed in patients with non-dipper hypertensive patients compared with patients with dipper-type hypertension, with nocturnal activity increased, sleep benefit decreased, nocturnal awakening points and sleep rhythm. Increased fragmentation, activity and sleep may be two important factors affecting blood pressure circadian rhythm. Therefore, it is of great significance to focus on the prevention of normal high blood pressure by focusing on the sleep quality of people with normal high blood pressure, especially improving sleep quality and improving blood pressure circadian rhythm.

5. Conclusion

For the intervention of high- normal blood pressure people, we prefer simple and convenient ear acupuncture therapy. More clinical studies have shown that: auricular acupressure as a typical method of non-pharmacological intervention, is a traditional method of Chinese medicine treatment, with dredge meridians, adjust the organs, balance yin and yang, prevent health care role[14]. Ping Li[15] found that ear acupressure treatment can significantly reduce the systolic blood pressure and diastolic blood pressure in patients with Phase I of simple hypertension, so as to achieve the purpose of controlling blood pressure. The ear has a close relationship with the body's organs and meridians. It is said in "Lingshu • Kouwen" that: "The ear gathers in the place of the pulse". At the same time, modern research found that there is a close relationship between auricular specificity and biomolecular activity, including gene mutations, gene expression and regulation. The electrophysiological characteristics of the auricular point reflect the specificity of the physiological function and pathological changes of the body, and its coincidence rate has a clinical significance[16]. Xuezhong Guan et al.[17] found that in addition to the distribution and dominance of the nerve structure in the auricle, an outstanding morphological feature is the distribution of vagal and glossopharyngeal nerves. These nerves contain parasympathetic

nerve fibers. In most patients with hypertension, enhancement of sympathetic nervous system activity is the initiating factor of hypertension. The enhancement of sympathetic nerves on the heart and blood vessels is the basis for maintaining blood pressure. Auricular acupressure in the treatment of hypertension may increase the excitability of the parasympathetic nerve to suppress the abnormal sympathetic state of the sympathetic nerve, thereby reducing the blood pressure effect[18]. In the autonomic nervous system, the sympathetic nerves are excited during the day, and the nighttime nerve tension is reduced, while the parasympathetic excitability is enhanced, which is one of the factors of the change of blood pressure rhythm. Therefore, auricular acupressure from the regulation of law and blood pressure to achieve the therapeutic goals may be related to the regulation of the autonomic nervous system, but also the auricular acupressure play a role in the adjustment of liver and kidney, the role of a possible mechanism of calming the nerves.

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Application of Nursing Risk Management in Assisted Reproductive Nursing

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Abstract: Objective: discuss and analyze the application effects of nursing risk management in assisted reproductive nursing. Methodology: this research was carried out by selecting 384 IVF-ET (In-vitro fertilization and embryo transfer) patients admitted by certain hospital during February 2016 to February 2018 as research objects, who are classified into general group and research group at random by numbering all randomly, with 192 objects per group for comparing adverse event rate and nursing quality of patients in these two groups. Result: the adverse event rate of patients in research group is 4.17%, while that in general group is 10.94%, with the adverse event rate in research group lower than that in general group ($P < 0.05$); the overall scores of nursing quality in research group is (96.37±9.37), while that in general group is (79.34±8.25), with the overall score in research group significantly higher than that in general group ($P < 0.05$); the nursing satisfaction of research group and general group are 95.31% and 84.38% respectively, with the satisfaction in research group significantly higher than that in general group ($P < 0.05$). Conclusion: in assisted reproductive nursing, the application of nursing risk management plays a critical role, which can not only reduce the adverse event rate, but also effectively improve the nursing quality of patients, as well as improve the nursing satisfaction of patients, thus being proven that nursing risk management shall be widely applied in clinical nursing.

Keywords: Nursing risk management; Assisted reproduction; Application Effect

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1. Introduction

Assisted reproductive technology, also known as ART technology, is a kind of technology of manually controlling gametes, zygotes and embryos to get pregnant by employing medical technologies and methods [1]. During the assisted reproductive nursing process, patients who fail to get pregnant within a relatively long time will feel down under great mental stress [2], which may result in strained nurse-patient relationship. In order to relieve such kind of adverse nurse-patient relationship, the application of nursing risk management plays an important role during the assisted reproductive nursing process. Therefore, this research was carried out by selecting 384 IVF-ET patients admitted by certain hospital during February 2016 to February 2018 as research objects for discussing and analyzing the application effects of nursing risk management in assisted reproductive nursing, with the results reported as below.

2. Data and Method

2.1 General Data

This research was carried out by selecting 384 IVF-ET patients admitted by certain hospital during February 2016 to February 2018 as research objects, who are classified into general group and research group at random by numbering the patient admission number randomly, with 192 objects per group, under the premise that all patients signed the Informed Consent Form. Wherein, the age of patients in research group ranges from 30-37, with the average age being (31.21±0.11), infertility age ranging from 1-7, and average infertility age being (4.35±2.12). Wherein, the age of patients in general group ranges from 31-36, with the average age being (30.72±0.20), infertility age ranging from 1-5, and average infertility age being (4.27±2.34). Meanwhile, select 32 nurses of certain hospital at the same period as the research objects, and then train them accordingly. All selected nurses shall be

registered nurses, with the age ranging from 25-34 and the average age of (28.64±2.37). Compare the general information of patients in both groups, with difference free from statistical significance (P > 0.05), which are comparable.

2.2 Method

The nursing method employed for patients in general group is a kind of routine nursing, while the one employed in research group is nursing risk management. To be specific, the corresponding methods are as below: (1) Identification of nursing risks. In assisted reproductive nursing, the application of nursing risk management and risk identification is critical, which can provide evidences for formulating emergency plans. In order to carry out risk identification, it is necessary to establish the nursing risk management group firstly. Upon establishing the nursing risk management group, there is a need to select leaders and members, with 1 leader and 31 members. On this basis, formulate the corresponding nursing regulations, and organize related personnel to study for mastering the operation specifications completely. After that, carry out training for related personnel and then determine the training contents. During the training, intensive training on specialty shall be employed. Training contents mainly involve in specialty knowledge, professional skill, emergency skill and first aid knowledge. Upon completion of the training, review and examine the concrete work of related personnel, and record the cause of adverse events occurred in the nursing process. (2) Nursing risk management measures: based on identified nursing risks, analyze the arising cause by employing discussion and communication methods. At the same time, put forward different countermeasures against different reasons, such as enhancing communication with patients, and paying attention to improve the responsibility consciousness and professional levels of nurses. In a word, bring forward and improve the corresponding improvement measures against risks occurred in the nursing process. Later, continue to assess nurses, and then analyze, conclude, improve and re-apply. (3) Formulate and perfect risk management responsibility system and ensure that related nurses can improve their nursing level constantly via implementing such a system. (4) Build up the information file management system and expand the user authority for accelerating nurses to improve personal professional level constantly.

2.3 Observation Indicator

Observation indicators employed in this research mainly cover adverse event rate, nursing quality rating and nursing satisfaction. Wherein, nursing quality rating is carried out by employing the hospital-prepared scale, with assess-ment indicators mainly covering professional knowledge, skill, and service attitude and communication skills. The full score of each rating item is 100, and the higher the rating, the better the nursing quality. Furthermore, hospital-prepared nursing satisfaction questionnaire is also employed in this research for assessing the nursing satisfaction, with assessment criterion being ① strongly satisfied; ② generally satisfied; ③ unsatisfied; ④ very unsatisfied; The overall satisfaction = (① + ②) / (① + ② + ③ + ④) *100%

2.4 Statistical Treatment

Data processed via software SPSS19.0 shall be employed. Enumeration data shall be expressed in %, with $x2$ for validation; measurement data shall be expressed in $\bar{x}$±s, with t for validation. If P is < 0.05, it means the difference is of statistical significance.

3. Results

3.1 Comparison of Adverse Event rate of Patients in Both Groups

According to the research on adverse event rate of patients in both groups, we can find that there are 8 adverse events occurred in total 192 patients in research group, with the adverse event rate being 4.17%, while 21 adverse events occurred in total 192 patients in general group, with the adverse event rate being 10.94%. The data of adverse event in research group is much lower than that in general group, and it means the difference is of statistical significance (x2=6.301, P=0.001, P < 0.05).

3.2 Comparison of Nursing Quality Rating of Patients in Both Groups.

According to the research on nursing quality of patients in both groups, we can find that the overall scores of nursing quality in research group is (96.37±9.37), while that in general group is (79.34±8.25), with the overall score in research group significantly higher than that in general group. It means the difference is of statistical significance (t=7.435, P=0.006, P < 0.05).

3.3 Comparison of Nursing Satisfaction of Patients in Both Groups

According to the research on nursing satisfaction of patients in both groups, we can find that there are 183 patients being satisfied about the nursing in 192 patients in research group, with the satisfaction being 95.31%, while 162 patients being satisfied about the nursing in total 192 patients in general group, with the satisfaction being 84.38%, with the satisfaction in research group significantly higher than that in general group. It means the difference is of statistical significance (x2=12.596, P=0.000, P < 0.05).

4. Conclusion

Along with the improvement of medical level and consciousness of rights safeguarding, better requirements on nursing level and nursing quality of nurses have been put forward. Nursing risk management mainly refers to keeping patients free from impairment of biological functions and mental function, disfunction or deficit and death within the permissible range of laws and regulations under the nursing process [3]. During the assisted reproductive nursing process, adverse nurse-patient relationship is prone to occur in the nursing process considering the specificity of nursing objects, which may further result in patient-hospital disputes [4]. The main reasons shall be attributed to the more prominent existence of ethical, legal and psychological issues involved in reproductive medicine, which is also a quite controversial subject. Till now, there is no unified nursing method available for assisted reproductive nursing in China [5]. In regard to assisted reproductive nursing, different scholars have carried out study from different perspectives, such as personalized nursing, humanistic care and informatization management. By retrieving the currently available literature, we can find that there are few researches from the perspective of nursing risk management. Therefore, this research contends that the application of nursing risk management in the assisted reproductive nursing is of great significance and value. Therefore, this research was carried out by selecting 384 IVF-ET patients admitted by certain hospital during February 2016 to February 2018 as research objects for studying the application effects of nursing risk management in assisted reproductive nursing, with the results reported as below.

According to the research in this Paper, the adverse event rate of patients in research group is 4.17%, while that in general group is 10.94%, with the adverse event rate in research group lower than that in general group. It means the difference is of statistical significance ($P < 0.05$). This means that the application of nursing risk management in the assisted reproductive nursing can effectively lower the adverse event rate. According to the further analysis of adverse events based on this finding, we can find that the factors resulting in the occurrence of adverse events can be attributed to nurses, patients and hospital. Among which, nurses and hospital are the key factors, therefore, some scholars carried out research in this regard. Taking 82 patients as research objects, Huan Lv, Yuhui Zhang, Qiong Chang, et al. (2016) analyzed risk factors of nursing risk management in the assisted reproductive nursing. According to their research, the main risk factors are generated from nurses, patients, hospital and society [6]. Wherein, nurses and patients are the most important influencing factors, which is consistent with the research results in this Paper. Therefore, when applying the nursing risk management in the assisted reproductive nursing, special attention shall be paid to the training of nurses, which can be helpful for improving the professional level of nurses to a certain degree, thus lowering the adverse event rate. What's more, from the perspective of patients, when applying the nursing risk management, proper nursing methods are also employed for the adverse events caused by patients, such as psychological nursing, which can further relieve the doctor-patient relationship to a certain degree, thus lowering the adverse event rate. Furthermore, these scholars also analyzed the adverse event rate, which was, however, carried out from the perspective of season, with the result also showing that the application of nursing risk management can also lower the occurrence of adverse events. Therefore, the application of nursing risk management in the assisted reproductive nursing is of great clinical value.

Meanwhile, this research makes a comparison on nursing quality of patients in both groups, we can find that the overall scores of nursing quality in research group is (96.37±9.37), while that in general group is (79.34±8.25), with the overall score in research group significantly higher than that in general group. It means the difference is of statistical significance ($P < 0.05$). This means that the application of nursing risk management in the assisted reproductive nursing can also improve the nursing quality of patients. The nursing risk management employed in this research also carried out management of patients, such as improving the understanding of patients on ART. The advancement of patient quality will certainly boost the nursing quality significantly. This theory has been proven via research carried out in this Paper, and some scholars also carried out research in this regard. According to their research, the nursing for patients in the assisted reproductive center can enhance the understanding rate of patients on ART, which can further improve the nursing quality of patients[7]. The foregoing results also serve as the basis for further research. Some scholars also analyzed from the perspective of the rate of wrong implementation of doctors' orders, with the results showing that the nursing risk management is of great significance [8-9]. By retrieving the currently available literature, we find that researches carried out by some scholars are also helpful for the research in this Paper. According to the investigation to patients, we can find that the application of risk management nursing, compared with routine nursing, can better improve the nursing quality of patients. Therefore, the application

of nursing risk management in the assisted reproductive nursing is of great significance and value.

Finally, this research also compared the nursing satisfaction of patients of both groups. According to the results, the satisfaction on nursing of research group and general group are 95.31% and 84.38% respectively, with the satisfaction of research group much higher than that of general group. It means the difference is of statistical significance ($P < 0.05$). This means that the application of nursing risk management in the assisted reproductive nursing can also improve the nursing satisfaction of patients. This shall be attributed to the application of nursing risk management in the assisted reproductive nursing, which can lower the adverse event rate, improve the nursing quality and boost the nursing satisfaction of patients. Some scholars also carried out research in this regard, with the results consistent with the research one in this paper[10]. Therefore, the application of nursing risk management in the clinical nursing is also of great value and significance.

In conclusion, in assisted reproductive nursing, the application of nursing risk management plays a critical role, which can not only reduce the adverse event rate, but also effectively improve the nursing quality of patients, as well as improve the nursing satisfaction of patients, thus being proven that nursing risk management shall be widely applied in clinical nursing.

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One Case of Autopsy Pathological Analysis of Acute Pancreatitis Combined with Hemorrhage in Pericardial Cavity

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Abstract: Senile male, physically fit at usual, he died suddenly without any clinical symptoms. By autopsy dissection, it was found that large amount of bleeding was presented in pericardial cavity, the abdominal cavity and thoracic cavity had a small amount of hemorrhage, partial pancrea tissue had coagulation necrosis accompanied with infiltration of neutrophile granulocyte and degeneration and necrosis of liver cell accompanied with acute or chronic inflammation cell infiltration. Laboratory examination of the patient when he was alive suggested that liver function and coagulation function had obstacles, there was not any timely clinical process, and he died suddenly. Autopsy examination results suggested that acute pancreatitis caused a large quantity of bleeding in pericardial cavity, which led to cardiac tamponade and it cause acute circulation failure, which initiated cardiac arrest and then death. Coronary heart disease may exert certain facilitation effect in the death process. Patients with pancreatitis, especially the senile and pancreatitis patients with coronary artery disease, should be evaluated and prevented ahead of schedule, for those patients who had coma suddenly, it should be thought that it had possibility of combining with hemorrhage in the interior of pericardial cavity, the patient's doctor should try his or her best to reduce death rate.

Keywords: Acute pancreatitis; Hemorrhage in pericardial cavity

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1. Introduction

Acute pancreatitis could cause death due to its severe comorbidity, the most common at early stage was multiple organ function failure, and the most common at later stage was infection.[1] Of these, the symptom with high death rate was hemorrhage in abdominal cavity, those with almost 100% death rate was hemorrhage in pericardial cavity, the occurrence rate of both was relatively low, especially the latter. Those with autopsy report were even rarely seen, at present, 1 case of autopsy analysis on severe symptomatic acute pancreatitis leading to hemorrhage of pericardial cavity was reported.

2. Clinical Data

The deceased is male, 58 years old. He underwent time-choosing surgery due to "ring-like hemorrhoid", he could not answer to call when making preoperative preparation, ECG monitoring suggested cardiac arrest, blood pressure could not be measured, SPO2 could not be measured, the patient then died due to ineffective rescue.[2] When the patient was alive, he only had clinical symptoms of hemorrhoid; he had no extenuation, fatigue, no chilly, no fever, no nausea and vomit, no low fever and night sweat. He had regular physical examination annually, he was physically fit, he denied" hepatitis, tuberculosis and typhoid

fever" and other infectious disease history and contact history, no "coronary heart disease, diabetics" disease history. He denied trauma and surgical history.

3. Autopsy

3.1 Systematic Anatomy Results

The surface of the corpse showed that the development was normal; skin had no cyanosis and ecchymosis. Bilateral thoracic cavity had 300ml and 840ml light red fluid separately; there was few blood coagulation patches. Bilateral lung surface section was grey and grey white in color with soft nature. There was 900ml blood in pericardial cavity, it was flowed out in eject (Figure 1.2). Cardiac apex surface had an irregular wound in the diameter of 0.5cm, which had not been passed through left ventricle;[3] heart surface partial region was coarse. The anterior descending branch of left coronary artery, the main branch of left coronary artery, the main branch of right coronary artery and aortic arch had local atherosclerosis plaque formation, lumen cavity I-III grade narrowing. 30ml light red fluid was visible in abdominal cavity. Regions surrounding pancrea had no visible hematocele and effusion, the surface section of pancrea was light yellow in color, no hemorrhage and necrosis were observed, the nature was moderate. Liver surface section was dark red in color, the nature was moderate. Spleen surface section was dark red

in color, the nature was moderate. Brain tissue had edema; various brain chambers had no bleeding.

3.2 Pathological Examination Results

3.2.1 Pancreas (Fig 5.6.7)

Macroscopic view: The volume was slightly greater, the mass was slightly increased, and its nature was slightly harder.

Microscopic view: Pancrea tissue structure was in existence, part of the region was autocytolysis, coagulation necrosis with infiltration of large quantity of neutrophile granulocytes were observed in the local, no hemorrhage was observed.

3.2.2 Liver (Fig 3.4)

Macroscopic view: Volume was increased slightly, the surface section was dark red in color, the local was red alternated with yellow, and no hardening change was observed.

Microscopic view: Liver cell was degenerated and necrosis with dilation and congestion of blood sinus, hemorrhage as well as infiltration of large quantity of neutrophile granulocyte and monocyte as well as inflammation cells.

3.2.3 Heart

Macroscopic view: The size of heart was normal, the crevasse of heart apex surface could not be passed through heart chambers, and the surrounding region had suspicious scar formation. The section of heart muscle was grey red in color with moderate nature. The thickness of right ventricle was 0.8cm; the tunica externa had fat-hyperplasia. Anterior descending branch of left coronary artery, main branch of left coronary artery, main branch of right coronary artery and aortic artery had visible formation of atheromatous plaque; its lumen narrowing was 70%, 50%, 10% and 1% separately, no tear and interlayer formation were observed in main aortic arch.

Microscopic view: Anterior descending branch of left coronary artery, main branch of left coronary artery, main branch of right coronary artery and aortic artery had intima thickening, cavity surface fibroplasia, hyaloids formation and fibrous cap, the lower part of fibrous cap had amorphous athero-material and cholesterol crystallization deposition with calcification. Pericardium visceral vessel was dilated with congestion as well as infiltration of relatively more inflammation cells, which were mainly composed of lymphocytes and monocytes. Visceral layer of pericardium had significant congestion, scattered small lesion hemorrhage with infiltration of inflammation cells, which were mainly composed of lymphocytes and

monocytes. Tunica adventitia fat of right ventricle had hyperplasia and it was thickened and extended toward myocardial matrix, which reached 1/3-2/3 of the external layer of myocardial wall, myocardial structure was in existence, myocardial infarction was not observed, there were small-lesion shaped hemorrhage in local region.

3.2.4 Bilateral lung

Macroscopic view: the surface was smooth, which showed grey color, its nature was soft; the local nature was slightly solidified. An old scar was observed in the region below lung pleura of left lower lobe of the lung, its diameter was 1cm, and grey-white moderate-natured mass in the size of 3cm × 2cm × 2cm could be observed in region adjacent to bronchus of left lower lobe, which was fused with surrounding lymph nodes.

Microscopic view: vessel of lung interstitial tissue and alveolar wall blood capillary were dilated with congestion, power-stained edema fluid or bleeding were visible in alveolar cavity of local regions of left and right upper lung. A large quantity of large nuclear and dark stained tumor cells that were arranged in micro papilla were observed in mass adjacent to bronchus, immunohistochemical examination showed that TTF-1 (+) and there was formation of tumor embolus in vessels.
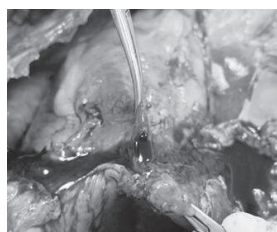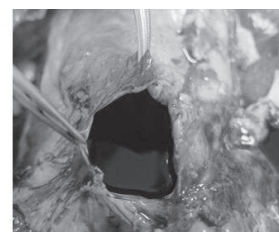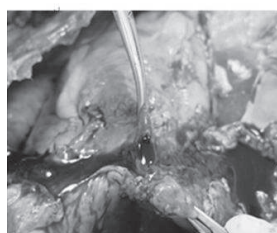

Figure 1

Figure 2
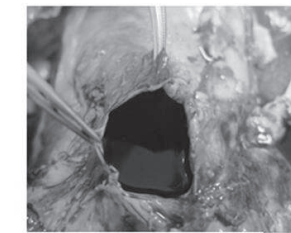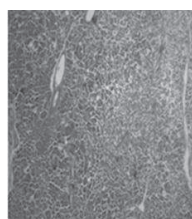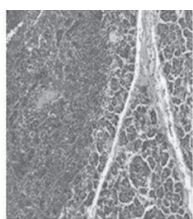

Figure 3

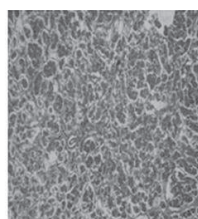**Figure 4**

| Figure 5 | Figure 6 | Figure 7 |

Fig. 1 and Fig. 2 showed that the interior of pericardium cavity had large quantity of hematocele, which was about 900ml.

Fig. 3 and Fig. 4 showed degeneration and necrosis of liver cells accompanied with dilation, congestion, hemorrhage and infiltration of large quantity of neutrophile granulocytes of blood sinus, portal area was accompanied with infiltration of lymph cells and monocyte as well as other inflammation cells.

Fig. 5, Fig. 6 and Fig. 7 showed the existence of pancrea tissue structure, partial region has autolysis, coagulation necrosis with infiltration of large quantity of neutrophile granulocyte could be observed in the local, and hemorrhage was not observed.

3.3 Laboratory Examination

At 1 day prior to his death, blood draw test results showed: ① Liver function: alanine aminotransferase (ALT) 1,472 IU/L (reference value: 0-40 IU/L), asparagus aminotransferase (AST) 635IU/L (reference value: 0-40IU/L), γ-glytamyl transferase (GGT) 120U/L (reference value:0-50U/L), Lactic dehydrogenase (LDH) 2012 U/L (reference value: 104-245 U/L), HBDH 1287Iu/L (Reference value: 72-190 IU/L). ② Coagulation function: Prothrombin time (PT) 23.1S (reference value: 9-17S), international standardized ratio (PT-INR) 2.05 (reference value:0.8-1.31S), Active partial thrombin time (APTT) 46.5 (20-40 sec), fibrinogen (FBG) 1.6g/L(reference value:2.0-4.0g/L). ③ Blood routine: White blood cell (WBC) 14.5×109/L (Reference value: 4-10×109/L), Large scale platelet ratio (P-LCR) 44.40% (Reference value:13-43%）.

3.4 Autopsy Conclusion

Combined with autopsy dissection, pathological examination, laboratory examination and clinical data analysis, it was regarded that the deceased had acute pancreatitis with large quantity blood effusion in pericardial cavity, which lead to cardiac tamponade and caused acute circulation failure, then cardiac arrest and death. Coronary heart disease may exert certain facilitation effect in death process.

4. Discussion

The deceased of this case was 58 years old, the onset of disease was acute, and he had no abdominal pain, jaundice, high fever and other symptoms when he was alive, he had sudden cardiac arrest, conscious loss, large vessel beat cease, the illness status was dangerous and he died rapidly. Autopsy dissection had found that pericardial cavity of the deceased had 900ml blood effusion, which far exceeding the 150ml diagnosis criteria of acute pericardial effusion, acute pericardial hematocele of effusion could lead to ventricle diastole suffocating, decreased cardiac output, which limiting blood returning to heart and heart beat and causing acute circulation failure as well as cardiac arrest and this is the most direct reason of death. By dissection, it was found that the deceased had relatively serious foundation disease, i.e. coronary artery artherosclerosis cardiac disease, the narrowing of anterior descending branch lumen of left coronary artery reached III grade, the narrowing of main branch lumen of left and right coronary artery also had II grade and I grade; it had artery arch artherosclerosis (atheromatous plaque), because of this, relatively more time should be spent at first sampling to find whether there was formation and break of aneurysm and interlayers, the heart itself had no break, all these were negative findings. At the same time, during sampling of lung tissue, it was found that there were swelling lymph nodes in left lung hilum, after multiple sampling and microscopic testifying, it was left lung micro-papilla type adenocarcinoma with acinous adenocarcinoma accompanied with metastasis of lung hilum and mediastinum lymph nodes, however, there was no metastasis and infiltration of pericardium. Macroscopic and microscopic observation on other organs fond that coagulation necrosis with infiltration of large quantity of neutrophile granulocytes were observed in pancrea tissue, liver cell degeneration and necrosis accompanied with dilation, congestion and hemorrhage of blood sinus as well as infiltration of large quantity of neutrophile granulocytes, portal area was accompanied with infiltration of lymph cells, monocytes as well as inflammation cells, which suggesting it had acute pancreatitis (necrosis type, no hemorrhage), acute liver necrosis. At the same time, 1 day prior to death, the laboratory examination results suggesting impairment of coagulation dysfunction, liver function impairment, it was possible that after the necrosis of pancrea tissue, large quantity of pancrea enzyme was released, which induced pericarditis and effusion by blood flow or lymph path, concurrently, it initiated the release of IL6, IL8, NO,TN-

Fαand other factors, which affected the liver function and coagulation function of organizm and thereby aggravated hemorrhage of pericardial cavity and thoracic cavity.

Severe acute pancreatitis (SAP) belongs to the special type of acute pancreatitis, which is an acute abdominal symptom with dangerous status, more comorbidity and death rate, which accounting for 10%-20% of whole acute pancreatitis. With the development of SAP surgical therapy, the curative rate was improved, however, total death rate was still as high as about 17%. The main clinical manifestations were persistent abdominal pain, jaundice, shock, high fever, breathe abnormality, consciousness change, abdominal tract hemorrhage, ascites, skin and mucosa bleeding and etc., there were few patients, especially the senile or weak patients without abdominal pain or only slight abdominal pain , which were easily to be missed in diagnosis. And senile severe pancreatitis patients themselves' organ storage function was low, often combined with one or several chronic disease, once pancrea had necrosis or infection, the illness status was usually aggravated rapidly.

Acute pancreatitis combined with fake cyst and abdominal cavity hemorrhages were relatively frequently observed in clinical practice, however, cases combined with pericardial hemorrhage was rarely encountered. Han Aijun et al[4] had found that hematocele of cardiac cavity was not coagulated but accompanied with hemolytic phenomenon; however, specific bleeding amount and pathogenesis mechanism were not reported. The bleeding amount was large in this case, it was rarely observed.

5. Conclusion

Some scholars had conducted early stage evaluation on disease cause and severity of severe acute pancreatitis, the results found that senile severe acute pancreatitis were mainly composed of gallbladder origin type or idiopathic acute pancreatitis type, furthermore, senile patients often had concurrent hypertension, coronary artery disease, diabetics and brain infarction as well as other diseases, the occurrence rate and death risk of comorbidities of whole body were higher than those with non-senile acute pancreatitis[5-8]. It is suggested that for the elderly, especially pancreatitis patients with coronary artery disease history, evaluation and prevention should be undertaken ahead of schedule, for patients who had sudden syncope, doctors should think the possibility of comorbidity with hemorrhage in pericardium cavity and attempts should be made to reduce death rate as far as possible.

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Effect of Detail Nursing Intervention on Headache and Negative Emotion in Patients with Subarachnoid Hemorrhage

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Abstract: objective: to explore the effect of detail nursing intervention on headache and negative emotion in patients with subarachnoid hemorrhage. Methods: the clinical data of 100 cases of subarachnoid hemorrhage from January 2015 to January 2017 of neurosurgery in our hospital were randomly divided into the control group and the experimental group, Detail nursing intervention in experimental group, Comparison of two groups of clinical nursing effects. Results the duration of hospitalization and headache in the experimental group were shorter than those in the control group. Fewer headaches than control group (P<0.05) Less headache than control group (p<0.05). Results: the results of SAS and SDS scores were significantly lower than those in the control group (p<0.05) after nursing in the experimental group. Conclusion: the patients with subarachnoid hemorrhage receive detailed nursing intervention; contribute to the alleviation of their headache symptoms, Relieves the patient of negative emotional problems

Keywords: Detail nursing intervention; Subarachnoid hemorrhage; Headache; Negative emotions

1. Introduction

Subarachnoid hemorrhage is a common neurosurgical disease; the main cause of the disease is the rupture of blood vessels in the brain and at the bottom of the brain. The blood enters the subarachnoid cavity to induce the corresponding symptom; the occurrence of this disease will greatly reduce the quality of life of patients. Headache is the most common type of complication in patients with subarachnoid hemorrhage, affected by disease and complication, patients are also prone to negative emotional problems such as anxiety and depression, and has adverse effects on clinical treatment and nursing care. Therefore, the enhancement of subarachnoid hemorrhage in patients with detailed nursing intervention has a positive impact on relieving headaches and negative emotions. This study analyzed the effect of detail nursing intervention on headache and negative emotion in patients with subarachnoid hemorrhage.

2. Data and Methods

2.1 General data

The clinical data of 100 patients with subarachnoid hemorrhage from January 2015 to January 2017 were analyzed retrospectively. Male 62 cases, female 38 cases, aged 42~76, average (58.4±15.3) years old, Hunt-Hess Grading: Grade I 20 cases, Grade II 40, Grade III 40 cases. Randomly divided into control group and experimental group, 50 cases in each group, two groups of patients sex, age, Hunt-Hess The grading results were not statistically significant (p>0.05).

2.2 Methods

The control group received routine care including health education, oral care, posture nursing and vital signs monitoring, the experimental group underwent detailed nursing intervention, Specific measures: first, dietary intervention. The nursing staff should develop a healthy diet plan for the patients, To high fiber, high protein, high calorie food mainly, For patients with cough symptoms, can be replaced by nasal feeding supplementary nutrition, To enhance the patient's ability to resist, Every 200ml, 4~6 times a day. Second, language function training. Nursing staff should actively communicate with patients, Instruct patient to exercise language function actively, and actively participate in social activities, for activities of interest to them, actively open their hearts, promote the patient language response, reduce the incidence of depression. Third, limb function exercise. Nursing staff to assist patients in the early stage of passive movement of upper and lower limbs, as well as joint massage and flexion and extension activities, for patients with spastic symptoms, can slowly stretch contracture muscles, prevent excessive force induced muscle or joint injury, to ensure that patients gradually recover the active exercise ability, exercise gradually, from small to large, To achieve the best results. Fourth, prevention and care of complications. For patients with benign periods, In order to reduce the incidence of recurrent bleeding or increase bleeding, the need to reduce the number of patients moving, and brisk movement, for

the patients with irritability symptoms, appropriate use of sedatives, pressure site to place air cushion or cotton pads, timing roll over, gently pat the back, reduce the incidence of lung infection. Fifth, psychological intervention. Nursing staff should monitor the mental state of patients, strengthen the psychological intervention work, prevent patients from being stimulated, and actively communicate with the patients of their interesting topic, let patients' families participate in nursing work, provide psychological support for patients, at the same time, to provide patients with comfortable and warm medical environment, so that they maintain a good psychological status, and actively cooperate with the treatment.

2.3 Observation Index

First, comparative analysis of two groups of patients after nursing, time of hospitalization, duration of headache, number of headaches and degree of headache, and other observation results, the use of visual analogue scale (VAS) to assess the patient's headache, using a 10cm long horizontal marker, painless for 0 points, severe pain of 10 points, the higher the score, The more severe the pain.

Second, using the Depression Self-Assessment scale (SDS) and the Anxiety Self-Assessment Scale (SAS) to evaluate the negative emotions at different points in the two groups, a total of 20 indicators were set, 4 were graded, the SAS score was greater than 50, and depression was the SDS score greater than 54.

2.4 Statistical Analysis

The clinical data are used SPSS17.0 software processing analysis, counting data with X2 test, measurement data Use (x±s), T test, if p<0.05, the difference is statistically significant.

3. Evaluation of Results

3.1 Headache Situation

In the experimental group, the duration of hospitalization and headache lasted shorter than the control group, the number of headaches is less than the control group, the degree of headache is lighter than the control group, the two groups have significant statistical significance (p<0.05). As shown in table 1.

3.2 Negative Emotions

There was no significant difference in the scores of SAS and SDS between the two groups before nursing (P > 0.05). The scores of SAS and SDS after the nursing in the experimental group were significantly lower than those in the control group, and the difference between the two groups was statistically significant (P < 0.05). As shown in Table 2.

Table 2. Two Groups of Patients Did Not Point to Negative Emotional Analysis at the Same Time [score, x + s]

Group	Time	SAR	SDS
Experimental group (n = 50)	Before nursing	59.45 + 5.23	60.08 + 5.28
	After care	45.23 + 4.12*	45.12 + 4.42*
Control group (n = 50)	Before nursing	59.67 + 5.83	60.11 + 5.62
	After care	51.12 + 4.63	51.18 + 4.88

Notes: Compared with the control group, * represents P < 0.05.

4. Discussion

Subarachnoid hemorrhage is a kind of brain surface or bottom cerebral vascular lesions induced by blood flow into the subarachnoid space of clinical syndrome, patients will have varying degrees of headache symptoms, and reduce the quality of life of patients, and produce all kinds of negative emotional problems. The results of medical research confirmed that patients with subarachnoid hemorrhage by ventricle. The effects of epidural and brain areas such as vascular rupture and bleeding of the blood into the subarachnoid space, resulting in changes of intracranial pressure and CSF lesions, and further increase the[1-2]. Induced headache symptoms at the same time, patients after subarachnoid hemorrhage, intracranial pressure will gradually Rise, and thus stimulate the cerebrospinal fluid, disruption of the blood cell structure, and generation of vasoactive substances and 5- induced release of serotonin and other substances, which is the main cause of subarachnoid hemorrhage[3-4]. Headache symptoms in patients with progressive headache for children is the weight of psychological trauma, easy to induce a series of physiological and psychological problems, and serious to improve the effect of treatment and prognosis of the disease. Usually, the clinical use of dehydrating agent and analgesic treatment of subarachnoid hemorrhage patients with headache, however, affected by the anxiety of patients with depression,

Table 1. Analysis of headache after nursing in two groups [X±s]

Group	Number of examples (examples)	Time of hospitalization (d)	Duration of headache (d)	Number of headache (Times)	Degree of headache (score)
Experimental group	50	19.45 + 3.15*	12.29 + 2.04*	9.78 + 2.16*	2.78 + 0.75*
Control group	50	27.12 + 4.27	19.45 + 4.15	20.45 + 3.42	6.01 + 1.04

Notes: Compared with the control group, * indicates p<0.05.

the effect of the treatment of pain is poor, therefore, the Department of Effective clinical nursing has a positive effect on the improvement of the symptoms of headache and the reduction of negative emotions in patients with[5-6].

5. Conclusion

To sum up[7-11], patients with subarachnoid hemorrhage receive detailed nursing intervention, which helps to relieve the symptoms of headache and relieve the negative emotional problems of the patients.

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Conflict of interests: Researcher A is an employee of XXX. Researcher B has received grants from XXX.

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Contribution: Researchers A and B researched literature and conceived the study. Researcher A was involved in protocol development, gaining ethical approval, patient recruitment and data analysis. Researcher B wrote the first draft of the manuscript. All authors reviewed and edited the manuscript, and approved the final version of the manuscript.

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Some examples:

- Negotiation research spans many disciplines[3,4].
- This result was later contradicted by Becker and Seligman[5].
- This effect has been widely studied[1-5,7].

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XX. References

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