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Chemical Composition and Effect of Aqueous Extract of *Alpinia Oxyphylla* Miq. on Inhibition of *Salmonella Typhi*

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Abstract: Objective: To investigate the separation and extraction process of the primary chemical constituents of *Alpinia oxyphylla* Miq. against *Salmonella typhi*, and its antibacterial efficacy, providing theoretical basis for novel gastrointestinal epidemic drugs and interventions against pathogen transmission. **Methods:** *Alpinia oxyphylla* Miq.: purified water-to-material ratio 1:5, refluxed at 100°C for 6 hours, repeated three times. *Alpinia oxyphylla* Miq. extract was concentrated by freeze-drying at a 30:1 solid-to-liquid ratio to obtain the final extract. The efficacy of the extraction method and antibacterial activity were assessed using the disk diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined to establish the minimum therapeutic thresholds. Gas chromatography-mass spectrometry (GCMS) was employed to identify the primary bioactive constituents and their relative concentrations in the *Alpinia oxyphylla* Miq. aqueous extract. **Results:** The water extract of *Alpinia oxyphylla* Miq. demonstrated potent antibacterial activity against *Salmonella typhi* ($P < 0.001$), with an inhibition zone diameter of 8.43 ± 0.11 mm, MIC of 0.25 g/ml, and MBC of 0.5 g/ml. The extraction process was established as follows: *Alpinia oxyphylla* Miq. : purified water ratio 1:5, reflux heating at 100°C for 6 hours, repeated three times, followed by freeze-drying at 30:1 concentration. This yielded an extract rich in , primarily comprising octadecanoic acid (44.27%), palmitic acid (26.18%), caproic acid (8.15%), 10-hydroxydecanoic acid (7.83%) as the main components, supplemented by 3-hydroxy-dodecanoic acid (1.78%), cis-13-eicosenoic acid (1.58%), valeric acid (1.21%), heptanoic acid (1.13%), nonanoic acid (1.06%), oleic acid (0.87%), and butyric acid (0.47%). **Conclusion:** This extraction process for novel antimicrobial compounds from *Alpinia oxyphylla* Miq. demonstrates high stability, operational feasibility, and precise composition and efficacy. It provides a practical theoretical foundation for developing novel natural medicines against infectious diseases caused by *Salmonella typhi*, while offering further insights for antimicrobial drug development.

Keywords: *Alpinia Oxyphylla* Miq; *Salmonella Typhi*; Extraction; Antibacterial Activity

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1.Introduction

1.1 Research Background and Importance

Alpinia oxyphylla Miq., also known as Yizhizi or Yizhi, is the dried mature fruit of *Alpinia oxyphylla* Miq. (Zingiberaceae), primarily produced in Guangxi, Yunnan, Hainan, Guangdong, and Fujian provinces. It ranks among the Four Great Southern Medicinal Herbs. It possesses a warm nature and pungent flavour, containing active constituents such as zingerone, kaempferol, euryzine A, euryzine B, and euryzine alcohol. It exhibits effects of warming the spleen to arrest diarrhoea and consolidating essence to reduce urination. It is frequently employed for spleen-cold diarrhoea, abdominal cold pain, excessive salivation, kidney deficiency with enuresis, frequent urination, and seminal emission with turbid discharge. Modern applications include clinical use and fundamental research in diarrhoea, Alzheimer's disease, cancer, kidney disease, and bone disorders^[1-5]. *Salmonella typhi* is one of the most prevalent serotypes within the *Salmonella* genus. As a zoonotic, foodborne pathogen affecting both humans and animals, it evades host immune clearance through immune escape mechanisms, leading to persistent infections that pose a severe threat to human health and public safety^[6-10]. While extensive research exists on chemically synthesised drugs targeting *Salmonella typhi*, these present safety concerns and high production costs. Furthermore, research into natural medicines and bioactive compounds against this pathogen remains underdeveloped, significantly limiting its prevention, control, and treatment. Consequently, exploring novel natural medicines against *Salmonella typhi* infection holds considerable significance.

1.2 Research Objectives

This study employed a common and straightforward liquid solvent heating extraction process to isolate the active components of *Alpinia oxyphylla* Miq. against *Salmonella Typhi*, followed by high-fold concentration. Chemical composition and content analysis were conducted using GCMS. Bacteriostatic activity was evaluated using the zone of inhibition as an efficacy indicator, while MIC and MBC values were determined to establish dose-response relationships. This provides a scientific basis for the development and clinical application of new drugs based on the antimicrobial efficacy of natural products.

2. Materials and Methods

2.1 Materials

Salmonella Typhi CMCC (B) 50071 (Shanghai Luwai), *Alpinia oxyphylla* Miq. (Beijing Tongrentang), 0.22 µm needle filter, McFarland turbidity tubes (Hunan Bickman), Nutrient agar medium, Nutrient broth medium (Jinan Baibo), 204178 - Digital Vernier Caliper (SYNTEK, Germany), SPX-80B Biochemical Incubator (Tianjin Saidelisi), THZ-100 Constant Temperature Shaker Incubator (Shanghai Yetuo), Agilent 7890 B-5977 Gas Chromatography-Mass Spectrometry System (Agilent Technologies, USA), RV 10 auto pro V rotary evaporator (Germany, Eka), 6 mm × 0.7 mm sterile blank antimicrobial susceptibility discs (UK, Oxoid), WP-UPS-10/20 fully automatic water purifier (Sichuan, Watpu).

2.2 Methods

2.2.1 Separation and extraction process for active constituents from *Alpinia Oxyphylla* Miq. Aqueous Extract

Alpinia oxyphylla Miq. was pulverised and sieved through an 80-mesh screen. The powder-to-purified water ratio was 1:5. The mixture was soaked at room temperature for 2 hours, Reflux heated at 100°C for 6 hours, repeated three times. Centrifuged at 10,000 rpm for 1 hour; combine supernatants and freeze-dry at a 30:1 ratio to solidify the test drug. For use, dissolve in purified water to a concentration of 1 g/ml for subsequent experiments.

2.2.2 GCMS Analysis of Bioactive Components in *Alpinia Oxyphylla* Miq. Aqueous Extract

Gas chromatography employed an HP-5MS column (30 m × 0.25 mm × 0.25 µm) with a constant flow rate of 1 mL/min using helium as carrier gas. Column temperature followed the programmed ramping schedule in Table 1. Injection port temperature was 280°C, with a 1 µL injection volume and 2:1 split ratio. Mass spectrometry employed an electron impact ionisation source with an electron energy of 70 eV, an ion source temperature of 220°C, a transfer line temperature of 280°C, and a solvent delay of 3 minutes. Data were acquired in full scan mode over the range of 10–650 amu. GCMS data were analysed using Mass Hunter (VB.07.00) software for compound identification via integration. Search parameters were: SNR (signal-to-noise ratio) 2; Sharpness threshold 25%; Absolute height threshold 500 points; Relative height 0.1%. Automatic library searching utilised the NIST 17 database with a score threshold of 60; absolute height threshold set to 100 points; relative height threshold set to 0.5%.

Table 1: Temperature-programmed conditions

Temperature ramp rate	Temperature	Hold time/min	Hold time
	40	0	0
5	100	3	15
20	300	3	28

2.2.3 Efficacy of *Alpinia Oxyphylla* Miq. Aqueous Extract in inhibiting *Salmonella typhi*

Sterile solution (concentration 1 g/ml) was obtained by passing the solid-state final product aqueous solution through a 0.22 μm needle filter twice. *Salmonella Typhi* was diluted with sterile water to a concentration of 1.5×10^7 cfu. Using a pipette, 20 μL of the bacterial suspension was dropped onto 9 cm nutrient agar plates and spread evenly. A sterile blank antibiotic susceptibility disc (6 mm diameter) was then placed on each plate. A 20 μL pipette volume of sterile drug solution was applied to fully saturate the disc for the treatment group. A purified water group was established as a control using the same method. Both groups were incubated in a 37°C incubator for 24 hours, after which the diameter of the inhibition zone was measured using calipers.

2.2.4 Dose-response relationship of *Alpinia Oxyphylla* Miq. Aqueous Extract in inhibiting *Salmonella typhi*

Dissolve 2 g of the solid final product in 2 mL sterile broth. Filter twice through a 0.22 μm needle filter. Prepare ten dilutions using a half-serial dilution method (initial drug concentration: 1 g/mL). Dilute *Salmonella typhi* in sterile water to a concentration of 1.5×10^8 cfu using sterile water. Pipette 100 μL (neglecting this minute volume) into each group to achieve a bacterial concentration of 1.5×10^7 cfu. Incubate at 37°C for 24 hours, then determine the MIC by observing turbidity. For all clear groups, mix thoroughly, then pipette 20 μL of the suspension onto 9 cm nutrient agar plates, spreading evenly. Incubate the plates at 37°C for 24 hours, then observe colony growth to determine the minimum bacterial concentration (MBC).

2.2.5 Statistical Methods

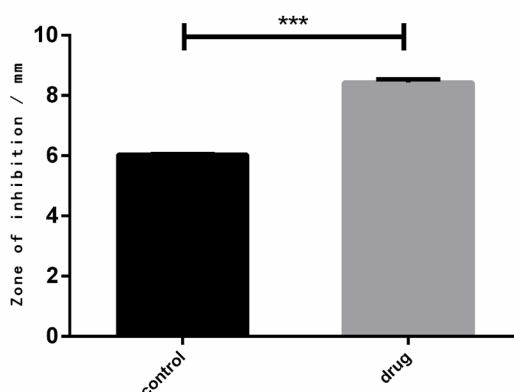
Statistical analysis was performed using GraphPad Prism 6.01 software. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Inhibitory Effect of *Alpinia Oxyphylla* Miq. Aqueous Extract on *Salmonella Typhi*

The inhibition zone diameter in the treatment group was 8.43 ± 0.11 mm, while that in the control group was 6.04 ± 0.01 mm, demonstrating a significant difference ($P < 0.001$). This indicates that the water extract of *Alpinia oxyphylla* Miq. effectively inhibits *Salmonella Typhi*, as shown in Figure 1.

Figure 1: Statistical diagram of inhibition zones (note: *** $P < 0.001$)



3.2 GCMS Analysis of Bioactive Components in Aqueous Extract of *Alpinia Oxyphylla* Miq.

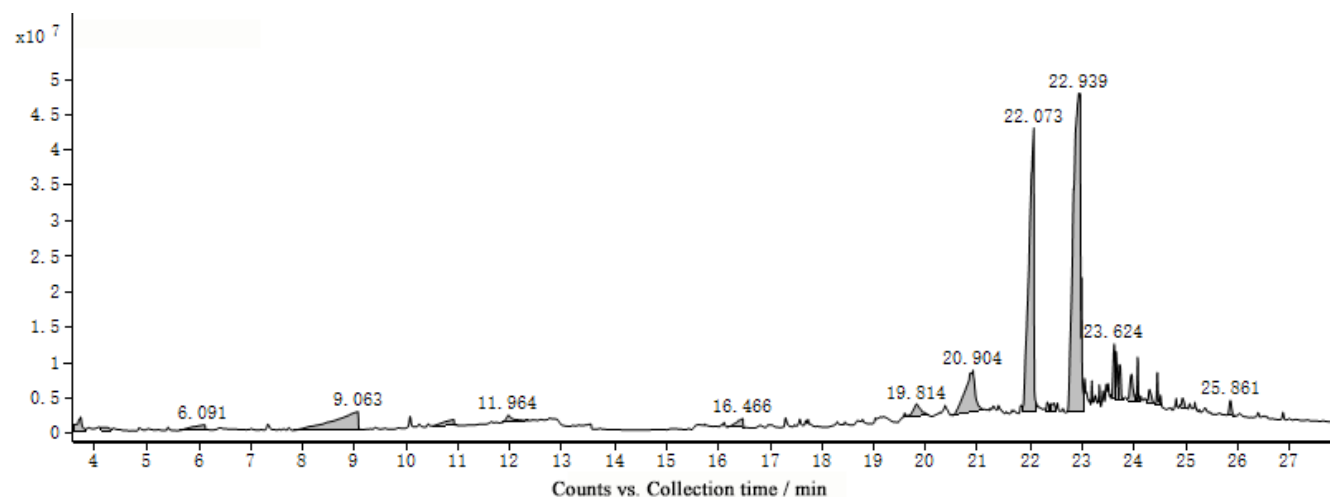
Analysis revealed four principal fatty acids: octadecanoic acid (44.27%), palmitic acid (26.18%), caproic acid (8.15%), 10-hydroxydecanoic acid (7.83%) as the four principal fatty acids, each constituting over 5% of the total. Additionally, seven minor fatty acids were identified: 3-hydroxy-dodecanoic acid (1.78%), cis-13-eicosenoic acid (1.58%), pentanoic acid (1.21%), heptanoic acid (1.13%), nonanoic acid (1.06%), oleic acid (0.87%), and butanoic acid (0.47%) as seven secondary representative organic acid components. These collectively constitute the anti-*Salmonella typhimurium* system of the water

extract of *Alpinia oxyphylla* Miq., as shown in Table 2 and Figure 2.

Table 2 : GCMS Analysis Results

Name	Molecular Formula	Retention time/min	Peak Area Percentage/%
cis-Vaccenic acid	C18H34O2	22.939	44.27
n-Hexadecanoic acid	C16H32O2	22.073	26.18
Hexanoic acid	C6H12O2	9.063	8.15
10-Hydroxydecanoic acid	C10H20O3	20.904	7.83
3-hydroxy-Dodecanoic acid	C12H24O3	19.814	1.78
cis-13-Eicosenoic acid	C20H38O2	23.66	1.58
Pentanoic acid	C5H10O2	6.091	1.21
Heptanoic acid	C7H14O2	10.903	1.13
Nonanoic acid	C9H18O2	16.466	1.06
Oleic Acid	C18H34O2	24.071	0.87
Butanoic acid	C4H8O2	4.215	0.47

Figure 2 : GCMS base peak chromatogram



3.3 Dose-response Relationship between Aqueous Extract of *Alpinia Oxyphylla* Miq. and *Salmonella Typhi*

MIC and MBC assays yielded MBC at 0.5 g/ml and MIC at 0.25 g/ml, indicating that higher concentrations of *Alpinia oxyphylla* Miq. extract are required to inhibit *Salmonella typhi*, demonstrating a clear dose-response relationship.

Conclusion

Digestive system infections severely impact human health, not only through direct tissue damage caused by pathogens but also through associated complications or comorbidities. The persistent emergence of drug-resistant pathogens and frequent outbreaks place a continuous burden on society and public finances, making the pursuit of more effective, low-toxicity, and economical therapeutic agents crucial [11-16]. Natural products and their derivatives, already successfully applied clinically, offer inherent advantages such as safety, accessibility, high efficacy, and low toxicity, making them ideally suited to address this pressing issue. Therefore, the effective isolation and extraction of antibacterial active components from these sources, coupled with the evaluation of their antimicrobial effects, can lay the theoretical foundation for traditional Chinese medicine in combating digestive system infections.

This study employed a straightforward technique of reflux extraction with water to isolate water-soluble components from *Alpinia oxyphylla* Miq.. Utilising GC-MS omics analysis, it identified a diverse array of antibacterial active constituents, primarily comprising organic acids such as octadecanoic acid, palmitic acid, hexanoic acid, and 10-hydroxydecanoic acid. Organic acid compounds, being common substances with broad sources and high safety profiles, exhibit well-established antibacterial efficacy. Their effectiveness has been demonstrated in studies on digestive system infections such as *Helicobacter pylori* and *Salmonella* [17-19]. The extraction of numerous organic acid compounds in this study provides technical assurance for the practical application of this process route. Furthermore, significant antibacterial activity against *Salmonella typhi* was observed ($P < 0.001$), with an inhibition zone diameter of 8.43 ± 0.11 mm. The minimum inhibitory concentration (MIC) was determined to be 0.25 g/ml, with MBC at 0.5 g/ml. This indicates a positive correlation between inhibitory strength and drug concentration, demonstrating the feasibility of applying this extraction process to typhoid *Salmonella* drug development and providing reference for its rational clinical application.

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Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Research Progress of Berberine in the Prevention and Treatment of Atherosclerosis

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Abstract: Atherosclerosis (AS) is the core pathological basis of Cardiovascular Disease (CVD) worldwide. Its occurrence and development involve endothelial dysfunction, lipid deposition, chronic inflammation and abnormal proliferation of smooth muscle cells. Berberine (BBR), also known as berberine, is an isoquinoline alkaloid extracted from traditional Chinese medicine such as *Coptis coptidis* and *Phelloberia angustifolia*. It has traditionally been used for antibacterial and anti-inflammatory treatment. In recent years, it has been found that it has multi-target metabolic regulation and anti-inflammatory properties, showing significant potential in the prevention and treatment of AS. This article systematically reviews the research progress of berberine in the treatment of AS by improving endothelial function, regulating lipid metabolism, inhibiting inflammatory response, regulating smooth muscle cell phenotypic transformation, and anti-oxidative stress, and discusses the current status and challenges of its clinical application.

Keywords: Berberine; Atherosclerosis; Research Progress

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1.Introduction

Arteriosclerosis is a chronic progressive disease characterized by lipid deposition, plaque formation, and luminal stenosis in the arterial intima, which is the main cause^[1] of CVD such as myocardial infarction and stroke. Studies have shown that CVD causes about 18 million deaths in the world every year, accounting for 32%^[2] of the total deaths. Statins play a positive role in reducing low-density lipoprotein cholesterol and stabilizing plaque, but their important side effects, such as elevated liver enzymes and muscle toxicity,^[3] greatly limit their clinical promotion and use. Therefore, it is of great clinical significance to find safe and effective adjuvant therapy drugs.

Berberine (molecular form C₂₀H₁₇NO₄, molecular weight 336.36), γNO₄, is a natural component in plants such as

Ranunculaceae and Berberidaceae, which is mainly used to treat intestinal infection and diarrhea^[4]. Later studies have confirmed that berberine has hypoglycemic and lipid-regulating effects^[5], and with the deepening of research, the mechanism of berberine in metabolic diseases has gradually become clear^[6]. In recent years, with the recognition of the complex pathological network of AS, the research on the intervention of berberine through multiple targets has become a hot topic. This article focuses on the pathological mechanism of AS, systematically summarizes the anti-AS effect and mechanism of berberine.

2. The main pathological mechanisms of atherosclerosis

The occurrence of atherosclerosis (AS) is a complex process involving multiple factors and interrelated pathological links. The core process of AS is endothelial dysfunction, which is manifested as endothelial cell (ECs) injury leading to reduced nitric oxide (NO) bioavailability and impaired vasodilation function, and at the same time, the expression of adhesion molecules (such as VCAM-1 and ICAM-1) is up-regulated, which promotes the adhesion and infiltration of circulating monocytes into the vascular intima. These macrophages, together with the damaged ECs themselves, take up oxidized low-density lipoprotein (ox-LDL) through scavenger receptors (such as SR-A and CD36) on their surface, leading to intracellular lipid accumulation and transformation into foam cells, forming lipid striations, which are the hallmark of early lesions. This lipid deposition environment continuously stimulates and drives the chronic inflammatory response: activated macrophages secrete large amounts of proinflammatory factors (such as TNF- α and IL-6), which further recruit more inflammatory cells and maintain the inflammatory state, while inducing increased expression of metalloproteinases (MMPs) and destroying the stable structure of the plaque. In the process, membrane in vascular smooth muscle cells (SMCs) key phenotypic transformation, by deflating, into synthetic migrated to vascular intima. It is worth noting that oxidative stress runs through the process. The excessive production of reactive oxygen species (ROS) not only directly aggravates ECs injury, promotes the oxidation of lipids (especially LDL) to ox-LDL, but also amplifies inflammatory signaling transduction^[7], forming a self-reinforcing vicious cycle that jointly promotes the initiation, progression, and finally formation of unstable plaques in AS.

3. Multiple target mechanisms of berberine intervention on atherosclerosis

3.1 Introduction to Berberine

Berberine is a naturally occurring alkaloid compound found in the roots, rhizomes, and stem bark of several plants, including *Coptis chinensis* (Goldthread) and *Berberis* species (such as Barberry). It has a long history of use in traditional Chinese and Ayurvedic medicine, primarily for treating bacterial diarrhea and gastrointestinal infections. Modern research has revealed that berberine possesses a wide range of pharmacological activities. It is now widely recognized as a multi-functional agent with demonstrated effects on cardiovascular health, metabolic regulation (including diabetes and high cholesterol), anti-inflammatory responses, and anti-cancer properties. Its ability to interact with multiple molecular pathways makes it a promising therapeutic compound for complex diseases like atherosclerosis.

3.2 Improve endothelial function and protect vascular barrier

Endothelial dysfunction is the key initiating link of atherosclerosis (AS). Studies have shown that berberine can effectively improve endothelial function through multiple pathways. On the one hand, berberine significantly enhanced the bioavailability of nitric oxide (NO), which has vasodilator and protective effects. The main mechanism is that berberine promotes the phosphorylation of eNOS at the key site (serine 1177) through the PI3K/Akt signaling pathway, thereby enhancing the enzyme activity and increasing the synthesis and release of NO. At the same time, berberine can also inhibit the uncoupling process of eNOS, reduce the production of harmful superoxide anion, and further ensure the availability^[8] of NO. Li et al.^[9] found that pretreatment of human umbilical vein endothelial cells (HUVECs) with berberine (10 μ mol/L) effectively reversed the downregulation of eNOS mRNA expression induced by oxidized low-density lipoprotein (ox-LDL) and significantly increased NO level by 32%. On the other hand, berberine could effectively inhibit the expression of inflammation-related vascular endothelial adhesion molecules. Under inflammatory stimulation, I κ B kinase (IKK) is usually activated, leading to the phosphorylation and degradation of the inhibitory protein I κ B, which allows NF- κ B to translocate into the nucleus to initiate the transcription^[10] of adhesion molecule genes such as VCAM-1 and ICAM-1. Berberine can inhibit IKK activity, reducing I

kappa B predominate phosphorylation and degradation, preventing the nf-kappa B of nuclear transfer and downstream target gene expression. Animal model experiment proved that, in the high fat diet of apolipoprotein E knockout mice (ApoE^{-/-}), giving berberine (100 mg/kg/d) intervention after 8 weeks, the aorta tissue VCAM 1 protein expression of less intervention model group significantly reduced the amount of 45%^[11].

3.3 Regulation of lipid metabolism and reduction of ox-LDL deposition

Disorders of lipid metabolism, especially hypercholesterolemia, are the core risk factors that promote the development of atherosclerosis (AS). Studies have confirmed that berberine can play a regulatory role by intervening multiple key links of lipid metabolism. In terms of source regulation, berberine can effectively inhibit the synthesis^[12] of fatty acid and triglyceride (TG) in the liver. Berberine inhibited the activity of its downstream target acetyl-coa carboxylase (ACC) through enhancing the phosphorylation of AMPK α at ser108 in A dose-dependent manner. This cascade of activation leads to A decrease in malonyl-coa production, which ultimately inhibits fatty acid synthase (FASN) expression and reduces lipid deposition^[13] in the liver.

Berberine can significantly up-regulate the expression levels of ABCA1 and ABCG1 in macrophages, effectively promote the efflux of intracellular cholesterol to HDL particles, thereby reducing the formation^[14] of foam cells. Zhang et al.^[14] observed that treatment of RAW264.7 macrophages with 20 μ mol/L berberine resulted in a 2.1-fold increase in ABCA1 mRNA expression and a significant 38% reduction in intracellular total cholesterol content.

Berberine can selectively inhibit the activity of some strains of intestinal flora involved in bile acid unconjugation, reduce the production of secondary bile acids, and promote the reabsorption of primary bile acids in the hepato-intestinal circulation. This activates the farnesoid X receptor (FXR) signaling pathway in the liver, which in turn inhibits the liver's own cholesterol synthesis^[16]. Notably, the abundance of beneficial mucoid Akkermansia muciniphila in the gut was significantly increased^[17] after berberine intervention. The enrichment of Akkermansia muciniphila can strengthen the intestinal mucosal barrier function (in part due to the short-chain fatty acid SCFAs produced by it) and reduce the release of harmful substances such AS endotoxin into the blood circulation, which can induce the production of inflammatory factors in the liver and indirectly promote the process of AS.

3.4 Inhibit chronic inflammation and block the inflammatory cascade

Chronic inflammation is the core pathological process that drives the initiation, progression and even plaque instability of atherosclerosis (AS). Studies have shown that berberine can effectively inhibit the synthesis and release of key pro-inflammatory factors. In macrophages and vascular smooth muscle cells, berberine can significantly reduce the mRNA transcription level and protein expression of TNF- α , IL-6, IL-1 β and other inflammatory mediators. On the one hand, berberine can inhibit the activation of nuclear factor- κ B (NF- κ B) pathway (such as reducing the phosphorylation of I κ B α inhibitor protein, thereby hindering the nuclear translocation and transcriptional activation of NF- κ B dimer), and on the other hand, it can reduce the phosphorylation level^[18] of mitogen-activated protein kinase (MAPK) pathway members (such as p38 and ERK1/2). It was found that in the ApoE^{-/-} mice model of atherosclerosis, berberine intervention significantly reduced the serum TNF- α concentration by 52% and the aortic IL-6 mRNA expression level by 40%^[19].

Berberine can inhibit the maturation and differentiation of dendritic cells (DCs) and weaken their antigen presentation ability, thereby reducing excessive immune activation. At the same time, berberine can also promote the differentiation and expansion of regulatory T cells (Tregs) with anti-inflammatory function and enhance the immune tolerance^[20] of the body. In addition, berberine can also inhibit the formation of neutrophil extracellular traps (NETs), an important link in the inflammatory cascade of neutrophils, which helps to reduce NETS-mediated vascular endothelial injury and local inflammation amplification^[21].

Berberine significantly down-regulated the mRNA and protein expression of matrix metalloproteinases, especially MMP-2 and MMP-9, induced by inflammation, mainly by inhibiting the NF- κ B pathway as described above, while upregulating the expression of its endogenous inhibitor, tissue inhibitors of metalloproteinases. The regulation of MMP/TIMP balance is beneficial to protect the integrity of collagen and other components in the fibrous cap of plaque and enhance the stability^[22] of plaque. In support of this mechanism, human aortic smooth muscle cells (HASMCs) treated with 50 μ mol/L berberine

showed that the up-regulation of MMP-9 mRNA induced by tumor necrosis factor- α (TNF- α) stimulation was effectively inhibited by 60%^[23].

3.5 Regulation of phenotypic transformation of smooth muscle cells and inhibition of plaque progression

The transformation of smooth muscle cells (SMCs) from a contractile type that maintains vascular tone to a synthetic type with high proliferation and migration ability is a key pathological step that drives the enlargement of atherosclerotic plaques. Platelet-derived growth factor (PDGF) plays an important role in the phenotypic transformation, proliferation and migration of SMCs through the activation of the downstream PI3K/Akt and MAPK/ERK1/2 signaling pathways. Berberine can directly inhibit the tyrosine phosphorylation of PDGF receptor (PDGFR), thus significantly interfere with the activation of the receptor, and finally block the transmission of its downstream signaling cascade, which creates conditions^[24] for SMCs to maintain a relatively quiescent phenotype. Berberine can significantly increase the expression levels of key cyclin-dependent kinase inhibitors such as p21 and p27, effectively arrest the cell cycle progression (especially the transition from G0/G1 phase to S phase), arrest the cell cycle progression in the less active phase, and finally inhibit the pathological expansion^[25] of SMCs. In the rabbit model of atherosclerosis, treatment with berberine (50 mg/kg/ day for 4 weeks) resulted in a 48%^[26] reduction in proliferating cell nuclear antigen (PCNA) positive SMCs proliferation index in the aortic intima.

3.6 Anti-oxidative stress and reduction of oxidative damage

Excessive oxidative stress directly damages vascular endothelial cells (ECs), accelerates the oxidation of low-density lipoprotein (LDL) and amplifies the inflammatory cascade, which plays a complex role in the process of atherosclerosis (AS). Studies have shown that berberine significantly activates the antioxidant defense pathway of nuclear factor erythroid 2-related factor 2 (Nrf2) in cells. The results showed that berberine (20 μ mol/L) significantly increased the mRNA expression level of HO-1 and effectively reduced the intracellular ROS load^[27,28] by 50%. At the same time, the isoquinoline ring conjugated system and quaternary ammonium group in the molecular structure of berberine can effectively provide electrons, quickly neutralize reactive oxygen species such as superoxide anion and hydroxyl radicals, and play an immediate antioxidant protection role^[29] in the cellular microenvironment.

4. Clinical study of berberine in the prevention and treatment of atherosclerosis

4.1 Efficacy of intervention alone or combined with statins

A number of randomized controlled trials have confirmed that berberine monotherapy or combination therapy can significantly improve the pathological indicators related to AS. A clinical trial in patients with hyperlipidemia and carotid plaque showed that oral berberine 1500 mg (500 mg thrice daily) for 12 months significantly reduced serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, as well as carotid intima-media thickness (IMT). It is worth noting that the curative effect with atorvastatin (20 mg/day) standard treatment group effect, prompt berberine has with statins is quite stable^[30] plaques.

Further study in type 2 diabetic patients with AS showed that after 16 weeks of treatment with berberine combined with metformin, serum oxidized low-density lipoprotein (ox-LDL) levels were significantly reduced, and endothelial function was significantly improved as measured by brachial flow-mediated vasodilation (FMD). This finding confirmed the synergistic intervention value^[31] of berberine and metformin on the progression of diabetes-related AS.

4.2 Safety and tolerability

Berberine has good clinical safety, and the common adverse reactions are mild gastrointestinal discomfort (such as diarrhea and nausea), with an incidence of about 5%-10%, and most of them are transient^[32]. No serious adverse events^[33] caused by significant interactions were found when berberine was used in combination with existing AS treatment drugs (such as statins and antiplatelet drugs). However, it should be noted that berberine may affect the metabolism of some drugs, and relevant indicators^[34] need to be monitored when combined with AS in clinical practice.

5. Challenges and prospects

Although berberine has shown unique multi-target intervention advantages in the prevention and treatment of atherosclerosis

(AS), its clinical translation still faces key challenges. The primary constraint is its inherent low bioavailability. After oral administration, berberine is susceptible to the metabolism of the intestinal first-pass effect, resulting in a systemic bioavailability of only about 1%-3%^[35], significantly restricting its therapeutic potential. In order to break through this bottleneck, researchers are actively exploring new delivery strategies, including the use of nanoparticles such as liposomes or polymer micelles, the design of prodrug molecules such as berberine-oleate complex, and the combination of probiotics to improve the intestinal absorption environment^[36]. Among them, the relative bioavailability of berberine-oleic acid nanoparticles in the mouse model has been increased by 4.2-fold^[37] compared with the free drug, showing significant technical prospects. Secondly, the dose optimization for clinical application needs to be clarified urgently. However, long-term high doses (>1000 mg/d) may increase the risk^[38] of gastrointestinal adverse reactions. Therefore, it is urgent to establish an accurate dose-response relationship and safety boundary through large-scale and long-term studies. Thirdly, the extensive effects of berberine on multiple cell populations, such as vascular endothelial cells, macrophages, and smooth muscle cells, constitute the basis of the therapeutic effect, but also increase the difficulty of mechanism analysis. In the future, it is necessary to integrate cutting-edge technologies such as single-cell transcriptomics and spatial metabolomics to fine characterize the cell type-specific signaling network and provide a theoretical foundation for the development of intervention strategies based on precise regulation of pathways.

6. Conclusions

Berberine has shown significant clinical application potential by improving endothelial function, regulating lipid metabolism, inhibiting inflammatory response, regulating SMCs phenotypic transformation and anti-oxidative stress. Although the problems of bioavailability and dosage optimization still need to be solved, with the progress of preparation technology and the in-depth study of mechanism, berberine is expected to become an important adjuvant drug for the prevention and treatment of AS and provide a new strategy for the comprehensive management of CVD.

7. Contributions

Zhang P: Responsible for literature retrieval, data extraction, full-text writing. Xu JL and J X: Participated in literature screening and verification of key data, and assisted in analyzing controversial points. Z W : Guided the selection of topics and framework design, reviewed and validated the scientific rigor and logic of the full text, and assumed ultimate responsibility for the paper.

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Research Status of the Mechanism of Action of Danshen Dropping Pills on Diabetic Retinopathy

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Abstract: Diabetic retinopathy (DR) is one of the common complications in patients with diabetes, which seriously affects the patient's vision and quality of life. With the increase in the number of patients with diabetes, the prevention and treatment of DR has become an urgent clinical problem to be solved. In recent years, Danshen dripping pills, as a traditional Chinese medicine preparation, have received widespread attention for its unique pharmacological effects. Studies have shown that Danshen Dropping Pills has a significant regulatory effect on retinal vascular damage, oxidative stress, inflammatory response and cell apoptosis. Current research results show that Danshen Dropping Pills can reduce retinal damage and improve the vision of diabetic patients through multiple mechanisms. However, in-depth research on its specific mechanism of action is still needed to better understand its potential application in the prevention and treatment of DR. This article aims to explore the mechanism of action of Danshen Dropping Pills in diabetic retinopathy by reviewing relevant literature and provide a theoretical basis for clinical application.

Keywords: Danshen Dropping Pills; Diabetic Retinopathy; Mechanism of Action; Oxidative Stress; Inflammatory Response

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1.Introduction

Diabetic retinopathy (DR) is the most common eye complication of diabetes. As the incidence of diabetes increases globally, the incidence of DR is also increasing year by year. The main pathological characteristics of DR include retinal microvascular damage and neovascularization, which may eventually lead to severe visual impairment or even blindness^[1]. In recent years, Salvia miltiorrhiza dropping pills, a traditional Chinese medicine preparation made from Salvia miltiorrhiza extract, have attracted attention for its microcirculation improvement, antioxidant and anti-inflammatory effects. Studies have shown that Danshen Dropping Pills show significant efficacy in the treatment of DR, especially in reducing retinal damage and improving vision^[2]. This article will focus on exploring the mechanism of action of Danshen Dropping Pills in diabetic retinopathy, with a view to providing new ideas for clinical treatment.

The occurrence of diabetic retinopathy is closely related to multiple factors, including oxidative stress caused by hyperglycemia, chronic inflammatory response, and microvascular dysfunction^[3]. These factors work together to cause damage to retinal endothelial cells and neurons, thereby affecting vision^[4]. In this context, Danshen Dropping Pills may play an important role in the treatment of DR by resisting oxidative stress and inflammatory responses through its active ingredients. Studies have shown that the main active ingredients in Salvia miltiorrhiza, such as tanshinone and

salvianolic acid, have significant antioxidant and anti-inflammatory effects and can effectively reduce oxidative damage and inflammatory response in the retina, thereby improving the pathological changes of DR^[5].

In recent years, more and more studies have focused on the mechanism of action of Danshen Dropping Pills on DR. Studies have found that Danshen Dropping Pills may exert its therapeutic effect by regulating multiple signaling pathways in the retina, especially pathways related to oxidative stress and inflammation^[4, 6]. In addition, Danshen Dropping Pills may also reduce the pathological damage of DR by improving microcirculation and promoting the regeneration of retinal blood vessels^[7]. In summary, in-depth study of the mechanism of action of Danshen Dropping Pills in diabetic retinopathy will provide important basis for its clinical application and point the way for future research directions.

2.Main body

2.1 The main components and pharmacological effects of Danshen dripping pills

2.1.1 Effect of tanshinone

Tanshinone is one of the main active ingredients in Danshen dripping pills and has significant pharmacological effects. Studies have shown that tanshinone can exert its biological activity through multiple mechanisms, including antioxidant, anti-inflammatory and anti-fibrotic effects. Specifically, tanshinone can inhibit oxidative stress and reduce intracellular reactive oxygen species (ROS) levels, thereby protecting cells from damage^[8]. In addition, tanshinone has also been found to regulate multiple signaling pathways, such as the PI3K/Akt and MAPK pathways, which play an important role in cell proliferation and survival^[9]. In the study of diabetic retinopathy, tanshinone is believed to improve retinal microcirculation and reduce retinal damage, thus having potential therapeutic effects on diabetic retinopathy^[10].

2.1.2 Role of flavonoids

Flavonoids are another important component in Danshen dripping pills and have a wide range of biological activities. Studies have shown that flavonoids can exert their protective effects through mechanisms such as anti-oxidation, anti-inflammation and improving blood circulation. For example, flavonoids can significantly reduce the level of inflammatory factors and inhibit the release of cytokines such as TNF- α and IL-6, thereby reducing the inflammatory response^[11]. In addition, flavonoids have also been shown to promote the function of vascular endothelial cells and improve blood vessel relaxation, which is particularly important for the treatment of diabetic retinopathy^[12]. By regulating endothelial function and inhibiting platelet aggregation, flavonoids help improve microcirculation, thereby protecting retinal nerve cells^[13].

2.1.3 Contribution of other active ingredients

In addition to tanshinone and flavonoids, Danshen dripping pills also contain a variety of other active ingredients, such as water-soluble phenolic acid compounds (such as danshenic acid) and polysaccharides. These ingredients work together to enhance the overall efficacy of Danshen dripping pills. Studies have shown that water-soluble phenolic acid compounds have good antioxidant and anti-inflammatory effects, and can improve the pathological state of diabetes-related complications by inhibiting oxidative stress and inflammatory reactions^[14]. In addition, the polysaccharide components in Danshen dripping pills are also believed to enhance the body's immune function and promote cell repair, which in turn plays an auxiliary role in the prevention and treatment of diabetic retinopathy^[15]. The synergistic effect of these ingredients makes Danshen dripping pills show good prospects in the treatment of diabetic retinopathy.

2.2 Effect of Danshen Pills on retinal vascular injury

2.2.1 Improving microcirculation

As a traditional Chinese medicine preparation, Danshen dripping pills have significant effects on improving microcirculation. The occurrence of diabetic retinopathy is closely related to microcirculation disorders. Improvement of microcirculation can effectively reduce the occurrence of retinal ischemia and hypoxia, thereby reducing retinal damage. Studies have shown that Danshen dripping pills can promote blood flow and improve microcirculation by dilating blood vessels and reducing blood viscosity. This improvement not only helps increase the oxygen supply to the retina, but also promotes the clearance of metabolic products, thereby reducing retinal damage and pathological processes. In addition, Danshen dripping pills may further improve microcirculation and enhance the stability and function of retinal blood vessels by regulating the function of endothelial cells^[16].

2.2.2 Promote the recovery of endothelial cell function

Endothelial cells play a vital role in maintaining blood vessel integrity and function, and hyperglycemia caused by diabetes can lead to impairment of endothelial cell function. The application of Danshen dripping pills can promote the functional recovery of endothelial cells and improve their living environment. Studies have found that Danshen dripping pills can reduce endothelial cell damage caused by diabetes through antioxidant and anti-inflammatory mechanisms, promote cell proliferation and migration, thereby enhancing blood vessel repair capabilities. In addition, Danshen dripping pills may also regulate relevant signal pathways, promote the functional recovery of endothelial cells, improve blood vessel permeability and reactivity, and further reduce the occurrence and development of retinopathy^[17]. The study of these mechanisms provides important theoretical basis and clinical guidance for the treatment of diabetic retinopathy with Danshen dripping pills.

2.3 Effect of Danshen Dripping Pills on Oxidative Stress

2.3.1 Antioxidant mechanism

As a traditional Chinese medicine preparation, Danshen dripping pills are mainly composed of tanshinone and Danshen polysaccharide, which has significant antioxidant effects. Studies have shown that Danshen dripping pills can enhance the expression of intracellular antioxidant enzymes by activating the Nrf2/ARE signaling pathway, thereby improving the antioxidant capacity of cells. This mechanism reduces the production of nitric oxide (NO) by inhibiting the expression of inducible nitric oxide synthase (iNOS), thereby reducing the level of oxidative stress and reducing cell damage. In addition, Danshen dripping pills can also directly scavenge free radicals, block free radical chain reactions, and reduce the incidence of oxidative damage^[18]. This multi-target antioxidant mechanism makes Danshen dripping pills show good prospects in the treatment of diseases related to oxidative stress.

2.3.2 Protection against oxidative damage

In multiple studies, Danshen dripping pills have been shown to effectively protect cells from oxidative damage. For example, Danshen dripping pills can significantly improve the survival rate of HepG2 cells in a hydrogen peroxide (H₂O₂)-induced oxidative damage model and reduce intracellular reactive oxygen species (ROS) levels. This protective effect is closely related to its promotion of the activity of antioxidant enzymes such as the superoxide dismutase SOD and glutathione peroxidase GPx^[19]. In addition, Danshen dripping pills also improve mitochondrial function and reduce intracellular lipid peroxidation, further reducing oxidative damage. These research results show that Danshen dripping pills have important clinical application value in the prevention and treatment of diseases related to oxidative stress such as diabetic retinopathy^[20].

2.4 Regulation of Danshen Dripping Pills on Inflammatory Response

2.4.1 Inhibition of inflammatory factors

The role of Danshen dripping pills in regulating inflammatory reactions is mainly reflected in the inhibition of multiple inflammatory factors. Studies have shown that Danshen dripping pills can significantly reduce the levels of multiple pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF- α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6). These factors play a key role in the pathogenesis of diabetic retinopathy, and their over-expression can lead to inflammation and damage to the retina^[21]. By inhibiting the expression of these inflammatory factors, Danshen dripping pills can reduce the inflammatory response of the retina, thereby protecting the function and structure of retinal cells. In addition, Danshen dripping pills have also been found to further reduce the production of inflammatory factors by inhibiting the activation of the nuclear factor κ B (NF- κ B) signaling pathway^[22]. The study of this mechanism provides a theoretical basis for the application of Danshen dripping pills in diabetic retinopathy.

2.4.2 Regulation of immune cells

The regulatory effect of Danshen dripping pills on immune cells is also an important part of its anti-inflammatory effect. Studies have found that Danshen dripping pills can regulate the functions of macrophages and lymphocytes and promote their transformation into an anti-inflammatory phenotype. For example, Danshen dripping pills can promote the polarization of M2-type macrophages, which are known for their anti-inflammatory and tissue repair properties^[23]. In addition, Danshen dripping pills can also inhibit the activation of M1 type macrophages and reduce the pro-inflammatory factors they secrete, thereby reducing the level of systemic inflammation. This regulation of immune cells not only helps reduce the

inflammatory response in diabetic retinopathy, but may also promote retinal repair and regeneration by improving the local microenvironment^[21]. Therefore, the role of Danshen dripping pills in regulating the function of immune cells provides a new perspective and possible treatment strategies for their treatment of diabetic retinopathy.

2.5 Effect of Danshen dripping pills on apoptosis

2.5.1 Regulation of apoptosis signaling pathways

Danshen dripping pills have shown a significant regulatory effect on apoptosis signaling pathways in the treatment of diabetic retinopathy. Studies have shown that Danshen dripping pills can exert their anti-apoptosis effect by regulating multiple signaling pathways such as PI3K/Akt, STAT, TGF- β and Fas. Specifically, the PI3K/Akt signaling pathway plays a key role in cell survival and apoptosis. Danshen dripping pills activate this pathway to promote cell survival and inhibit apoptosis^[24]. In addition, Danshen dripping pills also inhibit the expression of apoptosis related proteins such as Bax and Caspase-3 and enhance the expression of Bcl-2, thereby reducing the degree of apoptosis^[25]. This regulatory mechanism is not only obvious in retinal cells, but has also been verified in many other cell types, indicating that Danshen dripping pills have extensive cytoprotective effects.

2.5.2 Improvement in cell survival rate

The use of Danshen dripping pills significantly improved cell survival, especially in diabetic retinopathy models. Studies have shown that Danshen dripping pills can effectively reduce the apoptosis rate and promote cell proliferation and survival. This phenomenon is closely related to its regulation of apoptosis signaling pathways. Danshen dripping pills inhibit the expression of apoptosis related factors and enhance the activity of intracellular anti-apoptosis factors, thereby improving cell survival^[26]. In the experiment, the survival rate of cells treated with Danshen dripping pills was significantly higher than that of the control group. This result provided theoretical support for its clinical application in diabetic retinopathy. In addition, Danshen dripping pills may further promote cell survival and reduce pathological damage by improving the metabolic status of cells and enhancing antioxidant capacity^[25]. These research results provide important experimental basis for the treatment of diabetic retinopathy with Danshen dripping pills.

3. Discussion

The application of Danshen dripping pills in the treatment of diabetic retinopathy (DR) is gradually becoming a research hotspot. This article reviews how Danshen dripping pills can effectively slow down the progression of DR by improving retinal microcirculation, anti-oxidation, anti-inflammation and regulating apoptosis. The interaction of these mechanisms provides an important basis for our understanding of the therapeutic potential of Danshen Pills.

However, although the current research results show that Danshen dripping pills have good prospects in the treatment of DR, the safety and effectiveness of clinical applications still need further in-depth discussions. There may be certain differences in results between different studies, which reflects the influence of factors such as study design, sample selection and evaluation criteria. Therefore, future research should focus more on standardized clinical trial design to better compare the results of different studies and ensure that the most effective treatment options are provided for diabetic patients.

At the same time, researchers should also pay attention to the combined application of Danshen dripping pills with other anti-diabetic drugs to explore their synergistic effects. Comprehensively considering the views and findings of various studies and reasonably balancing different treatment strategies will help improve the management level of DR. In addition, long-term follow-up studies are also extremely important to evaluate the long-term effects and safety of Danshen Pills in different patient groups.

4. Conclusion

Danshen dripping pills have shown good application potential in the treatment of diabetic retinopathy, but more reliable data support is needed to fully understand its clinical role. Future research should not only focus on the mechanism of the drug itself, but should also be closely integrated with clinical practice to bring more scientific and effective treatment options to diabetic patients.

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Conflict of Interests

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Parathyroid Cyst Presenting as a Cervical Mass: A Case Report and Updated Review of the Literature

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Abstract: Background: Parathyroid cysts (PCs) are rare lesions that often resemble other cervical cystic conditions, making preoperative diagnosis challenging, especially in resource-limited settings. **Methods:** We report a case of a parathyroid cyst diagnosed and treated at Chongqing University Fuling Hospital in 2020. Clinical presentation, imaging, cytological features, pathological findings, differential diagnosis and management strategies were analyzed in conjunction with an updated review of the literature. **Conclusion:** PCs represent a rare subset of parathyroid lesions and are most often non-functioning. Their imaging and cytological characteristics frequently resemble those of thyroid cysts, which may lead to misdiagnosis. Measurement of parathyroid hormone levels in aspirated cyst fluid is essential when a PC is suspected. Surgical excision remains the definitive treatment. This case underscores the diagnostic challenges and highlights the importance of heightened clinical awareness, particularly in primary and rural healthcare settings.

Keywords: Parathyroid Cyst; Cervical Cystic Lesion; Diagnosis; Benign Lesion

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1.Introduction

Parathyroid cysts (PCs) represent an uncommon subset of parathyroid lesions characterized by the accumulation of serous or proteinaceous fluid within the cyst cavity, first described in 1880 and with the earliest documented surgical excision reported in 1905^[1]. A comprehensive meta-analysis of all documented parathyroid cyst cases published between 1905 and 2016 identified 359 patients, revealing a female predominance and indicating cervical swelling as the most frequently reported presenting symptom^[2]. These lesions are conventionally categorized into functioning and non-functioning types, the vast majority are non-functioning and are identified incidentally during routine cervical or thyroid imaging or surgical exploration, with no biochemical evidence of primary hyperparathyroidism^[3]. Conversely, functioning PCs are typically observed in older male individuals and present with overt clinical and laboratory manifestations of primary hyperparathyroidism. Their cystic fluid contains markedly elevated concentrations of parathyroid hormone (PTH), and cyst rupture may precipitate an acute parathyroid crisis^[4].

Accurate preoperative identification of PCs remains challenging. On fine-needle aspiration cytology (FNAC), non-function-

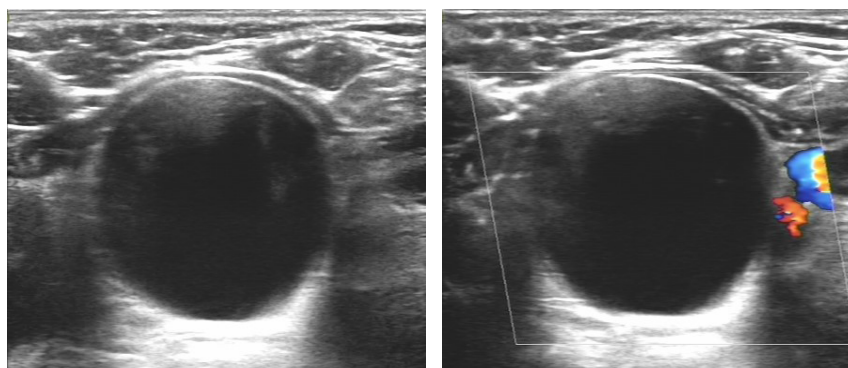
ing PCs often mimic cystic thyroid nodules because cytological specimens typically lack recognizable parathyroid elements. This diagnostic ambiguity is less evident in functioning PCs, in which hypercalcemia and elevated serum PTH levels prompt targeted endocrine evaluation and help establish a definitive diagnosis^[5]. Although the overall prognosis of PCs is excellent and long-term outcomes are generally favorable, accurate diagnosis and meticulous procedural handling are essential. Radiologists and surgeons must exercise particular caution during evaluation and intervention, as accurate recognition and appropriate management are often needed to achieve optimal therapeutic outcomes. Herein, we describe a case of a non-functioning parathyroid cyst diagnosed in a rural hospital in China and provide a review of the current literature. This report aims to enhance awareness among community-level clinicians and radiologists and to highlight the diagnostic challenges and clinical implications associated with this uncommon entity.

2.Case Presentation

A 46-year-old female patient was admitted to the Department of Breast and Thyroid Surgery of our hospital on March 25, 2020, due to a one-week history of an anterior neck mass. One week prior, she palpated a walnut-sized mass in the anterior cervical region, which did not move with swallowing. The mass was non-tender and showed no ulceration. She denied fever, night sweats, weight loss, palpitations, personality changes, or appetite alterations, and reported no hoarseness or choking when drinking. She also denied bone pain, kidney stones, or any history of neck irradiation.

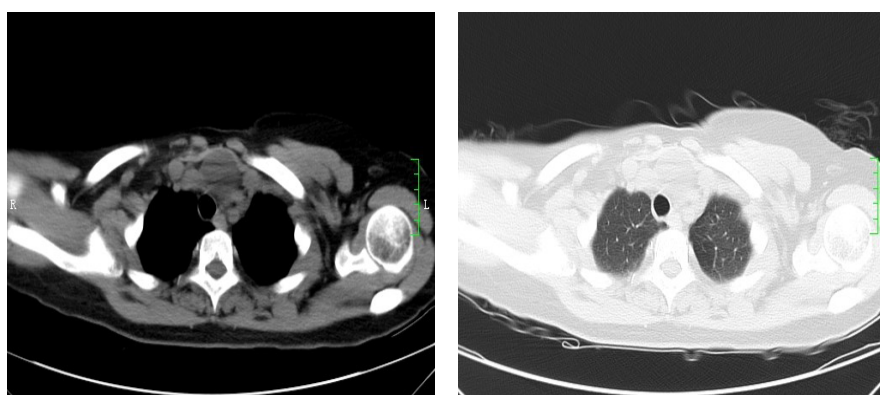
On admission, physical examination revealed mild swelling in the anterior neck and a relatively large palpable mass with medium firmness, clear borders, and good mobility. Outpatient ultrasonography showed an anechoic mass measuring 33.3 mm × 30.5 mm located in the midline anterior neck above the sternal notch, between the bilateral common carotid arteries. The lesion had a regular shape, well-defined margins, good sound transmission, and slightly irregular inner walls. Color Doppler Flow Imaging (CDFI) detected no obvious blood flow signals within the cyst or along the cyst wall (Figure 1).

Figure 1. A well-defined cystic mass located above the sternal notch in the anterior cervical region. CDFI shows no obvious blood flow signals within the cyst or along the cyst wall.



Contrast-enhanced cervical CT revealed a cystic, water-density lesion measuring approximately 34.4 mm × 31.7 mm located in the lower portion of the left thyroid lobe, with no enhancement on contrast imaging (Figure 2).

Figure 2. A cystic, water-density lesion located in the lower portion of the left thyroid lobe, with no enhancement observed on contrast-enhanced imaging.



The patient was admitted to our hospital for further evaluation and treatment. Preoperative routine examinations after admission revealed no significant abnormalities. On March 27, the patient underwent cervical exploration and excision of the neck mass under general anesthesia. Intraoperative findings showed that the mass had medium firmness and was predominantly cystic, measuring approximately 3.0 cm × 2.0 cm. The capsule was intact, the boundaries were well-defined, and there was no invasion of surrounding tissues. Postoperative pathological examination indicated a benign cystic lesion in the neck, with partial parathyroid tissue identified in the cyst wall (Figure 3).

Figure 3. A benign cystic lesion in the neck, with focal parathyroid tissue identified within the cyst wall. Bar=100um.



3. Discussion

Parathyroid cysts constitute a rare form of parathyroid pathology, accounting for approximately 0.8%–3.41% of parathyroid lesions and an estimated 0.075% prevalence in the general population. Although most commonly encountered in individuals in the fourth to fifth decades of life, occurrences in adolescents have also been documented. The present case provides additional insight into the clinical spectrum of PCs, particularly within the setting of a resource-limited rural hospital in China and contributes to the understanding of their presentation and management.

Our patient, a middle-aged woman, aligns with the demographic characteristics described in prior studies. The cyst in this case was relatively small and non-functioning, consistent with benign clinical behavior. Existing literature reports a mean cyst diameter of 4.81 ± 2.88 cm, with documented sizes ranging from 0.5 to 15 cm^[2, 6]. Cysts smaller than 2.5 cm are frequently identified incidentally during imaging examinations or cervical surgical procedures. In this instance, the lesion was self-detected by the patient, suggesting that even modestly sized cysts may become clinically apparent depending on their anatomical location^[7].

The pathogenesis of PCs remains incompletely elucidated. Several hypotheses have been proposed, including derivation from embryologic remnants of the third or fourth branchial pouches, coalescence of pre-existing microscopic parathyroid microcysts, retention of glandular secretions, persistence of vestigial Kursteiner canals from fetal development, and cystic degeneration within pre-existing parathyroid adenomas^[8]. Increasing reports have described parathyroid adenomas undergoing cystic degeneration and presenting as PCs^[7]. Therefore, researchers propose that cystic degeneration of parathyroid adenomas should be added to the etiological mechanisms as an additional potential contributing factor^[9].

Although parathyroid cysts (PCs) are infrequently encountered in routine clinical practice, they should be included in the differential diagnosis of cystic lesions of the neck and superior mediastinum^[10]. When radiologic findings raise suspicion for a parathyroid origin and malignancy has been reasonably excluded, fine-needle aspiration with measurement of PTH levels in the aspirated fluid is particularly informative, as it assists both in excluding other potential etiologies and in establishing a provisional diagnosis of a PC^[11]. Surgical excision is widely regarded as the definitive treatment for cystic parathyroid lesions, a consensus supported by accumulating evidence in the literature^[12]. In clinical practice, evaluation of serum calcium and phosphorus concentrations, combined with diagnostic aspiration to confirm parathyroid origin and assess for possible malignancy, is recommended prior to proceeding with operative management^[13].

This report is subject to the inherent limitations of a single case description. The observations may not be generalizable to the broader PC population, and the absence of long-term follow-up constrains conclusions regarding recurrence risk and

long-term outcomes. Nevertheless, the case underscores the importance of recognizing this uncommon entity, particularly in primary care and community hospital settings where diagnostic resources may be limited.

4. Conclusion

Due to the limited availability of basic research and large-scale retrospective analyses, the pathogenesis of PCs remains poorly defined and continues to warrant further investigation. Nonetheless, the growing number of published reports has progressively enhanced clinical awareness of this entity. By presenting a confirmed case from our institution and reviewing the current literature, we aim to further improve diagnostic accuracy and therapeutic decision-making practicing in rural healthcare settings in China.

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Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Thyrolipoma: A Case Report and Review of the Literature

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Abstract: Background: Thyrolipoma is a rare benign thyroid neoplasm composed of mature adipocytes within a follicular adenoma. Most lesions are asymptomatic, though some may enlarge and compress adjacent structures. **Methods:** We report a 30-year-old man with an incidentally detected thyroid nodule. Ultrasound showed a well-circumscribed, hyperechoic lesion, and shear-wave elastography indicated medium to high stiffness. The patient underwent right hemithyroidectomy, and histopathology confirmed thyrolipoma. **Conclusion:** Surgical excision is curative, with excellent long-term outcomes and no reported malignant transformation. Advanced imaging can aid preoperative assessment, and regular follow-up is recommended to monitor for recurrence or new lesions.

Keywords: Adipose Tissue; Thyrolipoma; Neck Swelling

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1.Introduction

Fatty tissue is not normally present within the thyroid gland. Fat-containing thyroid lesions can be classified as neoplastic or non-neoplastic. Thyrolipomatosis is extremely rare and is characterized by diffuse infiltration of adipose tissue throughout otherwise normal thyroid parenchyma, with very few cases reported, whereas thyrolipoma, also termed as adenolipoma or thyroid hamartoma, represents the most common neoplastic type and is defined by the presence of mature adipocytes interspersed within a follicular adenoma^[1]. Although most lesions are small and asymptomatic, some may enlarge to the extent that they compress adjacent cervical structures, resulting in dyspnea or dysphagia^[2, 3]. Notably, exceptionally large papillary thyroid carcinoma associated with diffuse thyroid lipomatosis cases has also been documented^[4, 5].

The pathogenesis of fat-containing thyroid lesions remains uncertain. Proposed mechanisms include developmental aberrations occurring during thyroid capsule formation and hypoxia-driven metaplastic transformation of fibroblasts^[2]. In addition, loss of succinate dehydrogenase subunit B (SDHB) expression and large SDHB gene deletions have been documented in both follicular and adipocytic components, suggesting an underlying disturbance in lipid metabolic pathways^[6].

The diagnosis of these lesions relies on an integrated assessment that includes clinical findings, imaging characteristics, and histopathological confirmation. Because thyrolipoma is uncommon, radiologists may be unfamiliar with its features and may fail to include it in the differential diagnosis. Nonetheless, timely recognition of this entity is essential for guiding appropriate management and ensuring favorable patient outcomes, a consideration that is especially relevant in resource-limited or rural

clinical settings. This case report aims to provide additional clinical insight and to summarize the existing literature on this rare lesion.

2.Case Presentation

A 30-year-old man was referred to our institution after a thyroid nodule was incidentally detected during routine health screening 10 days earlier. The patient was asymptomatic except for the palpable neck mass. He denied exophthalmos, fever, night sweats, weight loss, palpitations, or changes in appetite or personality. No hoarseness, dysphagia, or choking episodes were reported.

Physical examination revealed no facial edema. The neck was supple with the trachea in the midline, and a localized bulge was observed on the right side of the neck. Palpation identified a smooth, well-defined mass measuring approximately 2 cm × 5 cm, with medium firmness, no tenderness, and good mobility. The mass moved superiorly with swallowing. No palpable cervical lymphadenopathy was noted.

Ultrasound examination demonstrated mildly heterogeneous thyroid parenchyma with increased vascularity. A slightly hyperechoic, homogeneous lesion measuring 50.8 mm × 20.1 mm × 24.9 mm was detected in the right thyroid lobe. The lesion exhibited smooth borders, a regular shape, a longitudinal-to-transverse ratio <1, no calcifications, a uniform peripheral halo, and no posterior acoustic attenuation. Color Doppler flow imaging showed scant internal blood flow (Figure 1). Elastography indicated medium to high stiffness (Figure 2). The lesion was classified as TI-RADS category 3.

Figure 1 A slightly hyperechoic mass in the right thyroid lobe, with minimal internal vascularity detected on Doppler imaging.

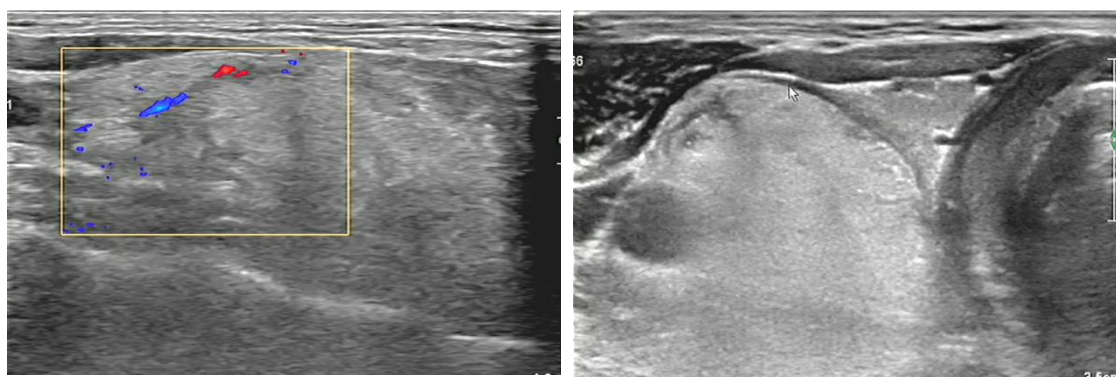
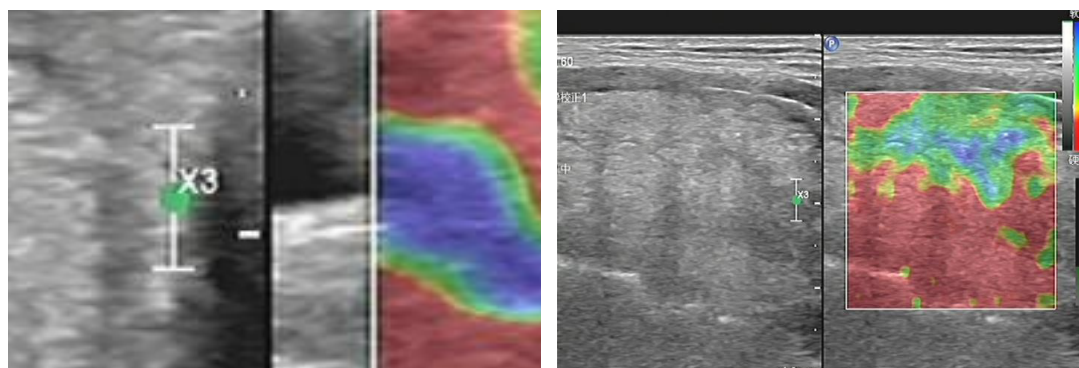


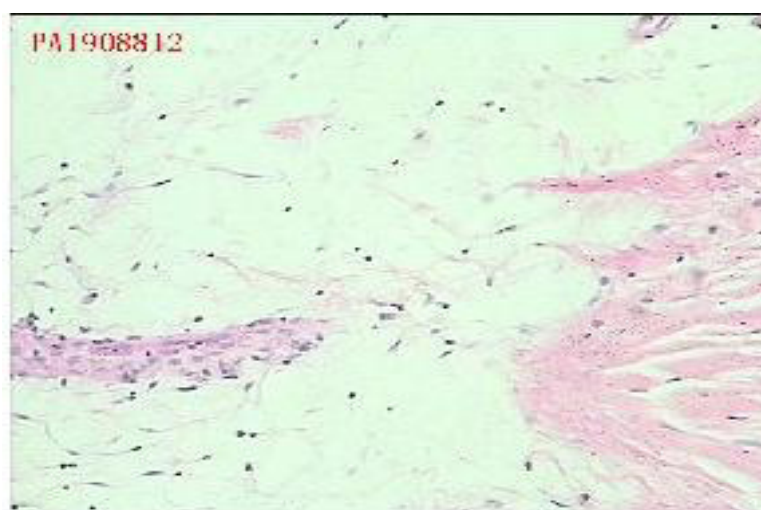
Figure 2 Shear-wave elastography demonstrating medium-to-high stiffness of the lesion.



Given the patient and family's preference for surgical management and the absence of contraindications in preoperative evaluations, a right hemithyroidectomy with exploration of the right recurrent laryngeal nerve was performed under general anesthesia. Intraoperatively, the right lobe was enlarged and appeared gray-red in color. Sectioning revealed a well-circumscribed yellow mass with a greasy texture and a clear boundary from the adjacent thyroid parenchyma.

Histopathological examination demonstrated abundant mature adipocytes within the lesion, confirming the diagnosis of thyroid lipoma (Figure 3). Postoperative recovery proceeded without any notable complications.

Figure 3 Histopathological examination showing abundant mature adipocytes within the lesion.



3. Discussion

Clinically, thyrolipoma presents similarly to other benign thyroid nodules. Most cases manifest as progressive neck enlargement or localized compressive symptoms, while thyroid function generally remains within the normal range. This entity can occur across a broad age spectrum, including in pediatric patients^[7]. In our case, the patient was a middle-aged man in whom a neck mass was incidentally detected during a routine physical examination, with no associated symptoms. Radiological evaluation revealed a well-circumscribed lesion without evidence of compression of adjacent structures.

Treatment of thyrolipoma disease typically consists of surgical excision, which is considered curative, and long-term outcomes are overwhelmingly favorable, with no documented instances of malignant transformation. For our patient, the cervical mass was incidentally detected during a routine physical examination. Following confirmation of the diagnosis, a hemithyroidectomy was promptly performed to achieve definitive management and to mitigate the risk of future enlargement or symptomatic compression. Several published cases have reported patients remaining asymptomatic for more than a decade after surgery; in one report, a woman remained symptom-free even 24 years following hemithyroidectomy^[8]. Our patient has not experienced any recurrence to date and will continue to undergo regular surveillance. Nevertheless, extended follow-up is advisable to detect any potential recurrence or the emergence of new lesions.

Ultrasound echogenicity of thyroid lesions generally correlates with their fat content, with low-fat lesions appearing hypoechoic and high-fat lesions hyperechoic. In the present case, the lesion was hyperechoic on two-dimensional ultrasound, exhibiting relatively homogeneous internal echoes, well-defined margins, a uniform peripheral halo, and no posterior acoustic attenuation. Reports on contrast-enhanced ultrasound or elastography of thyroid lipomas remain extremely limited^[9]. Here, we employed shear-wave elastography, providing novel imaging evidence regarding the elasticity characteristics of thyrolipoma. With the advancement of ultrasound technologies and the accumulation of reported cases, clinical recognition and understanding of this rare entity are progressively improving.

In conclusion, thyrolipoma is an important differential diagnosis to consider in cases of thyroid nodules, the need for surgery in these situations must be re-evaluated through additional research using cytological criteria.

Statements & Declarations

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Conflict of Interests

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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A Case of Spinal Cord Injury After Aortic Dissection Aneurysm Rupture Treated by Warm Acupuncture and Traditional Chinese Medicine

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Abstract: Spinal cord injury caused by aortic dissection aneurysm rupture is one of the common complications after aortic dissection rupture. This patient developed paraplegia due to gas and blood injury, stasis of arteries and arteries obstruction after aortic dissection aneurysm rupture. In order to treat the disease, the tutor from warm Yang tong Du, qi invigorate blood, kidney fill essence legislation, to the pathogenesis of the key as the breakthrough point, mainly with acupuncture, with traditional Chinese medicine treatment, in order to make qi and blood smooth operation, the essence of each other, promote the spinal cord and du repair. After half a year of treatment, the patient's lower limb motor and sensory functions returned to normal, and the bowel and urine functions returned to normal. The patient's daily life was significantly improved.

Spinal cord injury caused by aortic dissection aneurysm rupture is one of the common complications after aortic dissection rupture^[1]. The main clinical manifestations were limb motor sensory dysfunction, bladder and rectal dysfunction. At present, it is considered that the main causes may be related to the embolism of cardiopulmonary bypass, low perfusion, spinal cord ischemia and hypoxia, post-ischemic reperfusion injury and systemic inflammatory response caused by cardiopulmonary bypass^{[2][3]}. At present, there are few reports on the treatment of the disease with acupuncture and moxibustion of traditional Chinese medicine. The author followed her mentor Professor He Xingwei to cure 1 case of the disease mainly with warm acupuncture and moxibustion combined with traditional Chinese medicine, which is reported as follows.

Keywords: After Aortic Dissection Aneurysm Rupture; Spinal Cord Injury; Warm Acupuncture; Traditional Chinese Medicine

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1.The medical record

Patient, Moumou Li, female, 45 years old, office worker. On October 19, 2020, the patient suddenly had severe chest and back pain without obvious inducement, and was sent to a hospital in Nanchang for emergency treatment. Thoracic and abdominal CTA examination showed that aortic dissection aneurysm was ruptured, so "total aortic arch artificial vascular replacement combined with stent elephantia surgery combined with partial ascending aorta resection combined with artificial vascular replacement + aortic valvuloplasty" was performed under general anesthesia and cardiopulmonary bypass. After the operation, there were symptoms such as unstable blood pressure, arrhythmia, chest pain and chest tightness, accompanied by paralysis of both lower limbs, loss of pain, temperature and touch below the xiphoid plane, and dysfunction of bladder

and rectum. After symptomatic support treatment such as analgesia, hypotension and heart rate control, the hospital left the hospital after stable vital signs. The patient was admitted to the fourth department of acupuncture and moxibustion in our hospital on 2020-11-03 for acupuncture rehabilitation. Physical examination: the face and limbs are slightly swollen, the neck is slightly stiff, and about 10cm surgical wound can be seen at the sternum. The muscle strength of both lower limbs was level 1+, the big toe of both feet could be slightly flexed and extended, and the muscle bundles of both lower limbs vibrated occasionally. Pain, touch, temperature and position sensation decreased significantly below the chest 7 plane. The knee tendon and Achilles tendon reflexes were significantly weakened without pathological signs.

Diagnosis: Western medicine diagnosis: ① spinal cord injury; ② hypoproteinemia; ③ Aortic dissection rupture (after implantation); ④ Neurogenic bladder; ⑤ Neurogenic intestine; ⑥ Hypertension grade 3. TCM diagnosis: ① flaccidity (kidney qi deficiency, blood stasis syndrome); ② Difficulty in urination ; ③ Constipation; ④ The wind is dizzy.

Treatment: Warm Yang tong Du, qi huoxue, kidney fill essence.

Acupuncture acupoint selection: main acupoint: supine acupoint selection: Tianshu, Zhongwan, Jianli, Guiguai, DaJu, Shuidao, Qihai, Guan Yuan, Zhongji, Qihai, Liangqiu, Fengshi, Yinling quan, Yangling quan, Zusanli, Fenglong, Zulinqi, Taichong, Zhigou, Houxi, Shenmen, Danzhong. Lateral decubitus point selection: Jiaji point (chest 5-waist 5), Zhiyang, Shenshu, Dachang Shu, Yaoyangguan, Mingmen, Zhibian, Huiyang, Huantiao, Weizhong. (Except the abdominal and Du meridian points, all the other points were bilateral.)

Method of operation: Patients were taken supine position and lateral position acupuncture treatment. The acupoint skin was disinfected with 75% alcohol and needled with a 1.5-inch millimeter needle. The acupuncture angle and depth of various points are acupuncture according to the “meridian and acupoint science” in the textbook of the 12th Five Year Plan. In Zusanli, Qihai and Guanyuan, the method of lifting and inserting was adopted, and in Shenshu, the method of twisting and turning was adopted, and the needle at each point was about 1 minute. The rest of the acupoints are flat reinforcing flat purging technique, medium stimulation. Each time and each group of acupoints were kept for 30 minutes, once a day. The acupoints in supine position were first acupuncture and moxibustion. After treatment, the acupoints in lateral decubitus position were taken for acupuncture and moxibustion. In each position, AI Tiao warm acupuncture was applied immediately after the needle was inserted in the abdomen, Guanyuan, Qihai, Zusanli, or in the back, waist Yangguan, Mingmen, Zhiyang, etc. During warm moxibustion with moxa sticks (moxa sticks are fixed on moxa sticks suspension frame or operated manually, moxa sticks must be close to the needle handle or facing the acupoints, and the heat shall be subject to local flushing and the patient can tolerate), the ash of moxa sticks shall be removed every 3 ~ 5 minutes to prevent the ash from falling off and scalding the patient's skin. For each position, moxibustion was applied for 30 minutes, once a day.

Traditional Chinese medicine decoction: Prepared Astragalus membranaceus 20g, Angelica angelica 10g, Ligusticum chuanqi 10g, cooked ground 10g, fried Radix paeoniae alba 10g, Radix reed 15g, Lu Xiangcao 10g, Plantain plantain 10g, Codonopsis pilosula 15g, Poria cocos 15g, fried Atractylodes atractylodes alba 15g, Euphorbia officinalis 10g, Herba epimedii 10g, Cornus officinalis 15g, prepared glycyrrhiza glycyrrhiza 5g, Parsnip 10g, schisandra chinensis 10g, Cistanche 10g, tortoise board 10g. Take it in water, one dose a day, and warm it twice in the morning and evening (after taking it for 1 month, the patient refused to continue taking it because of fear of bitter medicine).

Due to the previous history of hypertension for 4 years, during the treatment period, nifedipine controlled-release tablets were used to control blood pressure, metoprolol succinate to control heart rate, Mecobalamin tablets to nourish nerves, and limb rehabilitation training was used to promote recovery.

2.Course of treatment: 6 times / week, 4 weeks as a course of treatment.

Treatment results: After 2 weeks of treatment, the proximal muscle strength of both lower limbs was grade 3, the distal muscle strength was grade 3, the muscle tension was low, and the sense of pain and temperature decreased. Urination can be solved by itself, but the control force is poor; After 4 weeks, the muscle strength of the left lower limb was 3 +, and the muscle strength of the right lower limb was grade 3. The pain and temperature of both lower limbs decreased. You can help stand. Urinate once every 2-4 hours. Occasionally, you have poor control when you are nervous and constipation; After 8 weeks, the left lower limb muscle strength was 4 + and the right lower limb muscle strength was 4. The pain and temperature

sensation of both lower limbs were improved, and the knee and ankle reflexes of both lower limbs were weakened. Urinate once every 3-4 hours, urinate clear and long, can endure for 3-5 minutes, stool control is fair, occasionally constipation, need to open plug dew to help discharge. After 12 weeks, the left lower limb muscle strength was grade 5 and the right lower limb muscle strength was grade 4 +, the pain and temperature sensation of both lower limbs were improved, and the knee and ankle reflexes of both lower limbs were weakened. The function of defecation and defecation is almost normal, with occasional dry defecation.

After half a year of treatment, the patient's lower limb motor and sensory functions were nearly normal. The muscle strength of the left lower limb was grade 5, and the muscle strength of the right lower limb was grade 5 -. The pain and temperature sensation of both lower limbs were close to normal. The defecation function returned to normal.

3. Comments

Aortic dissection aneurysm is caused by aortic intima tear, which enters the aortic middle membrane through the intima rupture opening and forms a dissection hematoma, which extends along the aortic wall to the surrounding area and causes severe acute and critical cardiovascular disease in both true and false cavities, also known as aortic dissection or aortic separation or aortic dissection hematoma^{[4][5]}. Aortic dissection rupture is often life-threatening. At present, surgical treatment is the most direct and important effective treatment, but it is often accompanied by complications such as spinal cord injury.

Paraplegia caused by spinal cord injury belongs to the category of "impotence disease" in traditional Chinese medicine. Its onset is due to exogenous warm poison, damp heat evil, internal emotional injury, diet injury, congenital deficiency, improper sexual intercourse, trauma and exposure to poisons, resulting in the loss of Qi, blood and semen in the five internal organs, resulting in the loss of maintenance of muscles, muscles and veins, which is called flaccid syndrome^[6]. The patient suffered from the injury of Qi and blood and obstruction of due to the superintendent tract the operation of aortic dissection aneurysm. On the one hand, the movement of Yang Qi in the governor's pulse is blocked, the meridians are blocked, the Yang Qi cannot go down, and the meridians and veins are out of nourishment, which can lead to adverse activities of both lower limbs; At the same time, the operation of the brain marrow divine machine needs the nourishment and transportation of the essence of Yang qi. When Yang qi is deficient, the essence is insufficient, and the vein is blocked, it will lead to the abnormal operation of the brain marrow divine machine. The brain marrow divine machine can not freely control the limb motor sensory function, and the limb motor sensory function can also be abnormal. In addition, the dysfunction of Yang Qi in the governor's pulse and the abnormal operation of the divine machine in the brain can lead to the loss of bladder gasification, the abnormal transportation of bladder water and fluid, and then accompanied by diseases and syndromes such as amenorrhea and urinary incontinence. On the other hand, the obstruction of the operation of the governor vessel can lead to the abnormal distribution of the essence of Qi and blood, the homology of Qi and blood, the biochemical operation of the essence of Qi and blood, and the inability to nourish the meridians and veins, which is also an important factor in the occurrence of impotence.

In view of the key pathogenesis of this disease, when from the warm Yang tong Du, yiqi huoxue, kidney fill essence legislation, to make qi and blood smooth operation, the essence of each other, and promote the repair of spinal cord and governor vessel. Therefore, the tutor pays attention to warm acupuncture combined with traditional Chinese medicine in the treatment of this disease. On the one hand, it can nourish the meridians and veins by Tonifying the essence of Qi and blood; By dredging the governor vessel and warming Yang Qi, it can not only nourish the meridians and veins, but also promote the normalization of the operation of the brain marrow divine machine, so as to restore the motor and sensory functions of the limbs dominated by the brain marrow divine machine, as well as the functions of bladder and rectum. Consequently, selecting Jiaji point near the governor vessel can dredge the local Qi and blood of the governor vessel; Taking Shenshu, Yaoyangguan, Mingmen, Zhiyang, Shenshu, Zhibian, Huiyang and Weizhong points to dredge the governor's pulse, tonify the kidney and fill the lean marrow, so as to normalize the metaplasia and operation of the essence of Qi, blood and body fluid, and then replenish the governor's pulse, which is conducive to the recovery of impotence syndrome^{[7][8]}. Following the principle of "treating impotence only takes the channel of Yangming", it takes Danzhong, Jianli, Zhongwan, Guanyuan, Zhongji, Qihai, Tianshu, Huilai, Liangqiu, Zusanli, Fenglong, Sanyinjiao, Xuehai and other points to replenish the essence of Qi and blood and dredge meridians. Select Yinling quan, Yangling quan, Huantiao, Taichong, zulinqi, Fengshi and other acupoints near to

dredge the vein. Moxa stick warm needle moxibustion is added at Yaoyangguan, Zusanli, Qihai and other points to replenish qi and Yang, dredge meridians, promote the operation of Yang, restore the normal operation of divine machine, and realize the recovery of motor sensory function of both lower limbs and bladder and rectum function. Traditional Chinese medicine selects drugs with the effects of Yi Qi Wen Yang, dredging veins, tonifying kidney and essence, diuresis and so on, so as to promote the repair of spinal cord.

This medical record is based on the legislation of Warming Yang and dredging governor, supplementing qi and activating blood, tonifying kidney and filling essence. It is treated with Warming Acupuncture and moxibustion combined with traditional Chinese medicine to warm yang, dredge governor vessels, replenish the essence of Qi and blood, and promote the normal operation of brain marrow divine machine, so that the brain marrow divine machine can regulate limb motor sensation, bladder, rectum and other functions can also return to normal.

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Conflict of Interests

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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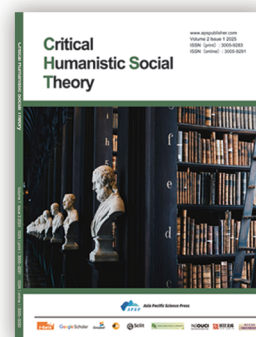
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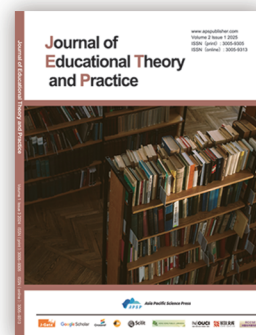
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