Aim and Scope

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Application of DNA Molecular Identification Method to Distinguish Ejiao and its Adulterants

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ABSTRACT

Objective: To identify Ejiao and its adulterants at the DNA level by using DNA molecular marker. Ejiao (Asini Corii Colla) is a commonly used medicinal material. However, its adulteration is a serious concern. Due to the morphological characteristics of Asini Corii Colla and its adulterants, traditional identification techniques often complex and professional, which is not conducive to the circulation management and safety of the medicinal materials. To improve the distinction between Asini Corii Colla and its adulterants accurately, this study identified and its adulterant samples based on the CytB sequence. Sequence characteristics, Basic Local Alignment Search Tool (BLAST) application, genetic distance, construction of phylogenetic tree showed the CytB sequence to accurately identify Asini Corii Colla from its adulterants. Furthermore, in this study, we designed a specific primer, based on the CytB sequence, and established a PCR detection system for rapid, sensitive, and specific identification of Asini Corii Colla. Compared to DNA barcoding technology, this method has shorter detection time, stronger specificity, and higher sensitivity, which lays the foundation for the rapid identification of Asini Corii Colla.

1. Introduction

Colla Corii Asini (Ejiao in Chinese), which is made from fresh or dry skin of Equus asinus L. after dehairing, also known as donkey skin glue. It is a precious Chinese traditional medicine widely used in China for thousands of years. As recorded in many classic monographs of Chinese traditional medicine and in ancient books, Ejiao displays great efficacy in enriching blood and staunching bleeding, being mainly used for the treatment of gynecological diseases, such as menoxenia and post-partum uterine bleeding [1]. It is no surprise that multiple pharmaceutical factories manufacture Ejiao under different brand in Chinese traditional medicine market. To market supervision department, there are many problems related to quality control of Ejiao, a major issue is its manufactur-
ten processes such as airing the skin of donkey, scraping, soaking, gutting, peeling, concentrating, gelling, cutting, airing and rubbing, etc. After that, Ejiao is further refined into liquid formulation, etc. Enzymatic hydrolysis, purification, drying and other complicated manufacturing processes are implemented in production [3]. The traditional methods of authenticity identification of Ejiao, such as macroscopical identification, microscopic identification and physical and chemical identification, depend on personal experience and professional knowledge [4]. Meanwhile, the preparation process of other animal skin glues is similar to that of Ejiao, and their appearance traits are similar as well, thus it is extremely difficult to distinguish Ejiao from other animal skin glues.

At present, Traditional Chinese medicinal materials are authenticated in three methods. The first method is macroscopical identification, which can only be applied to appearance identification but is very hard to authenticate some processed medicinal materials their appearance had been changed. The second method is microscopic identification, which is used to observe and authenticate microscopic features of tissue structure of Traditional Chinese medicinal materials, but cannot identify some Traditional Chinese medicinal materials having similar basic structure. The third method is physical and chemical identification, which is applied to identify effective components via physical and chemical methods, such as chromatography and spectrometry, but has low resolution and poor specificity to near-source chemical components [5].

With the development of biotechnology, the species identification methods are also constantly developed. It has gone through several stages such as morphological identification, cell-level identification, biochemical analysis identification, immunological identification, and molecular marker identification. For morphological identification, cell-level identification, biochemical analysis identification and immunological identification, there are some defects that may lead to failure or incorrect identification [6-7].

Molecular Marker Identification: This is an approach to detect the differences in DNA fragments between individuals or between populations to achieve the goal of identification. Molecular markers have many advantages: it is not affected by the environment or other factors, high polymorphism, and can provide complete genetic information, etc. The most commonly applied technologies of molecular markers are: Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphism DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Microsatellite DNA (STR), Single Nucleotide Polymorphism (SNP), etc. [7-9].

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism’s DNA to identify it as belonging to a particular species. It was first proposed in 2003 by researchers at the University of Guelph in Ontario, Canada [10]. The short genetic sequence from a standard part of the genome works efficiently to identify species the way a supermarket scanner distinguishes products using the black stripes of the Universal Product Code. Thus it was given the name “DNA barcode”. DNA barcodes vary among individuals of the same species, but only to a very minor degree. If the DNA barcode region is effective, the minor variation within species will be much smaller than the differences among species.

Mitochondrial DNA (mtDNA) is the genetic material that exists outside the nucleus in eukaryotic cells. It has a simple molecular structure. It does not undergo recombination with nuclear DNA and has no identical sequence with nuclear DNA. It has multiple copies, has a rapid evolutionary rate, and follows maternal inheritance. Cytochrome b gene (Cyt b) is one of the genes that is coded by mtDNA, and its gene product plays an important role in electron transfer in the respiration chain. Cyt b gene has a moderate evolutionary rate and a clear evolutionary pattern that makes it suitable for the studies on the phylogenetic evolution at the intra- and interspecific levels [11-13].

In the present study, to implement the feasible DNA barcode technique in the identification of Ejiao and its adulterants. Several Ejiao products had been collected on the market and tested their authenticity with approach of LC-MS/MS, according to Chinese Pharmacopoeia. Then, by comparing different genomic extraction methods, a suitable way for donkey-hide gelatin was optimized to obtain the genome with higher purity. Because mitochondrial genes are the only self-replicating genome in eukaryotes, its number via duplicate is thousands of times higher than that of nuclear genes, furthermore, it is more stable and resistant to degradation than nuclear genomes, the mitochondrial gene CytB gene was selected as the molecular marker to detect the collected samples in the study. Meanwhile, Bioinformatics analysis would be performed after obtaining the sequence by sequencing. Compared the legal detection with Chinese Pharmacopoeia, the CytB sequence could accurately identify Ejiao from its adulterants. This method has shorter detection time, stronger specificity, and higher sensitivity, which lays the foundation for the rapid identification of Ejiao. The study provides a theoretical basis for the establishment of standard, and provides technical support for further improving the molecular detection field of Ejiao.

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2. Experimental Materials and Reagents

Reagents

Formic acid, ammonium bicarbonate, NaCl, EDTA, chloroform, isooamyl alcohol and other reagents were purchased from Sinopharm Reagent Co., Ltd. β-mercaptoethanol, proteinase K, equilibrated phenol, C hexel-100, Agarose, DNA polymerase (Phanta™ Super-Fidelity DNA Polymerase Vazyme), DNA marker (Toneker 2000), DNA dye (Gel RED™ Nucleic Acid Gel Stain, Biotium), etc were purchased from Wuhan Shengya Biotechnology Ltd.

Experimental instruments


Chromatography Column

Shiseido (S201204-11, column length: 150 mm, column diameter 2.1 mm).

3. Results

3.1 The Legal Identification

Ejiao occupies an important position in China’s health product market, with an output value of nearly 500 billion yuan. Genuine Ejiao is made from the hide of Equus anatinum L. Due to the shortage of raw materials, the price of Ejiao is continually rising, adulteration occurs from time to time. The main ingredient of adulteration is cow hide glue. The legal identification method of Ejiao includes two parts. One is observation of the characteristics of Ejiao, and the other is comparing the consistency of the chromatographic peaks between sample and reference medicinal materials by using high performance liquid chromatography - mass spectrometry (HPLC-MS).

Sample collection

A total of 11 different samples used in this study are all purchased from Chinese market and enterprises, and stored at -20°C after collection. 8 out of 11 Ejiao products were obtained from 7 manufacturing companies, one donkey hide was provided by Hubei Kang Pharmaceutical Co., Ltd. one cowskin came from slaughterhouse in Wuhan. The source of samples showed in the Table 1 below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Manufacturer</th>
<th>Batch production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1</td>
<td>Hubei A Pharmaceutical Co., Ltd</td>
<td>150108</td>
</tr>
<tr>
<td>Y2</td>
<td>Shijiazhuang A Pharmaceutical Co., Ltd</td>
<td>140409</td>
</tr>
<tr>
<td>Y3</td>
<td>Shandong A Pharmaceutical Co., Ltd</td>
<td>15050181</td>
</tr>
<tr>
<td>Y4</td>
<td>Shandong B Pharmaceutical Co., Ltd</td>
<td>140811</td>
</tr>
<tr>
<td>Y5</td>
<td>Shandong B Pharmaceutical Co., Ltd</td>
<td>20150505</td>
</tr>
<tr>
<td>Y6</td>
<td>Shandong C Pharmaceutical Co., Ltd</td>
<td>20141101</td>
</tr>
<tr>
<td>Y7</td>
<td>Shandong D Pharmaceutical Co., Ltd</td>
<td>20141130</td>
</tr>
<tr>
<td>Y8</td>
<td>Shandong E Pharmaceutical Co., Ltd</td>
<td>20150611</td>
</tr>
</tbody>
</table>

Character identification

In order to identify the authenticity of Ejiao products, firstly, according to the 2015 edition of Chinese Pharmacopoeia, the character of Ejiao was identified by observation. The sharp of genuine Ejiao should be cuboid or cube. The color is brown to dark brown with luster. The material is hard and brittle with bright cross section, and the fragments are brown and translucent to light. The smell is weak and taste is slightly sweet.

Ejiao component identification

According to the “Chinese Pharmacopoeia” 2015 edition, whether the collected samples are authentic Ejiao depends on if the consistency has been fulfilled or not by comparing chromatographic peaks between sample and reference medicinal materials which was detected by HPLC-MS.

The result of the legal identification for these 8 collected Ejiao products are shown in the Table 2 below. They are all genuine products.

<table>
<thead>
<tr>
<th>Number</th>
<th>Character identification</th>
<th>Ejiao component identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1</td>
<td>qualified</td>
<td>qualified</td>
</tr>
<tr>
<td>Y2</td>
<td>qualified</td>
<td>qualified</td>
</tr>
<tr>
<td>Y3</td>
<td>qualified</td>
<td>qualified</td>
</tr>
<tr>
<td>Y4</td>
<td>qualified</td>
<td>qualified</td>
</tr>
<tr>
<td>Y5</td>
<td>qualified</td>
<td>qualified</td>
</tr>
<tr>
<td>Y6</td>
<td>qualified</td>
<td>qualified</td>
</tr>
<tr>
<td>Y7</td>
<td>qualified</td>
<td>qualified</td>
</tr>
<tr>
<td>Y8</td>
<td>qualified</td>
<td>qualified</td>
</tr>
</tbody>
</table>

The development of chromatography- spectrum technology has established the fingerprint information of various traditional Chinese medicines, which can provide abundant information for the identification and quality evaluation of traditional Chinese medicines. Legal identification of Ejiao implements this method because of its high specificity and effectiveness. However, this method
requires the use of large and expensive instruments, which is complicated to operate and has high requirements for the professional level of experimental personnel.

3.2 The Molecular Identification Test of Ejiao

The molecular identification test of Ejiao requires the extraction of DNA. The preparation process of Ejiao product mainly includes multiple steps such as donkey skin pretreatment, peeling, filtration, concentration, and gelation, which takes a long time. During this lengthy process, the degradation of DNA is very serious, increasing the difficulty of molecular detection. In order to obtain a higher concentration of DNA, a variety of extraction methods have been tested. After obtaining the DNA with higher purity, CytB gene was selected as the molecular marker, and the Ejiao samples were amplified, sequenced and analyzed.

3.2.1 Separating and Purifying DNA

Sodium Dodecyl Sulfonate (SDS) is a plasma surfactant, which can dissolve the cell membrane and nuclear membrane protein, rupture the cell membrane and nuclear membrane to release nucleic acid, and depolymerize the nucleic protein in the cell and denature the protein precipitation. Chelex-100 is a chemical chelating resin composed of styrene and divinylbenzene copolymer. It contains pairs of iminodiacetate ions, which can chelate polyvalent ions, especially, have higher affinity and chelation for high-valent metal ions. Glass milk technology uses the principle of combining DNA and silica under high-concentration salt conditions and eluting under low-salt conditions, so as to achieve the purpose of separating and purifying DNA [14].

There is a high amount of protein in Ejiao. Proteinase K is added during the extraction process to hydrolyze and digest proteins, especially histones that bind to DNA. Proteinase K requires a suitable temperature (65 °C) and proper time to hydrolyze proteins. On one hand, With the increase of reaction time, the protein hydrolyzes more thoroughly, on the other hand, a too long hydrolysis time will lead to partial nucleic acid depletion and proteinase K hydrolyzes in a poor ability. For improving the efficiency of DNA extraction, the time of proteinase K action should be optimized. Finally, three ways were tried for DNA extraction of donkey-hide gelatin.

Method a

Use proteinase K and Chelex-100 in DNA extraction, and purified DNA with glass milk.

(1) Transfer 200 mg Ejiao sample to a microfuge tube. Add 400 μL of digestion buffer (pH 8.0, consisting of 10 mmol/L Tris-HCl, 25 mmol/L EDTA, 100 mmol/L NaCl and 0.5% SDS) and 380 μL of 15% Chelex-100, invert the capped tubes to mix the contents. Incubate the solution at 50-60 °C water bath, occasionally inverting the tubes to make the solution completely dissolved. (2) Cooled the solution to room temperature, and added 20 mg/mL proteinase K 20 μL, mixed and incubated the solution in 56°C for 1 hour. (3) Centrifuge the solution at 12000 r/min for 10 min. Transfer the supernatant fluid to a new EP tube by aspiration. And add an equal volume of chloroform. Mix thoroughly, centrifuge at 12000 r/min for 10 minutes, and carefully transfer the supernatant to a new EP tube. (4) Add 5-20 μL glass milk and two times volumes of DNA Binding Buffer (6 mol/L NaCl), Incubate the solution at 50-60 °C water bath. Mix sample several times during incubation by inverting tube. (5) Incubate at room temperature for 5 minutes, and invert several times during this time. (6) Centrifuge the solution at 4000 r/min for 1 minute, and discard the supernatant. (7) Wash the DNA precipitate twice with 70% ethanol. Remove as much of the 70% ethanol as possible, using an aspirator. Store the pellet of DNA in an open tube at room temperature until the last visible traces of ethanol have evaporated. Add an appropriate amount of deionized water to completely dissolved DNA. Store the DNA solution at -20°C.

Method b

Use SDS alkaline lysis method and more than once use chloroform and isoamyl alcohol in DNA extraction.

(1) Transfer 200 mg Ejiao sample to a microfuge tube. Add 1ml of SDS extraction /lysis buffer (pH 7.2, 500 mmol/L NaCl, 100 mmol/L Tris-HCl, 50 mol/L EDTA, 100 mmol/L β- mercaptoethanol, 20% SDS) and 50 μL of 20 mg/mL proteinase K, invert the capped tubes to mix the contents. Mixed and incubated the solution in 56°C for 1 hour. (2) Centrifuge the solution at 12000 r/min for 1 minute, transfer the supernatant to a new EP tube. (3) Add an equal volume of equilibrated phenol, and gently mix the solution by slowly turning the tube end-over-end. Centrifuge the solution at 12000 r/min for 10 minutes, transfer the aqueous (upper) phase to a new EP tube. (4) Add an equal volume of phenol - chloroform - isoamyl alcohol (25: 24: 1), gently mix the solution by slowly turning the tube end-over-end. Centrifuge the solution at 12000 r/min for 10 minutes, transfer the aqueous (upper) phase to a new EP tube. (5) Add 0.2 volume of 10M NaAc and 2 volumes of ethanol, then swirl the tube until the solution is thoroughly mixed, Store the DNA solution at -20°C until the DNA forms a precipitate. (6) Centrifuge the solution at 12000 r/min for 10 minutes, and discard the supernatant. Wash the DNA precipitate twice with 70% ethanol.
Remove as much of the 70% ethanol as possible, using an aspirator. Store the pellet of DNA in an open tube at room temperature until the last visible traces of ethanol have evaporated. Add an appropriate amount of deionized water to completely dissolved DNA. Store the DNA solution at -20°C.

Method c

Use Deep-processed product genome extraction kit in DNA extraction.

The kit was purchased from Shanghai Shenggong Biological Co., Ltd. Ejiao sample was grinded with liquid nitrogen. DNA was extracted using Shenggong Deep-processed product genome extraction kit in accordance with the manufacturer’s protocol, and then was dissolved in 100 μL distilled water.

3.2.2 Comparison and Analysis of DNA Extraction Methods by Measuring the Concentration of the DNA

Measure the concentration of the DNA by Ultraviolet spectrophotometry to test the absorbance at 260 nm and 280 nm. If the value is about 1.8 to 1.9 at A260/A280, that means the purity of DNA is well. If the value is greater than 1.9 at A260/A280, that indicated the DNA may mix with some RNA. If the value is less than 1.8 at A260/A280, that suggested the DNA solution may contain some contamination of phenol or protein. Take sample Y 4 as an example, the absorbance is shown in the Table 3 below.

Table 3. Comparison and analysis of DNA extraction methods by measuring the concentration of the DNA

<table>
<thead>
<tr>
<th>Method</th>
<th>A260/A280</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1.846</td>
</tr>
<tr>
<td>b</td>
<td>1.751</td>
</tr>
<tr>
<td>c</td>
<td>1.802</td>
</tr>
</tbody>
</table>

By Comparison and analysis of DNA extraction methods, the DNA extracted in the method B had residues of phenols or proteins that were not removed. Method a and c may get higher purity DNA solution. Considering the simplicity of the operation, the method c was adopted, while increasing enzymatic reactions time of Proteinase K.

3.2.3 Selection of Molecular Markers and Sample Detection

Design of primers

Because mitochondrial genes are the only self-replicating genome in eukaryotes, and copy number is thousands of times higher than that of nuclear genes, it is more stable and resistant to degradation than nuclear genomes. Many studies have reported the use of mitochondrial CytB and 16SrRNA gene sequence analysis to identify animal species. Due to the serious degradation of DNA during the processing of Ejiao, mitochondrial genes are selected as molecular markers. 3 pairs of primers were designed for screening, as shown in the following Table 4 (primers are synthesized by Wuhan Aoke Dingsheng Biotechnology Co., Ltd.):

Table 4. The design of primers

<table>
<thead>
<tr>
<th></th>
<th>The design of primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CytB</td>
<td>Forward primer (5’-3’): CCA TCC AAC ATC TCA GCA TGA TG AAA</td>
</tr>
<tr>
<td></td>
<td>Reverse primer (5’-3’): GCC CCT CAG AAT GAT ATT TGT CCT CA</td>
</tr>
<tr>
<td>COI</td>
<td>Forward primer (5’-3’): GGT CAA CAA ATC ATA AAG ATA TTG</td>
</tr>
<tr>
<td></td>
<td>Reverse primer (5’-3’): TAA ACT TCA GGG TGA CCA AAA AAT</td>
</tr>
<tr>
<td>16sRNA</td>
<td>Forward primer (5’-3’): GCC TAT ATC AGA AGG TCA TAA AAG</td>
</tr>
<tr>
<td></td>
<td>Reverse primer (5’-3’): CAT GCC TGT GTF GGG TTA A</td>
</tr>
</tbody>
</table>
for 10 min to allow complete extension of PCR products.

The amplification procedures for CytB were started with predenaturing at 94°C for 2 min, followed by 40 cycles of denaturing at 94°C for 1 min, annealing at 53°C for 1 min and extension at 72°C for 1.5 min; the amplification was completed by holding the reaction mixture at 72 °C for 10 min to allow complete extension of PCR products.

**Detect PCR product by electrophoresis**

Detect PCR product by electrophoresis with 1.2% agarose, we found that we found that only CytB gene can be amplified, and other primers cannot be used to obtain fragments in the amplification procedure.

CytB fragment of the sample genome in amplification. As shown in the Figure 1 below (1.2 % agarose).

![Figure 1. Detect PCR product by electrophoresis (1 Y1, 2 Y2, 3 Y3, 4 Y4, 5 Y5, 6 marker, 7 Y6, 8 Y7, 9 Y8, 10 donkey hide, 11 one cowskin, 12 Ejiao reference medicinal material)](image1)

**3.2.4 Sequence Comparison**

Recovered the band of CytB from agarose gel, then sequenced the DNA, the sequence comparison result is shown in the Figure 2 below:

**3.2.5 Phylogenetic Analysis**

The phylogenetic tree result is shown in the Figure 3 below:

The phylogenetic tree shows that the CytB fragment of the sample and the cowhide are different dramatically and can be clearly distinguished. It is confirmed that the CytB gene can be used as a molecular marker to identify the donkey skin components of Ejiao and its products.

**4. Discussion**

A larger sample collection is needed to further test the effectiveness of the method. From an economic point

![Figure 2. The sequence comparison of CytB (E, Ejiao reference medicinal material; L, donkey hide; N, cowskin, Y1-YB, samples)](image2)

![Figure 3. The phylogenetic tree (E, Ejiao reference medicinal material; L, donkey hide; N, cowskin, Y1-YB, samples)](image3)
of view, the counterfeit manufacturers of Ejiao will use cheaper raw material, cowhide instead of donkey skin, to product Ejiao. So this project mainly focuses on detection method for distinguishing the cowhide from donkey skin. In addition, horse skin, mule skin, calf skin, calf skin, goat skin, etc. many other skins could be the raw material. The detection method needs to be further improved to enhance its applicability.

Although this project has initially completed the detection method for distinguishing Ejiao and its adulterants by the molecular marker gene (CytB), molecular identification technology cannot replace the existing detection technology. Species identification can ensure the “authenticity” of drugs, while the content determination of active ingredients or active parts can ensure the “effectiveness” of drugs, both of which are indispensable steps in drug quality control. Although molecular technology identification has broad application prospects, it will also encounter various challenges in the research. This technology cannot completely replace other existing identification technology methods, such as microscopy, physics and chemistry, chromatography and spectroscopy. Long storage time and improper storage condition will cause such phenomena as infestation, discoloration or mildew of Chinese herbal medicines, decoction pieces and Chinese patent medicines, which will seriously affect their quality and efficacy. This kind of situation also could affect the results of identification in molecular technology. For example, the DNA degradation too serious to be successful obtained its bar code sequence, or the extracted DNA may be contaminated by fungi due to mildew. Therefore, DNA barcoding technology should be combined with other methods to comprehensively evaluate the drug quality if the above situations are encountered in the actual drug testing work.

Acknowledgements

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References

1. Introduction

Breast cancer is a malignant tumor disease with a higher incidence in the female population. In recent years, with the advancement and improvement of timely diagnosis and overall treatment of breast cancer, the overall survival rate and survival time of patients have been greatly improved. At the same time, related complications of breast cancer treatment will interfere with the quality of life of patients and related problems. Began to gradually attract people’s attention \[1\]. In order to further improve the quality of life of patients, many studies have pointed out the related comorbidities and prevention measures of breast cancer treatment. Among them, dyskinesia of the affected upper limb is more common, which has developed into a major comorbidity in patients after breast cancer \[2\]. In the early stage of overall breast cancer treatment, surgical treatment (including breasts and armpits) and radiotherapy are likely to cause the production of scar tissue, fibrosis and soft tissue contraction in local locations. Such conditions can easily lead to dysfunction of the upper limbs of the patient and cause pain. Reduced range of motion, etc.,
continued development will lead to myofascial dysfunction, mucosal capsulitis, nerve function obstruction, which leads to pain and shoulder joint behavior obstruction, and upper limb function obstacles ultimately hinder the normal life and daily behavior of patients. First report the specific situation as follows.

2. Materials and Methods

2.1 General Information

For this study, a total of 40 female breast cancer patients who underwent surgical treatment were selected in our hospital from September 2017 to June 2019. According to different nursing methods by random method, all patients were divided into experimental group and control group, each with 20 cases. The diagnosis and classification of upper limb dysfunction were carried out according to the plan drawn up by Richards. The specific process is: ask the patient to stand facing the wall and lift his hands vertically. The difference between the hands to touch the high level indicates the difference in the upper limb lift level; arrange the patient’s hands to be reversed from the back to wear the bra to touch their own. The spine, the highest protrusion that the thumb can reach, is used to measure the level of abduction, external rotation, and internal rotation of the upper limbs. When any one of them is abnormal, it can be judged as the lack of ability of the side shoulder joint, that is, upper limb dysfunction.

The screening criteria are: (1) Meet the criteria: patients after breast cancer surgery (unlimited surgery); female patients aged 29-75 years; patients voluntarily join this study with informed consent. (2) Non-compliance: Patients with bleeding tendency such as severe blood diseases; patients with severe skin diseases; patients with allergies or history of drug allergy; patients with unconsciousness or mental illness.

2.2 Method

Patients in the control group were given training based on domestic and international standards for postoperative rehabilitation exercises for breast cancer. They were regularly and correctly guided by professional nurses every day, including non-resistance exercise training, resistance equipment exercise training, and music-rhythm rehabilitation exercises. The training time is 10 minutes, training 3 times a day, a total of half an hour, 2 times a week, giving a total of 8 treatments. At the same time, a certain amount of psychological care was given to solve the existing nursing problems in a timely and routine manner.

Patients in the experimental group were treated with MET on the basis of the control group, 40 minutes each time, 5 times a week. Among them, the main steps of the technique of relaxing muscle strength after isometric contraction are: the patient chooses a sitting position and asks him to place one hand below his back. If there is difficulty, he can put his hand on the same side of the greater trochanter. One of the therapist’s holds the patient’s elbow and places the other hand on the distal part of the forearm. Pull the distal end of the forearm away from the back as much as possible. The patient will make appropriate resistance. The isometric contraction is about 5-10 seconds. Slowly pull the arm to the new resistance. Limit, repeat the above actions 3-5 times. Perform eccentric contraction muscle strength in MET: Instruct the patient to choose a prone position and raise the arm to a comfortable limit. The therapist will hold the distal end of the humerus with one hand and place the other hand on the forearm. Try to use appropriate pressure Move the arm beyond the top of the head, the patient resists, slowly move the arm to the painless limit, continue to relax for about 10 seconds, maintain the new range without pain, if there is pain, it needs to be sent back slowly. Repeat 3-5 times, lower your arms, and perform this action 3-5 times. The total duration of the 2 groups of medical treatment was 4 months.

2.3 Observation Indicators

ROM is used as an objective indicator to detect the rehabilitation of patients’ upper limb function. A professional goniometer was used to detect the movement of the shoulder joints of the uninfected and affected upper limbs in the 4th and 12th weeks after surgery (shoulder extension, flexion, abduction, and adduction). Among them, the patient has no discomfort or pain, which means that the upper limbs are functioning normally.

2.4 Statistical Methods

Use SPSS 23.0 statistical software to conduct scientific and reasonable analysis and research on all the acquired data. Among them, the count data are compared with X2 detection. When \( P<0.05 \), it means that the difference is obvious and has statistical significance.

3. Results

Through early functional recovery training and MET technology intervention, 19 patients in the experimental group (up to 95% compliance rate) showed that the upper extremity function was up to standard (normal), while only 14 patients in the control group (70% compliance rate) showed that it was up to the standard. The difference is obvious and has statistical significance (\( P<0.05 \)). The specific conditions are shown in Table 1 below.
The main operation process of the muscle energy technology. The specific process was: Isometric contraction. The therapist performs external rotation isometric contraction and allows the same long contraction time for 5 seconds, the muscles relax after the isometric contraction. The therapist performs external rotation of the shoulder joint to the new blocked part of the movement, and then applies the same technique again, repeating the technique 3-5 times in the direction of external rotation. During the shoulder joint flexion, abduction, and internal rotation, the therapist repeats the above methods to complete the treatment process of muscle energy technology.

In summary, muscle energy technology can effectively accelerate the dissipation of edema of the affected limb of patients with upper limb dysfunction after breast cancer surgery, reduce the possibility of scar contracture, and avoid postoperative shoulder stiffness due to long-term immobilization and pressure bandaging. And upper limb dysfunction.

4. Discussion

Breast cancer is currently a major malignant tumor that endangers the physical and mental health of women around the world. There is a high incidence and mortality rate, and it continues to show a rising trend. Modified radical mastectomy for breast cancer is a common type of clinical surgery in the current surgical treatment of breast cancer, and patients often have a series of complications after surgery. According to the corresponding clinical data display and analysis, the current incidence of upper limb dysfunction after breast cancer surgery has reached 36%-65%, the affected side shoulder joint disorder has also reached 48%, and the incidence of subcutaneous effusion on the affected side is 10%. About -20% [5], upper extremity edema regurgitation usually occurs within 1-2 years after surgery, and upper extremity dysfunction is a common type of comorbidity after breast cancer surgery. The main symptom is that patients show varying degrees functional problems such as lymphedema, obstructed behavior of upper limbs and shoulder joints, and decreased muscle strength. It greatly interferes with the patient’s limb function, thereby affecting the patient’s normal life and social behavior.

MET technology is a manual treatment technique that actively participates in patients under the principle of painlessness, which can increase muscle length, flexibility, and increase muscle strength and stability. Patients in the experimental group were treated with muscle energy technology. The specific process was: Isometric contraction and relaxation (PIR), the patient’s shoulder joints were moved to the maximum extent, and appropriate resistance was applied to them to maintain the isometric contraction lasts for 5 seconds to effectively relax the internal rotator muscles; the contraction relaxation (CR) process is basically the same as PIR, but the difference is that it needs to simply complete repeated operations in the shield of the original resistance; interactive inhibition (RI); contraction Relaxation against contraction (CRAC) method. The patient received improved muscle energy training [6]. The main operation process of the muscle energy technology to improve the external rotation of the shoulder joint is to passively move the shoulder joint to the extreme position of the external rotation. The therapist increases the appropriate resistance to prevent the patient’s internal rotation isometric contraction and allows the same long contraction time for 5 seconds, the muscles relax after the isometric contraction. The therapist performs external rotation of the shoulder joint to the new blocked part of the movement, and then applies the same technique again, repeating the technique 3-5 times in the direction of external rotation. During the shoulder joint flexion, abduction, and internal rotation, the therapist repeats the above methods to complete the treatment process of muscle energy technology.

Funding

Item Number: 2018020778 project name: Clinical study of MET technique on the improvement of lymphedema and shoulder dysfunction after breast cancer surgery.

Item Number: 2019ZY3011 project name: Index analysis of the influence of Weisan acupuncture on sarcopenia.

Item Number: LHGJ20191057 project name: JAK/STAT signal regulation of macrophages involved in tendon remodeling and its mechanism.

References


675-678.


Discussion on Nursing Mode of Ecmo in Treating Severe Patients

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ARTICLE INFO

ABSTRACT

Objective: To evaluate the nursing effect of Ecmo treatment for severe patients. Methods: 66 patients treated with extracorporeal membrane pulmonary oxygenation were included in the experimental data. From August 2018 to August 2019, the patients were divided into experimental group and reference group by random digital table method, each group was 33 cases. Routine nursing and targeted nursing were performed to compare the complications of the two groups. Results: (1) The correlation index of extracorporeal membrane pulmonary oxygenation treatment before treatment was consistent, \( P>0.05 \), the oxygen saturation and oxygen partial pressure of 2 h, 4 h after treatment in the experimental group were higher than those in the reference group, compared with the reference group, the carbon dioxide partial pressure of 2 h, 4 h after treatment in the experimental group was lower, showing statistical significance of data test (\( P<0.05 \)). (2) The incidence of infection, bleeding, coagulation, embolism and hypotension in the experimental group (12.12%) was lower than that in the reference group (45.45%), showing statistical significance (\( P<0.05 \)). Conclusions: the specific nursing effect of extracorporeal membrane pulmonary oxygenation in severe patients can effectively improve the success rate of treatment, and the possibility of complications during treatment is low.

Keywords:
Extracorporeal membrane oxygenation
Ecmo
Critical
Nursing

1. Introduction

Extracorporeal membrane pulmonary oxygenation (Extracorporeal Membrane Oxygenation, ECMO) is an important guarantee of cardiopulmonary failure cardio-pulmonary bypass. It is a continuous life support therapy assistant technique. The venous blood of the patient is extracted from the body through the catheter and transported back to the patient’s body through the action of artificial lung and artificial heart [1]. During the course of extracorporeal membrane oxygenation treatment, the nursing work has been required. In this study, the nursing measures of extracorporeal membrane oxygenation for severe patients were analyzed and the application effect of targeted nursing was discussed.

2. Clinical Data and Methods

2.1 Clinical Information

For the treatment of severe patients with extracorporeal membrane pulmonary oxygenation from August 2018 to August 2019, 66 cases. The grouping method is the random number table method. They were experimental group (n=33) and reference group (n=33). In the experimental group, the ratio of severe female to male was 22 to 11, up to 68 years. The age limit is 38, and age mean was (43.29±13.78) years. Mechanical ventilation time longest 5 d, mechanical ventilation time shortest 2 d, mechanical ventilation time mean (3.59±0.58) d. In the reference group, the ratio of severe female to male was 20 to 13. Age limit 68 years old, age limit 37 years old, age average
is (43.25±13.76) years old. 6 d, maximum mechanical ventilation 2 d, minimum mechanical ventilation mean mechanical ventilation time was (3.62±0.60) d. To verify the data of age, sex and mechanical ventilation time in 2 groups of patients treated with extracorporeal membrane oxygenation, P>0.05, Comparable characteristics. Inclusion criteria: (1) All patients were severe pneumonia patients; (2) The patients could not correct hypoxemia after mechanical ventilation; (3) Patients or family members are aware of this study, Voluntary participation. Exclusion criteria: (1) malignant tumor patients; (2) Patients with mental illness [2].

2.2 Method

According to the routine nursing, the Bio line coated artery, vein intubation and extracorporeal membrane pulmonary oxygenation package were used to treat the femoral vein, centrifugal pump, oxygenation instrument and jugular vein. The flow rate of extracorporeal membrane oxygenation instrument is 2.5-4.2 L/min, the average operating time is 219 h, the oxygen concentration is adjusted to 30-40, the positive pressure at the end of respiration is 4-5 cmH2O, and the operating time is 219 h, moisture volume adjusted to 5-6 ml/kg [3]. 4 h Prothrombin time and arterial blood gas were tested h to ensure nursing care during extracorporeal membrane pulmonary oxygenation. Experimental group to carry out targeted nursing, specific measures include: first, vital signs monitoring, extracorporeal membrane pulmonary oxygenation treatment of severe patients vital signs monitoring. Keep the heart rate at 80 beats / min, to evaluate the blood pressure level and give the patient drugs to control blood pressure. For some patients with excessive respiratory frequency, the problem of blood oxygen saturation should be prevented, and the actual situation of patients should be given drug treatment. The arterial pressure level of the patients should be dynamically monitored, especially during the adjustment of the patient’s posture, and the pressure sensor system should be protected. Every 2 h the pupil of the patient was observed to prevent intracranial hemorrhage caused by heparin. Every 2 h the patient’s body temperature level is monitored to prevent the patient from hypothermia. Hypothermia will cause abnormal hemodynamic indexes and affect the patient’s life safety. If the patient’s conditions permit, the rectal temperature is detected and maintained at 35-36 degrees. Second, airway nursing, during sputum suction operation, should be strictly in accordance with the requirements of aseptic operation, as far as possible to implement shallow attraction, to prevent injury to patients’ airway mucosa. The patient’s airbag pressure is checked every 12 h and maintained at cmH2 25-30, give the patient heating, humidification management to ensure its airway mucosal humidification effect. Third, hemagglutination index monitoring, during extracorporeal membrane pulmonary oxygenation treatment, patients are more likely to develop thrombus, so during the treatment of patients will be systemic heparinization, but during the treatment is very likely to occur excessive anticoagulant problem, this should be targeted at the patient’s partial coagulation time monitoring, maintain its partial coagulation time of 150-200 s, platelet maintenance at 100×10^9 L above, observe the skin, mucous membrane, arteriovenous puncture position, drainage fluid, once bleeding, subcutaneous bleeding or blood substances, the patient should be informed in time to adjust the amount of heparin [4].

2.3 Observation Indicators

Blood oxygen saturation, oxygen partial pressure and carbon dioxide partial pressure were monitored 2h, 4h severe patients before and after treatment. The complications of extracorporeal membrane pulmonary oxygenation in severe patients were recorded, including infection, bleeding, coagulation, embolism, hypotension and so on.

2.4 Statistical Analysis

The data of all 66 cases of severe patients treated with extracorporeal membrane pulmonary oxygenation were expressed as (mean ± standard deviation) in SPSS 19.0 software, including: oxygen saturation, oxygen partial pressure, carbon dioxide partial pressure index before and after treatment, t test was carried out to meet the normal distribution. The counting data is expressed in the form n(%) cases, and the data is X² Test, including: extracorporeal membrane oxygenation treatment of severe patients with infection, bleeding, coagulation, embolism, hypotension and other complications. P<0.05, showing statistical significance of data test [5].

3. Results

Comparison of oxygen saturation, oxygen partial pressure and carbon dioxide partial pressure between the 2 h, 4 h before and after treatment of severe patients with extracorporeal membrane oxygenation P>0.05, the blood oxygen saturation and oxygen partial pressure in the experimental group were higher than those in the reference group. Compared with the reference group, the carbon dioxide partial pressure 2 h, 4 h the experimental group was lower after treatment (PP>0.05).

Comparison of complications such as infection, bleeding, coagulation, embolism and hypotension in severe patients treated with extracorporeal membrane oxygenation [6].
### Table 1. Comparison of blood oxygen saturation, partial pressure of oxygen and partial pressure of carbon dioxide 2h, 4h before and after treatment of severe patients with extracorporeal membrane oxygenation

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases (n)</th>
<th>(% blood oxygen saturation) Pre-treatment</th>
<th>2 h after treatment</th>
<th>4 h after treatment</th>
<th>Oxygen partial pressure (mmHg) Pre-treatment</th>
<th>2 h after treatment</th>
<th>4 h after treatment</th>
<th>Carbon dioxide partial pressure (mmHg) Pre-treatment</th>
<th>2 h after treatment</th>
<th>4 h after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental</strong></td>
<td>33</td>
<td>73.60±1.67</td>
<td>93.46±3.40</td>
<td>96.66±2.35</td>
<td>43.87±5.26</td>
<td>65.80±1.31</td>
<td>81.40±1.41</td>
<td>47.49±3.86</td>
<td>43.46±1.18</td>
<td>40.57±2.19</td>
</tr>
<tr>
<td><strong>Reference</strong></td>
<td>33</td>
<td>73.58±1.62</td>
<td>88.21±3.08</td>
<td>92.37±3.48</td>
<td>43.90±5.23</td>
<td>58.76±3.49</td>
<td>70.61±2.57</td>
<td>47.52±3.88</td>
<td>45.93±2.14</td>
<td>43.09±3.63</td>
</tr>
<tr>
<td>( t )</td>
<td></td>
<td>0.0493</td>
<td>6.5739</td>
<td>5.8688</td>
<td>0.0232</td>
<td>10.8487</td>
<td>21.1449</td>
<td>0.0314</td>
<td>5.8062</td>
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<tr>
<td>( P )</td>
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<td>0.9607</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.9815</td>
<td>0.0000</td>
<td>0.9749</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0011</td>
</tr>
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</table>

### Table 2. Comparison of complications such as infection, bleeding, coagulation, embolism and hypotension in patients with severe n(%) by extracorporeal membrane oxygenation

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases (n)</th>
<th>Infection</th>
<th>Bleeding</th>
<th>Blood clotting</th>
<th>Embolism</th>
<th>Low blood pressure</th>
<th>Total complications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental</strong></td>
<td>33</td>
<td>2 (6.06)</td>
<td>1 (3.03)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (3.03)</td>
<td>4 (12.12)</td>
</tr>
<tr>
<td><strong>Reference</strong></td>
<td>33</td>
<td>4 (12.12)</td>
<td>4 (12.12)</td>
<td>2 (6.06)</td>
<td>2 (6.06)</td>
<td>3 (9.09)</td>
<td>15 (45.45)</td>
</tr>
<tr>
<td>( X^2 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.9428</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0027</td>
</tr>
</tbody>
</table>

Compared with the complications of extracorporeal membrane pulmonary oxygenation in severe patients (see Table 2), the incidence of infection, bleeding, coagulation, embolism and hypotension in the experimental group (12.12%) was lower than that in the reference group (45.45%), showing statistical significance of data test (P<0.05).

### 4. Discussion

Extracorporeal membrane pulmonary oxygenation is the current advanced treatment of cardiopulmonary diseases, which can be divided into two forms. One is to carry out oxygenation after venous blood is induced, then to the vein, and generally to treat respiratory failure. The other is to oxygenate venous blood and return to the artery. The results showed that the incidence of complications such as infection, bleeding, coagulation, embolism and hypotension in the experimental group (12.12%) was lower than that in the reference group (45.45%), showing statistical significance of data test (P<0.05).

In the treatment of extracorporeal membrane pulmonary oxygenation, nursing staff should strictly abide by the principle of aseptic operation to prevent infection during the further period. Most of the patients treated by extracorporeal membrane pulmonary oxygenation are critical and critical. There are relatively many invasive pipelines, the immune system function of patients is seriously decreased, and the proportion of infection is high. Therefore, the requirement of clinical nursing is very high, nursing staff should strictly abide by the aseptic operation during the implementation of targeted nursing, and carry out nursing work in the aseptic state. If there is blood stains and sweat, it should be cleared to avoid the upward transmission of bacteria. In addition, for some patients who already have infection, we should apply blood culture, make clear the pathogenic bacteria of the patients, and give their targeted anti-infection treatment to ensure the safety of the patients. At the same time, the coagulation time of the patients should be monitored in nursing work, and the amount of heparin pump should be adjusted according to the actual situation of the patients. If the patients already have bleeding tendency, they should find out the cause immediately. And inform the doctor to deal with the problem of intracranial hemorrhage.

Combined with the above results, this study has obvious effect on the treatment of severe patients with extracorporeal membrane pulmonary oxygenation, and the blood gas related indexes of the patients have been significantly improved, and the incidence of complications during the treatment of patients has been reduced. Targeted nursing in extracorporeal membrane pulmonary oxygenation treatment has clinical value.

### References


[2] Lin Yan, Yu Chao, Gao Chunhua, et al. Nursing experience of one case of severe acute respiratory distress syndrome treated with ECMO combined with prone


Evaluation on the Influence of the Timing of Twin Pregnancy Termination on the Outcome of Mother and Infants

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Impact

ABSTRACT

Objective: To observe the maternal and infant outcomes of pregnant women with twins terminating their pregnancy at different timings. Methods: Among the twin pregnant women admitted to our hospital from August 2019 to August 2020, 50 primiparous women who opted to terminate their pregnancies at 5 different timings of “34——34+6 weeks”, “35——35+6 weeks”, “36——36+6 weeks”, “37——37+6 weeks”, “38——38+6 weeks” were selected as the research subjects. According to the timing of pregnancy termination, they were divided into 5 groups, each with 10 cases of pregnant women, and the impact of the timing of pregnancy termination on the outcome of the mothers and infants were compared. Results: The “37——37+6 weeks” group had the largest amount of postpartum hemorrhage, and the difference in Hb level before and after delivery was the largest. With the increase in gestational week, the weight of both large and small fetuses increased. In terms of neonatal diseases, the comparison between “34——34+6 weeks”, “35——35+6 weeks”, “36——36+6 weeks” and “37——37+6 weeks”, “38——38+6 weeks”, P<0.05, the comparison between “37——37+6 weeks” and “38——38+6 weeks”, P>0.05. Conclusions: The extension of the gestational week of twin pregnancies has no effect on postpartum hemorrhage, but it can improve the outcome of infants.

1. Introduction

According to the situation in recent years, the development of assisted reproductive technology has been getting better, and it has become more widely used, which has greatly increased the probability of twin pregnancy [1]. In fact, twin pregnancy is a high-risk pregnancy mode, the possibility of premature delivery or postpartum hemorrhage is relatively high, and the safety impact on the mother and fetus is relatively huge [2]. In order to promote the safety of twin pregnancy, it is necessary to conduct an in-depth analysis of the influence of the timing of twin pregnancy termination on the outcome of the mother and infants [3]. This study in our hospital took 50 primiparas with twin pregnancy as the research subjects, and observed and compared the effects of different termination timings on the maternal and infant outcomes. The report is as follows.

2. Information and Methods

2.1 General Information

The total number of pregnant women admitted to this study was 50, all of whom were primiparous with uncomplicated twin pregnancy. The admission time was from August 2019 to August 2020. According to the results of the lottery, 10 cases of pregnant women with general information P>0.05 were assigned to “34——34+6 weeks”, “35——35+6 weeks”, “36——36+6 weeks”, “37——37+6 weeks”, “38——38+6 weeks”,
weeks”, “38——38” each, the minimum ages were 27 and 25 years old respectively, the maximum ages were 36 years old and 38 years old respectively, and the average ages were (34.7 ± 3.7) years and (34.9 ± 3.6) years respectively.

2.2 Research Methods

5 different timings were chosen for the mothers to terminate the pregnancy, the 24h postpartum blood loss and the 24h hemoglobin (Hb) before and after delivery were observed and recorded, and the infants weights, 1—5min Apgar scores and the incidence of complications were determined.

2.3 Diagnosis Criteria

The diagnostic criteria were based on the relevant content of “Obstetrics and Gynecology” (edited by Xie Xing).

2.4 Statistical Analysis

Professional statistical software (SPSS 20.0) was used to carry out statistical analysis, X^2 was applied to check n (%), and t-test was applied to check (X ± s). If P<0.05, the difference between groups is statistically significant.

3. Results

3.1 Inter-group Comparison of Timing of Pregnancy Termination and Postpartum Hemorrhage

The “37——37” group had the highest postpartum hemorrhage, which was (428.50±380.31) ml, and the difference in Hb level before and after delivery was the largest, which was (13.56±14.97) g/L, as shown in Table 1.

According to Table 1, the inter-group comparison of the timing of pregnancy termination and the postpartum hemorrhage show P>0.05.

3.2 Inter-group Comparison of Large and Small Fetal Weight

The heavier fetus in twins is referred to as a large fetus; otherwise it is a small fetus. As the gestational age increases, the weight of the fetus increases, see Table 2.

According to Table 2, the inter-group comparison between large and small fetal weight is P>0.05.

3.3 Inter-group Comparison of Neonatal Morbidity

The number of morbid infants in “34——34” weeks”, “35——35” weeks”, “36——36” weeks”, “37——37” weeks”, “38——38” weeks” were 6 (60.00%), 4 (40.00%), 2 (20.00%), 1 (10.00%), and 0 cases respectively. See Table 3 below.

According to Table 3, the comparison between “34——34” weeks”, “35——35” weeks”, “36——36” weeks” and “37——37” weeks”, “38——38” weeks” shows P<0.05, while the comparison between “37——37” weeks” and “38——38” weeks” shows P>0.05.

<table>
<thead>
<tr>
<th>Weeks of Pregnancy</th>
<th>Postpartum Hemorrhage(ml)</th>
<th>Difference in Hb Level before and after Delivery(g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34——34** Weeks(n=10)</td>
<td>312.6±189.99</td>
<td>10.76±13.26</td>
</tr>
<tr>
<td>35——35** Weeks(n=10)</td>
<td>307.7±99.8</td>
<td>8.68±9.51</td>
</tr>
<tr>
<td>36——36** Weeks(n=10)</td>
<td>363.3±199.66</td>
<td>12.22±10.83</td>
</tr>
<tr>
<td>37——37** Weeks(n=10)</td>
<td>428.5±380.31</td>
<td>13.56±14.97</td>
</tr>
<tr>
<td>38——38** Weeks(n=10)</td>
<td>333.4±141.43</td>
<td>8.56±11.62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestation Week</th>
<th>Large Fetal Weight</th>
<th>Small Fetal Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>34——34** Weeks(n=10)</td>
<td>2261.12±217.70</td>
<td>2001.12±189.07</td>
</tr>
<tr>
<td>35——35** Weeks(n=10)</td>
<td>2598.76±288.73</td>
<td>2323.14±297.39</td>
</tr>
<tr>
<td>36——36** Weeks(n=10)</td>
<td>2611.39±299.36</td>
<td>2344.8±248.00</td>
</tr>
<tr>
<td>37——37** Weeks(n=10)</td>
<td>2813.76±272.86</td>
<td>2539.18±249.34</td>
</tr>
<tr>
<td>38——38** Weeks(n=10)</td>
<td>3021.55±405.01</td>
<td>2598.47±323.78</td>
</tr>
</tbody>
</table>
The effect of mode after delivery was the largest, which was ±14.97 g/L. As the gestational age increases, the weight of the fetus was basically mature at 34 weeks of gestation. When the pregnancy is terminated at this timing, the survival rate of the fetus is higher. From a practical point of view, there is excessive extension of uterine muscle fibers in women pregnant with twins, which leads to uterine contraction fatigue after delivery, resulting in a significant increase in the probability of postpartum bleeding. Moreover, the estimation of postpartum hemorrhage is more significantly affected by human factors, so there is a certain degree of uncertainty. Therefore, in this study, the difference in hemoglobin before and after delivery was also selected for observation in addition to postpartum hemorrhage. The results show that the timing of pregnancy termination does not affect the incidence of maternal postpartum hemorrhage.

This study showed that the “37—37th weeks” group had the highest postpartum hemorrhage, which was (13.56 ± 14.97) g/L. As the gestational age increases, the weight of the large and small fetuses both increase. The numbers of neonatal morbidity were 6 (60.00%) cases, 4 (40.00%) cases, 2 (20.00%) cases, 1 (10.00%) cases, and 0 cases in “34—34th weeks”, “35—35th weeks”, “36—36th weeks”, “37—37th weeks”, and “38—38th weeks” respectively. In terms of neonatal diseases, the comparison between “34—34th weeks”, “35—35th weeks”, “36—36th weeks”, “37—37th weeks”, and “38—38th weeks” shows P<0.05, while the comparison between “37—37th weeks” and “38—38th weeks” shows P>0.05.

To sum up, the difference in the timing of twin pregnancy termination will basically not affect the postpartum hemorrhage of the parturient. As for the infants with longer gestational age, heavier fetal weight, and at gestational weeks between 37 and 38 weeks, the morbidity is significantly reduced, showing that the timing of twin pregnancy termination can affect the outcome of infants.

4. Discussions

Twin pregnancies are becoming more and more common today. It can be divided into two types, namely “uncomplicated twin pregnancies” and “complicated twin pregnancies”, of which “complicated twin pregnancies” are twin pregnancies with multiple complications such as reverse arterial perfusion sequence syndrome, selective fetal growth restriction, and twin anemia-multiple blood sequence syndrome, etc. If there are no such complications, it is classified as “uncomplicated twin pregnancy”. Generally speaking, the delivery timing of twin pregnancy should be within 39 weeks, and if there are no complications, twin pregnancy termination between 37 and 38 weeks should be considered. Therefore, this study set the upper limit of the gestational week for twin pregnancies at 38 weeks, and the reason why 34 weeks was set as the lower limit of the gestational week for twin pregnancies was mainly because of the lung functions of the fetus was basically mature at 34 weeks of gestation. When the pregnancy is terminated at this timing, the survival rate of the fetus is higher.

From a practical point of view, there is excessive extension of uterine muscle fibers in women pregnant with twins, which leads to uterine contraction fatigue after delivery, resulting in a significant increase in the probability of postpartum bleeding. Moreover, the estimation of postpartum hemorrhage is more significantly affected by human factors, so there is a certain degree of uncertainty. Therefore, in this study, the difference in hemoglobin before and after delivery was also selected for observation in addition to postpartum hemorrhage. The results show that the timing of pregnancy termination does not affect the incidence of maternal postpartum hemorrhage.

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[5] Li FR. Influence of the timing of pregnancy termina-


Specific Targeting MRI of Chitosan Oligosaccharide Modified Fe₃O₄ Nanoprobe on Macrophage and the Inhibition of Macrophage Foaming Induced by ox-LDL

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1. Introduction

AS is a disease accompanied by an autoimmune response to low-density lipoprotein (LDL) that causes strokes, ischemic heart diseases, and peripheral vascular diseases etc., which has been one of the most usual chronic fatal causes in aged people. Early diagnosis, prevention, and further accumulation inhibition of atherosclerotic plaque have become the main directions of cardiovascular disease research.

In the medical imaging evaluation of atherosclerotic lesions, medical imaging apparatus such as ultrasound,
MRI, computerized tomography (CT), and nuclear medicine have made remarkable breakthroughs over time. Among them, MRI as a non-invasive diagnosis mode has been well applied to analyze the degree of stenosis, vessel wall thickness, and plaque size. It should be mentioned that although MRI has sufficient spatial resolution and good signal-to-noise ratio, however, it is not specific enough to characterize the composition of atherosclerotic plaques. Therefore, application of MR for the assessment of AS, especially at an early stage, has significant limitations. In recent years, the rapid development of molecular MRI nanoprobe has become an essential tool for studying AS under its advantages, including non-invasive, radiation-free, multiplanar imaging, multi-serial imaging, and high soft-tissue resolution, providing a new approach for the early detection of AS plaques.

Iron oxide as MRI contrast agent coupling with the targeting molecules has been used in vascular imaging, macrophage uptake, cell labeling, and cancer hyperthermia. Since common iron oxide nanoparticles are easily phagocytosed by peripheral phagocytes during circulation due to physical properties as well as biological characteristics, which may weaken their imaging performance. In order to enhance the biological compatibility and evade particle agglomerations, polymers, little molecules, surfactants, and others are commonly used to clad nanoparticles. Iron oxide magnetic nanoparticles have been employed as T₂ contrast medium, giving a negative comparison diagram in the ordinary way. In clinically, T₁-weighted images (T₁WI) need a longer repetition time (TR) and echo time (TE) than T₂-weighted images (T₂WI) as well as clearer T₂ images require more advanced MRI equipment, which all greatly increase the cost of clinical examination. Based on the presence of a high-intensity signal within the embolus or intraplaque hemorrhage caused by methemoglobin T₁ shortening, the plaque characterization with T₁WI in MR has facile plaque imaging. With the function of their sizes, superparamagnetic iron oxide nanoparticles (SPIO NPs) may provide a positive contrast in T₁WI. At the same time, it is different from gadolinium-containing contrast agents, which are cytotoxic and tend to accumulate in tissues organs. Iron oxide nanoparticles provide a safer gadolinium-free T₁ contrast agent for MR imaging. In this work, we synthesized PAA modified SPIO NPs as T₁ MRI contrast agent.

Macrophages have been the most extensively studied target to study AS plaques. Numerous studies have shown that macrophages were involved in the process of AS development and were closely related to plaque stability, playing an essential regulatory role in the atherosclerotic pathological process. In the early stage of the disease, inflammatory cells such as monocytes and macrophages enter the damaged blood vessel wall under the chemotactic action of various inflammatory factors and phagocytize lipids to become foam cells. In the late stage of the disease, macrophages, foam cells, lymphocytes, and mast cells are the main components of AS plaques, with macrophages and lymphocytes being the main cellular components in ruptured plaques. CSO are oligomers of chitosan and consist of 3 to 10 units of N-acetylgalactosamine or glucosamine. The CSO has been reported to interact with mannose receptors on the surface of macrophages through N-acetylgalactosamine structures. The mannose receptors on macrophages are consisted of extracellular cysteine-rich region (CR), type II fibronectin region (FN II), and C-type lectin-like region (CTLD). Especially, in the CTLD chains of extracellular mannose receptor, CTLD4 can recognize and bind the N-acetylgalactosamine residues of CSO in CTLD1 - 8. At the same time, with the synergistic participation of CTLD5-8, mannose receptors can bind to ligands more closely and firmly. Miraculously, CSO binding to macrophages significantly enhanced the abundant adenosine triphosphate-binding cassette transporter A1 (ABCA1) on the surface of macrophages, mediating cholesterol efflux out of the cell, and reversing the transport of cholesterol mediated by ABCA1 bound to Apolipoprotein A1 (ApoA1) and high-density lipoprotein (HDL), resulting in a significant decrease in intracellular cholesterol levels. It has also been found that CSO promotes intracellular cholesterol efflux while increasing the level of macrophage autophagy and further inhibiting macrophage frothing. Besides retaining the excellent biocompatibility and non-biototoxicity of chitosan, CSO keeps better water solubility and extral biological activities, including antibacterial, antifungal, antiviral, anti-tumor, exert fat, blood pressure control and hypocholesteremic effects, which have been widely used in antitumor and antioxidant applications. Meanwhile, CSO nanoparticles possess lower haemolysin activity, cytotoxicity and the high encapsulation efficiency made them as an effective carrier. Thus, CSO is expected to be used as the targeting molecule and therapy drug for AS.

In this report, we simply conjugated PAA modified Fe₃O₄ nanoparticles (PAA-Fe₃O₄) with CSO to fabricate the theranostic nanoprobe for AS. Such conjugation was realized via amide bonds between carboxyl groups on the surface of PAA-Fe₃O₄ and amino groups of CSO. CSO not only directs PAA-Fe₃O₄ to bind smoothly to the foaming macrophages via mannose receptors (Figure 1b) and achieves enhanced MR imaging by endocytosis (Figure 1c), but also effectively inhibits the further development of macrophages...
toward foam cells by promoting the out-cell transport of accumulated lipids within the macrophages and reducing the cholesterol content of the cells. The introduction of a CSO coating on the surface of iron oxide nanoparticles will greatly increase biocompatibility, thus facilitating the biomedical application of these nanoparticles and providing new ideas for the diagnosis and treatment of AS.

2. Materials and Methods

Materials

PAA was purchased from Aladdin Co., Ltd (Shanghai, China). Ferric chloride·Hexahydrate (FeCl₃·6H₂O) was acquired from Wokai Biotech Co., Ltd (Shandong, China). Ferrous sulfate·Heptahydrate (FeSO₄·7H₂O) was purchased from Meilunbio Co., Ltd (Shanghai, China). Both 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich (America). CSO (average molecular weight <1000) was ordered from Dibai Biotech Co., Ltd (Shanghai, China). RPMI1640 medium was got from Keygen Biotech Co., Ltd (Jiangsu, China). Ox-LDL was collected from Yuanye Biotechnology Co., Ltd (Beijing, China). The whole cholesterol detection kit was acquired from Suoqiao Biotech Co., Ltd (Beijing, China). Mouse mononuclear macrophage leukemia cells (RAW264.7) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

Synthesis of PAA modified Fe₃O₄ nanoparticles

The synthetic approach for PAA-Fe₃O₄ was referred to a literature previously reported by Kucheryavy et al. [25]. Briefly, a 20 mL amount of 4 mg/mL PAA solution was

Figure 1. Schematic illustration of the synthesis route of CSO-PAA-Fe₃O₄ nanoprobe (a). The mannose receptor on macrophage surface is composed of CR, FN II and CTLDs. Among them, CTLD4-8 can specifically recognize and bind the N-acetylglucosamine residue of CSO, (b). Endocytosis of CSO-PAA-Fe₃O₄ by macrophages (c).
first to remove oxygen by purging (≥50 min) with nitrogen and was heated to 100°C with a magnetic stirrer. After that, 0.4 mL of FeCl₃ & FeSO₄ solution (1.0 mol/L FeCl₃ and 0.5 mol/L FeSO₄) was quickly injected, followed by the addition of 9.0 mL of 28% ammonia solution. After stirred for 15 minutes, 0.6 mL of the FeCl₃ & FeSO₄ solution and 4.0 mL of ammonia solution were infused into the mixed solution every 15 minutes for four times. The obtained solutions were cooled to air temperature and dialyzed (MW=10000) for 72 hours in ultrapure water to remove unreacted raw materials. The PAA-Fe₃O₄ nanoparticles were stored at 4°C for use.

Conjugation of CSO to PAA-Fe₃O₄ nanoparticles

The carboxyl groups of PAA-Fe₃O₄ were first activated by EDC. In details, 200 mg EDC and 10 mL amount of PAA-Fe₃O₄ solution was magnetically stirred at indoor temperature for 15 min. Then, 1 g CSO, and 100 mg NHS were added to the mixed solution and reacted at 37°C for two hours. The obtained solutions were dialyzed (MW=3000) for 48 hours in ultrapure water. The obtained CSO-PAA-Fe₃O₄ solution was stored at 4°C before use.

Characterization of PAA-Fe₃O₄ nanoparticles and CSO-PAA-Fe₃O₄ nanoprobes

The transmission electron microscopy (TEM) images, fourier transform infrared (FTIR) spectra and surface zeta potential as well as the hydrodynamic diameters of fabricated nanoprobe were determined. For TEM characterization, 10 μL amount of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ solution was dropped onto carbon-coated copper grids, respectively. After dried, copper grids were ready for TEM observation and photography. For FTIR scanning, dried PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ powders were dispersed in potassium bromide (KBr) powder to prepare tablets. The TENSOR27 Fourier transform infrared spectrometer was used for scanning in the range of 500-2000 cm⁻¹. To analyze the surface zeta potential, 1 mL of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ solution was placed into the sample cell and analyzed by the Zetasizer Nano ZS90 nanoparticle potential analyzer, respectively.

Determination of iron concentration

The concentration of iron ions was tested by inductively coupled plasma-mass spectrometry (ICP-MS) (Optima 5300 DV, PerkinElmer, USA). 50 μL of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ solutions were mixed with 50 μL of concentrated nitric acid and placed in an oven at 80°C for 30 min, respectively. Then, 350 μL of 5% dilute nitric acid and 1.6 mL of H₂O were added to determine iron concentration by ICP.

T₁ relaxation determinations of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄

The T₁ relaxation time of PAA-Fe₃O₄ (0.066, 0.133, 0.266, 0.399, 0.532 mmol/L) and CSO-PAA-Fe₃O₄ (0.253, 0.337, 0.422, 0.506, 0.675 mmol/L) at different iron ion concentrations were measured by MR scanning. The scanning parameters were settled as follows. For T₁WI, TR 425 ms, TE 14.0 ms, reversal time 200-800 ms, matrix 384 × 224, field of view (FOV) 18 × 18 cm, layer thickness 3.0 mm, layer distance 1.5 mm. The original T₁-map image was processed by GE Aw4.2 workstation to obtain the T₁ relaxation time. The corresponding linear regression equations were plotted using the iron ion concentration as horizontal coordinates and the reciprocal of the samples’ T₁ relaxation time at different concentrations as ordinate to calculate the T₁ relaxation rates.

In vitro cell viability

RAW264.7 cells at logarithmic growth stage were inoculated in two 96-well plates at a concentration of 2 × 10⁴ cells/mL, followed by incubating at 37°C for 24 hours. PAA-Fe₃O₄ or CSO-PAA-Fe₃O₄ with different concentrations of Fe³⁺ (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, and 0.5 mmol/L) were incubated with RAW264.7 cells for 24 hours, respectively. After discarding the nanomaterials and washing the cells in 96-well plates with PBS for three times, a 100 μL of 5 mg/mL MTT solution was injected into each well and placed at 37°C for four hours. Finally, 100 μL of dimethyl sulfoxide (DMSO) was added to dissolve the purple crystals, and the absorbance value of each well was measured at 490 nm by the microplate analyzer.

Effect of CSO-PAA-Fe₃O₄ on the inhibition of macrophage foaming determined by Oil red O staining

6 mL of oil red O solution was diluted to 10 mL in a tube. After standing for 10 min, it was filtered in the dark. 60 μg/mL ox-LDL pretreated RAW264.7 cells were treated with PAA-Fe₃O₄ or CSO-PAA-Fe₃O₄ (Fe³⁺ concentration: 0, 0.15, 0.25, and 0.35 mmol/L) for 48 hours. After fixed with 4% paraformaldehyde for 15 min and rinsed twice with PBS, 500 μL oil red O working solution was added to each well. Dye in the dark for 40 min, and rinse with distilled water twice. The 24-well plates were the placed under an inverted fluorescence microscope (LEICA) for observation. The obtained images were further analyzed using ImageJ software.
Effect of CSO-PAA-Fe₃O₄ on the inhibition of macrophage foaming by TC determination

60 μg/mL ox-LDL pretreated RAW264.7 cells were treated with PAA-Fe₃O₄ or CSO-PAA-Fe₃O₄ (Fe³⁺ concentration of 0, 0.15, 0.25, and 0.35 mmol/L) for 48 hours. The TC amount in RAW264.7 cells were determined following the Total Cholesterol Assay kit instructions. Briefly, 400–500 million cells were collected and centrifuged at 1000 rpm for 20 min. After discarding the supernatant, 1 mL of isopropanol was added and the cells were ultrasonic crushed for 1 min (intensity 20%, ultrasonic 2 s, stop 1 s). Then, the supernatant after centrifugation was collected as TC liquid to be tested. For TC detection, 50 μL TC standard, 50 μL TC sample solution, and 150 μL OD (standard tube) / OD (assay tube) / cell volume (10⁶ cells) C (standard liquid) = 0.5 μmol/mL

In vitro specific MRI

RAW264.7 cells were incubated with PAA-Fe₃O₄ or CSO-PAA-Fe₃O₄ with different Fe³⁺ concentrations of 0, 0.25, and 0.35 mmol/L at 37 °C for two hours. After washed twice with PBS, trypsinized with EDTA-trypsin, and then suspended in 1% sepharose for MRI scanning. GE Signa 3.0 T whole-body magnetic resonance imager and a small animal coil were used for T₁WI scanning.

Statistical analysis

Statistical analysis was performed using SPSS software (version 24.0), and data conforming to a normal distribution are denoted by X ± S. Independent samples t-test was used for comparison between two groups, and one-factor ANOVA was used for comparison between multiple groups when the obtained data were by a normal distribution; otherwise, Welch ANOVA test was used. If results were statistically significant, differences were analyzed by the LSD method or Dunnett’s T3 test. It was used to indicate that the difference was statistically significant when aP-value of less than 0.05 (P<0.05).

3. Results and discussion

Preparation and characterization of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄

PAA was combined with Fe₃O₄ nanoparticles, and then CSO was linked through amide bonds to prepare CSO-PAA-Fe₃O₄ nanoparticles. Learnt from Figure 2a and 2b, PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ were spherically shaped and uniform in size. Both nanoparticles showed good dispersion properties, no adhesion, and exhibited a significant increase in particle size from 5.93 nm to 8.15 nm after the modification of CSO (Figure 2a and 2b). The zeta potentials were also changed with such modification from -31.7 mV for PAA-Fe₃O₄ to 25.13 mV for CSO-PAA-Fe₃O₄ (Figure 2a and 2b). In addition, the average hydrodynamic diameter of PAA-Fe₃O₄ was increased from 95.64 nm to 248.03 nm for CSO-PAA-Fe₃O₄ (Figure 2a and 2b). The T₁ relaxation times of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ at different concentrations were statistically significantly different (F=1311.83, P<0.05; F=1357.21, P<0.05). From the data in Figure 2a and 2b, it is apparent that the T₁ relaxation time of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ decreased significantly with the increase of iron ions concentration. And the r₁ relaxation rates of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ were calculated to be 5.317 m and 6.147 mM⁻¹·s⁻¹, respectively. Both of the r₁ relaxation rates are better than that of Gd-DTPA, commonly used in clinic, showing their possibility as MRI contrast agent. As Figure 2c shows, the FTIR spectra further testified the successful modification of CSO onto PAA-Fe₃O₄ nanoparticles. As shown in Figure 2c, the stretching vibration of C-N bond and tertiary alcohol C-O bond with the absorption peaks at 1321 cm⁻¹ and 1155 cm⁻¹ were observed on CSO-PAA-Fe₃O₄. A prominent absorption peak at 1073 cm⁻¹ coming from the stretching vibration absorption peak of C-O bond in the C-O-C structure on the CSO ring could also be detected, showing the existence of CSO in CSO-PAA-Fe₃O₄. All of the above changes indicated the successful fabrication of CSO-PAA-Fe₃O₄ nanoparticles.

Cytotoxicity assessment of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄

The biocompatibility of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ were assessed by MTT assay. As showed in Figure 3, there were no significant differences in the absorbance values of RAW264.7 cells neither treated with PAA-Fe₃O₄ nanoparticles, or with CSO-PAA-Fe₃O₄ ranging from 0.05–0.50 mmol/L Fe (F=2.138, P>0.05; F=1.904, P>0.05), indicating no significant cytotoxic influence of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ on RAW264.7 cell proliferation and desirable biocompatibility.

Oil red O staining to detect the lipidal aggregation in RAW264.7 cells

To show the influence of our fabricated CSO-PAA-
Figure 2. Characterizations of PAA-Fe₃O₄ (a) and CSO-PAA-Fe₃O₄ (b). (1-4) TEM observations, particle size distribution, Zeta surface potential distribution, and hydrodynamic diameters of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄. T₁-weighted phantom images of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ at different Fe³⁺ concentrations and the relaxation rate fit of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ (5). Fourier transform infrared absorption spectra of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ (c).
Fe₃O₄ nanoparticles on the lipidal accumulation of RAW264.7 cells, 8 groups were set as follows. RAW264.7 cells without any treatment were designated as the blank control group (BLK), and RAW264.7 cells pretreated with 60 μg/mL ox-LDL was set as the model group (NC). RAW264.7 cells pretreated with 60 μg/mL ox-LDL first, and then incubated with different concentrations of PAA-Fe₃O₄ or CSO-PAA-Fe₃O₄ were used as experimental groups. As illustrated in Figure 4, compared with the BLK group, the amount of the intracellular lipid accumulation was significantly increased in NC group. And no obvious change of intracellular lipid accumulation between the PAA-Fe₃O₄ groups and the NC group, showing the little effect of PAA-Fe₃O₄ on the inhibition of lipid accumulation in RAW264.7 cells. But when compared the PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ groups, the difference was signifi-

**Figure 3.** Cytotoxicity assessment of PAA-Fe₃O₄ and CSO-PAA-Fe₃O₄ against RAW264.7 (n=6).

**Figure 4.** The RAW264.7 cells were stained with oil red O, and the intracellular lipids were stained red. No significant lipid accumulation was observed in the blank control group (a), but significant lipidalaggregation in the model group was shown (b). In PAA-Fe₃O₄ groups, the lipidalaggregation was unchanged when the iron ion concentration increased (0.15, 0.25 and 0.35mmol/L) (c-e). The lipid accumulation gradually decreased with the increase of iron ion concentration in CSO-PAA-Fe₃O₄ groups (0.15, 0.25 and 0.35mmol/L) (f-h).
significant and a significant decrease in the intracellular lipid accumulation with a gradual increase of CSO-PAA-Fe₃O₄. Analysis using ImageJ software further presented such difference semi-quantitatively. The amount of intracellular lipid accumulation in the NC group was significantly higher than that in the BLK group (F=83.108, P<0.05). But there was no significant difference in the level of lipid accumulation between the NC group and the PAA-Fe₃O₄ groups (F=0.694, P>0.05) and CSO-PAA-Fe₃O₄ group with a Fe³⁺ concentration of 0.15 mmol/L (F=12.353, P>0.05). However, significant differences existed between the NC group and CSO-PAA-Fe₃O₄ groups with Fe³⁺ concentrations of 0.25 and 0.35 mmol/L (F=15.983, P<0.05) as well as between CSO-PAA-Fe₃O₄ groups (F=98.076, P<0.05). Such phenomena might come from the existence of CSO and the different amount of CSO in CSO-PAA-Fe₃O₄ nanoprobes.

Figure 5. ImageJ analysis of the staining area of RAW264.7 cells by Oil Red O. BLK: Blank group; NC: Model group; #: P level less than 0.05 compared with the model group; *: P level less than 0.05 by paired comparison (n=8).

Determination of the TC concentration of RAW264.7 cells

The absorbance values of RAW264.7 cells induced by 60 μg/mL ox-LDL with or without further incubation with PAA-Fe₃O₄ or CSO-PAA-Fe₃O₄ at different iron concentrations were determined. It was clearly displayed that compared with the BLK group, there was a remarkable increase in the content of TC in NC group (F=35.910, P<0.05). No statistically significant difference of the TC contents in RAW264.7 cells after incubation with PAA-Fe₃O₄ solutions of different iron concentrations (F=3.306, P>0.05). It presented a significant gradual decreasing trend of TC content in cells after incubated with increasing iron ion concentrations of CSO-PAA-Fe₃O₄ (F=35.128, P<0.05). In Figure 6B, there was noteworthy that CSO-PAA-Fe₃O₄ with 0.15 mmol/L Fe³⁺ did not produce a significant difference in the TC content between the CSO-PAA-Fe₃O₄ and NC groups, which is consistent with the results of the previous analysis of the intracellular lipid accumulation content. The difference in the content of TC in the CSO-PAA-Fe₃O₄ group at different concentrations was statistically significant (P<0.05). CSO-PAA-Fe₃O₄ with 0.25 mmol/L and 0.35 mmol/L Fe³⁺ down-regulated the content of TC in RAW264.7 cells to 62.6% and 56.5%, respectively.

Figure 6. TC concentrations in RAW264.7 cells incubated with different concentration ranges of PAA-Fe₃O₄ (a) and CSO-PAA-Fe₃O₄ (b). BLK: blank group; NC: model group; #: P level less than 0.05 compared with the model group; *: P level less than 0.05 by paired comparison (n=6).

Specific MRI of RAW264.7 cells in vitro

As Figure 7 showed, there was a significant difference in T₁ relaxation time between the blank and experimental groups shown by T₁-map color plots. In the Fe³⁺ concentration of 0.25 mmol/L, the T₁ relaxation times of PAA-
Fe₃O₄ and CSO-PAA-Fe₃O₄ group were (800.6 ± 14.30) ms and (647.2 ± 21.91) ms, and the T₁ relaxation time of the PAA-Fe₃O₄ group was significantly longer than that of the CSO-PAA-Fe₃O₄ group. One-factor ANOVA analysis compared the T₁ relaxation times of the BLK group, 0.25 mmol/L iron ion concentration of PAA-Fe₃O₄ group, and CSO-PAA-Fe₃O₄ group, and the differences were statistically significant (F=2418.877, P<0.05). Additionally, the T₁ relaxation times of CSO-PAA-Fe₃O₄ treated cells decreased with the increase of Fe³⁺ concentration (0.15 mmol/L, 0.25 mmol/L, and 0.35 mmol/L), which were (739.4 ± 31.59) ms, (647.2 ± 21.91) ms, and (565.9 ± 26.38) ms, respectively. The results showed that there were statistical differences in T₁ relaxation time between the BLK group and CSO-PAA-Fe₃O₄ group for each Fe³⁺ concentration (F=2310.838, P<0.05). The above MR imaging results showed that the cellular binding amount of CSO-PAA-Fe₃O₄ was significantly higher than that of PAA-Fe₃O₄ at the same Fe³⁺ concentration, and the cellular binding amount of CSO-PAA-Fe₃O₄ increased with the increase of nanoprobes. CSO-PAA-Fe₃O₄ nanoprobes could target RAW264.7 cells and might come from the specific binding of CSO to the mannose receptor of RAW264.7 cells and the endocytosis to achieve the targeted MRI of RAW264.7 cells.

4. Conclusions

In conclusion, targeted MRI and therapeutic nanoprobe based on CSO-PAA-Fe₃O₄ was successfully designed and was successfully developed. In the process of ox-LDL induction of macrophages, these nanoprobes could effectively enter the interior of macrophages and effectively inhibit the transformation of macrophages into foam cells. CSO-PAA-Fe₃O₄ exhibited good T₁-weighted macrophage targeting MRI capability and a high therapeutic effect on the inhibition of foamy macrophages formation.

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Conflict of Interest

The paper authors state that there is no conflict of benefits regarding the publication of this article.

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Effect of Metformin on Lactate Metabolism in Normal Hepatocytes under High Glucose Stress in Vitro

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ABSTRACT

To study the effect of metformin on lactate metabolism in hepatocytes in vitro under high glucose stress. Method: LO2 hepatocytes was cultured in vitro, hepatocytes were randomly divided into blank control group, 25 mmol/L glucose solution, 27 mmol/L glucose solution, 29 mmol/L glucose solution, 31 mmol/L glucose solution, 33 mmol/L glucose solution, 35 mmol/L glucose solution treatment group, after determining the optimal concentration as 31 mmol/L, use 30 mmol/L metformin solution, and then divided into blank control group, normal hepatocytes + the optimal concentration of glucose solution, normal hepatocytes + metformin solution, calculate the number of hepatocytes on cell count plate respectively in the 12 h, 24 h, 48 h, and use the lactic acid kit to determine the lactic acid value of the cell culture medium of normal liver cells + optimal concentration glucose solution and normal liver cells + optimal concentration glucose solution + metformin solution at 12 h, 24 h, and 48 h, respectively. Results: There was no significant change in the lactic acid concentration but significant increase in the number of surviving hepatocytes in the high-glycemic control group compared with that in the high-glycemic control group without metformin. Conclusions: Metformin has no significant effect on lactic acid metabolism of hepatocytes under high glucose stress in vitro, and has a protective effect on hepatocytes under high glucose stress. Based on this, it is preliminarily believed that metformin is not the direct factor leading to diabetic lactic acidosis.

1. Introduction

With the change of people’s lifestyle and habits, the incidence of diabetes is on the rise. At present, 1.01 million new diabetic patients are added every year in China. Of the 59 million people projected to reach 2025, more than 90 per cent are II diabetes (T2MD) [1-3]. China has the largest number of diabetics [4]. Because the development of diabetes involves many factors, the pathogenesis is more complex, and there is a variety of damage mechanisms interaction, can not be cured. Traditional antidiabetic drugs play a good role in controlling blood glucose and delaying
complications, but there are still some limitations and adverse reactions. Metformin is an oral antidiabetic drug of biguanidine. Because of its remarkable antidiabetic effect and clinical application value, metformin is widely used in clinic. Metformin is also regarded as the first-line antidiabetic drug in the world. HOME and DDP experimental studies have demonstrated the safety and efficacy of metformin in the treatment of diabetes [3]. However, with the increase of clinical application of metformin, some side effects have been gradually shown during its use as an antidiabetic drug, among which lactic acidosis (LA) is a concern of clinicians. Patients with lactic acidosis caused by taking metformin, is called as Metformin-related lactic acidosis (MALA). MALA is a rare and severe adverse reaction during metformin treatment because metformin hindered the pathway of lactic acid to glucose in mitochondria, causing Too much lactic acid produced or too little enaminated in the body, leading to metabolic disease, which has a very high mortality rate [6-7]. Studies have shown that metformin-induced lactic acidosis may be associated with severe disease in diabetic patients themselves [8]. It is mainly related to renal insufficiency, hypoxia and so on. Based on this, this paper will explain the effect of metformin on lactic acid metabolism in normal hepatocytes under in vitro high glucose stress, and then explore whether metformin has a direct correlation with lactic acid metabolism. To provide reference for clinical treatment of diabetes and the rational use of metformin.

2. Materials and Methods

2.1 Main Reagents and Instruments

Reagent: Cell selected LO2 hepatocyte strain, DMEM medium, trypsin, penicillin-streptomycin double antibody mixed solution, metformin, 10% fetal bovine serum, lactate assay kit (all purchased from Haikou Ruike Biotechnology Co., Ltd).

Instruments: Microscope, Ultra-clean Biological Table, CO2 Cell incubator.

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<td>2.46 Soil 0.79</td>
<td>3.27 Soil 1.38</td>
<td>3.48 Soil 1.42</td>
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<td></td>
<td>3.89 Soil 1.42</td>
<td>4.79 Soil 1.68</td>
<td>5.24 Soil 2.17</td>
<td>5.89 Soil 2.64</td>
<td>6.23 Soil 2.79</td>
<td>8.98 Soil 2.86</td>
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2.2 Experimental Methods

2.2.1 Cell Culture

LO2 cells were used in a DMEM complete culture medium of 10% fetal bovine serum, cultured in 37℃, 5% CO2 incubator. The culture box was cultured to the cell wall, and the conventional cell culture was changed once ~2 days. When the cell coverage reached 80% and 90%, take a transgenral culture, repeat operation. Freeze a bottle of subcultured cells to avoid accidental lack of LO2 hepatocytes.

2.2.2 A Comparative Experiment 1.2.2 Grouping Model

Establishment of high sugar model

High glucose pretreatment of LO2 hepatocytes was performed before the experiment, was performed (Glucose, G) the optimal concentration test, G concentration gradient is set as 0 mmol/L, 25 mmol/L, 27 mmol/L, 29 mmol/L, 31 mmol/L, 33 mmol/L, 31 mmol/L calculated out as optimal experimental concentration, The lactic acid content was measured by lactic acid kit.

Establishment of the metformin model

Metformin hydrochloride tablets were diluted to 30 mmol/L to cell culture medium and pretreated LO2 hepatocytes for, the culture time is 12 h, 24 h, 48 h.

Establishment of intervention model

The metformin hydrochloride medium with 30 mmol/L concentration and glucose solution with 31 mmol/L concentration were added to the culture bottle. After 12 h, 24 h, 48 h of culture, the lactic acid content was calculated by lactic acid kit.

2.2.3 Proliferation of Hepatocyte by Cell Count

The cells were decomposed by trypsin, digested for a period of time and then added to the culture medium to terminate the digestion. Then the culture medium was removed to the counting board with the liquid transfer gun, and the cells were counted strictly according to the rules of cell counting.
2.2.4 Hepatocyte Proliferation was Determined by Cell Counting Method

After adding metformin solution and high sugar treated cell solution into carbon dioxide cell incubator for 12 h, 24 h, 48 h, the lactic acid value in cell culture medium was determined by lactic acid kit, so as to judge the effect of dimethyl double strand on cells with high sugar.

2.3 Statistical Methods

The statistical analysis uses SPSS25.0 statistical software to process the data. The sample mean is used by sample t test, and the multiple mean is analyzed by single factor variance to. P<0.05 was statistically significant.

3. Results and Analysis

The G concentrations of the most suitable hepatocytes LO2 high glucose stress were explored after this experiment, as shown in Table 1, and the optimum experimental concentrations were obtained when the G concentration was 31 mmol/L.

The control group and experimental group were equipped with 30 mmol/L of metformin in culture medium. Normal hepatocytes, high glucose + normal hepatocytes, metformin + normal hepatocytes, high glucose and metformin + normal hepatocytes were designed respectively. The changes of lactate concentration measured in 12 h, 24 h, 48 h of culture were shown in Table 2.

Metformin itself does not produce lactic acid in hepatocytes. In high glucose environment, according to the experimental results of 2 and 4 groups, before metformin was added, The concentration of lactic acid in the culture medium of high glucose + normal hepatocytes increased from 1.42 mmol/L to 3.48 mmol/L, then increased to 5.89 mmol/L after 24 h, The increase rate of cell number began to decrease significantly after 48 h, lactic acid concentration also increased to 9.289 mmol/L. After metformin was added, there was no significant change in lactate concentration and cell number compared with those under high glucose stress.

4. Discussion

A large number of studies have shown that lactic acidosis is a rare and serious complication of diabetes, most of which occurs in patients with biguanidine and accompanied by liver and kidney insufficiency, heart failure and so on [9-10]. Recent studies have found that the association of lactic acidosis (Metformin Lactate Acidosis,MALA) caused by metformin with a normal therapeutic dose is rare, but improper clinical use may also lead to elevated plasma lactate levels and even lactic acidosis (Lactate Acidosis,LA).1 [11]. The liver is an important organ of glucose metabolism. The liver can absorb and use glucose, then reduce blood glucose and can convert glucose into liver glycogen and store it. Increased insulin resistance in cirrhotic patients affects glucose metabolism and causes hepatogenic diabetes. Diabetes also affects the liver, especially in patients with type 2 diabetes, and is prone to liver damage and nonalcoholic fatty liver disease [12]. Metformin, as a traditional antidiabetic agent, can promote glucose metabolism, increase its anaerobic fermentation, increase the level of lactic acid and lead to lactic acidosis. In addition, metformin can inhibit the utilization of lactic acid by liver and muscle, inhibit gluconeogenesis, thus reducing glucose production, thus increasing the risk of lactic acid poisoning [13-15].

In this study, metformin had little effect on lactate metabolism in hepatocytes in high glucose environment. After adding different levels of metformin to the experimental group and the control group, there was no significant difference in lactate content. However, different levels of metformin can promote cell proliferation.

<table>
<thead>
<tr>
<th>Table 2. Effects of metformin on lactate metabolism in normal hepatocytes under high glucose stress</th>
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<td>Group 1 (normal hepatocytes)</td>
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<tr>
<td>Concentration mmol/L of glucose added</td>
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<tr>
<td>Add metformin concentration (mmol/L)</td>
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<td>Concentration of lactic acid (mmol/L)</td>
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<td>Number of surviving hepatocytes (m)</td>
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<td>Group 3(normal group + metformin)</td>
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<tr>
<td>Concentration mmol/L of glucose added</td>
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<td>Add metformin concentration (mmol/L)</td>
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<td>Concentration of lactic acid (mmol/L)</td>
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<td>Number of surviving hepatocytes (m)</td>
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DOI: https://doi.org/10.30564/jams.v4i2.3094
cose environment can inhibit the proliferation of hepatocytes, which may be due to the STC2 expression induced by high glucose, and the overexpression of STC2 can further enhance the inhibition ability of hepatocyte proliferation induced by high glucose. Moreover, studies have shown that high glucose can promote the secretion of TNF-α, IL-6, and other inflammatory cytokines and regulate the expression of apoptosis-related molecules B lymphoma 2 and Bax, thus inducing hepatocyte apoptosis. Metformin can promote the proliferation of hepatocytes, probably because it can inhibit the secretion of inflammatory cytokines and the activity of nuclear transcription factor κB (NF-κB) through AMPK dependent pathway, so as to promote cell proliferation.

To sum up, metformin has no great effect on lactate metabolism in hepatocytes in high glucose environment, but different concentrations of metformin have protective mechanism on hepatocytes and can promote cell proliferation.

References


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Analysis of the Role of General Practitioners Services in Rural Areas during the COVID-19 Epidemic

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ABSTRACT

Since the outbreak of the COVID-19, various regions of China have been rapidly deployed under the leadership of the Central Committee of the Party to actively prevent and control the COVID-19. The rural areas of my country have weak links to the prevention and management of public health emergencies. Problems include lack of medical and health resources and farmers’ low awareness of epidemic prevention. Situations that correspond to the prevention and management of the COVID-19 are more serious. As the patient’s first contact and “gatekeeper” in the fight against the epidemic, the general practitioner is responsible for the “first visit-subsequent ongoing intervention”. This article is about the prevention and control of the COVID-19 epidemics and epidemic prevention in terms of dissemination of knowledge, informed crowd control, joint prevention and control, and standardized management of people. This is a summary of the efforts of general practitioners. Quarantine at home, interactive referrals to medical consortiums, special care for contracted families. The function during the management period aims to analyze the role played by general practitioners during the epidemic and to provide new ideas for the prevention and management of the epidemic. Provide more targeted general practitioner-style services in rural areas to promote the implementation and improvement of health and poverty alleviation. The health level of the rural population provides a theoretical standard.

Keywords:
General practitioner
SARS-CoV-2
COVID-19
Epidemic prevention and management

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), discovered in Wuhan in 2019, is a new type of coronavirus that has never been discovered in humans. It is highly contagious and the source of infection is primarily the patient and the main route. Infections for respiratory droplets and contact infections, all susceptible people are humans. As soon as the epidemic broke out, efforts were made to prevent and control the COVID-19s across the country, making the situation even more severe as the main battlefield for the prevention and control of infectious diseases in vast rural areas. On January 30, the Legislature announced that it would do a better job in preventing and managing the epidemic of the COVID-19 in rural areas. It noted that current epidemic prevention and management is at a critical time, and that rural epidemic prevention is one of the priorities of current prevention and management work. There is an urgent need to take more effective, orderly, scientific and thorough measures to prevent the spread of the infection. The “Notice” fully understands the importance, urgency, complexity and seriousness of epidemic prevention and management work in rural areas, and plays the role of major local medical and medical institutions and local physicians and townships.

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It needs to be fully demonstrated. Responsible personnel should properly perform screening, medical follow-up, pre-examination, triage, and referrals in rural areas. If you have a fever from the area of the epidemic or if you suspect an infection, you should immediately refer to a fever clinic. High-level hospitals that support epidemic tracking and facilitation close contact with confirmed and suspicious cases occurrence areas implement home health care observation measures and enhance health care for returnees and floating populations[1]. The general practitioner is the gatekeeper of the resident’s health and provides contracted residents with general practitioner services, chronic illness management, appointment reservations, and two-way referrals through ongoing health care. Based on the above advantages, general practitioners may be good at contract resident service management and personalized health education, with the government sector conducting major population screening, implementing standardized management and services for isolated observers. You can effectively help you get the job done. This article summarizes the function of general practitioners in the prevention and management of the COVID-19 epidemic.

2. The Role of General Practitioner Contract Services in the COVID-19 Epidemic

2.1 Disseminate Knowledge of the COVID-19 Epidemics and Epidemic Prevention

General practitioners are often the patient’s first contact and, therefore, the “gatekeeper” of the fight against the epidemic. As a trusted and respected community member, general practitioners have established different ways of contacting residents of the jurisdiction and have implemented different channels for publicity and education. General practitioners can also improve their understanding and awareness of epidemic risks. And, through negotiations and connections with governments and leaders, inform the public of the risks of epidemics and the potential to reduce them in an accurate and true way.

New coronavirus science education with timely disclosure of knowledge on prevention and control of COVID-19 epidemics using various communication platforms such as patient health education groups, general practitioner contract groups, and chronic disease management groups will be carried out. Tell residents about new coronaviruses and the spread of respiratory symptoms, self-examination and self-prevention methods. Residents said, “Be cautious and don’t panic. Wash your hands frequently and wear a mask. Don’t party and hang out.”

General practitioners use WeChat to record relevant videos and teach residents “7 steps to wash their hands” and how to wear the correct mask, leading them to personal protection. Provide online psychological counseling to feared residents so they can overcome their fears. Disseminate knowledge about the legal system to residents, raise awareness of legal compliance, and actively report whether or not they have a history of residence in Hubei Province.

General practitioners, along with street offices and police stations, set up epidemic prevention and management promotional points in residential areas and distributed COVID-19 epidemic prevention and management materials, pamphlets, and cartoons to residents. Manage and enhance epidemic prevention and self-defense[2].

General practitioners and community managers use blackboard newspapers, slogans, bulletin boards, LED electronic screens, local loudspeakers, etc., to publicize their knowledge of epidemic prevention and management and promote epidemic prevention and management. It is deeply rooted in people’s hearts and is done scientifically. Guide jurisdiction residents to establish proper preventive management concepts, standardize preventive and management behavior, and improve an individual’s preventive awareness and protective capacity.

2.2 Actively Participate in Joint Prevention and Management, Deploy Informed Crowds, and Stay at the Forefront of Epidemic Prevention and Management

General practitioners teams and townships, communities, village executives, community police, road management personnel, community security, etc., joint prevention to establish epidemics in village groups, communities, settlements, stations, docks, roads, etc. And form a community management work group Prevention and management of entrance and other location checkpoints, carpet tracking and screening of the COVID-19 endemic areas and suspected COVID-19 returnees, major prevention and management target and general prevention and management target discovery, categorized guidance and accurate prevention achievement and control.

Manage epidemic prevention and management through grids, screen layers, and with the help of members of the joint prevention and management team, people returning from endemic areas, or patients with COVID-19 and fever promptly refer or isolate suspects and take relevant control measures. General practitioners participate in joint prevention and manage to make epidemic prevention and management more efficient, truly establish epidemic prevention and management teams, build strong epidemic prevention and management lines of defense. And you can prevent the epidemic. The objectives and effects of epi-
demic, protection of susceptible people, and achievement control of epidemic prevention \[1\].

2.3 Standardize the Management of Isolated People at Home

Home quarantine medical observation is a detailed study at home for subjects who have a history of exposure to epidemics but have the longest incubation period of infectious diseases and therefore have no clinical symptoms based on the evaluation and judgment of medical staff. It refers to a means of medical observation. Major medical and health institutions (requires one person) single room), dedicated personnel will follow up on their health status. Avoid or control the suspicious spread of the new coronavirus \[1\]. The purpose of quarantine observation is to prevent patients from staying and spreading in society through physical quarantine, blocking the source of infection, blocking the route of infection, and avoiding the formation of second and third generation cases. After mastering the list of key populations, members of the Center’s General Practitioner Command and Management Working Group encrypt the list form and assign it to the corresponding general practitioners according to regional management principles. General practitioners do a good job. Being subject to personal protection, come home in time for a village committee executive or police visit, give health notices, temperature measurements, cautions for isolation observations, and have a body temperature (axillary temperature) of 37.3 ° C or higher. Report or arrange for respiratory symptoms such as cough, transfer to a designated fever clinic by the command and management working group, depending on the living conditions and physical condition of the main group of people without respiratory symptoms such as fever, it decide to adopt home-based isolation observation or intensive isolation observation.

On-site inspection on the first day of home observation (detention for inspection): “Notice”, “Health tips”, issuance of promotional leaflets, signing of “Home quarantine observation commitment”, filling out “Health information registration form” distribute mercury thermometers, disinfectant tablets, masks, and medical waste bags, direct their use, and perform initial temperature measurements and symptom investigations, to provide contact information for designated contacts, keep records; separate toilets and other isolation stations notifying home protection, disinfection, nutrition, and use.

Guidance for Home Isolation Medical Observation: (1) The subject of home isolation medical observation must be alone in the room, avoiding going to public places as much as possible, and at the same time, the room and public area are good. Make sure ventilation; if conditions do not allow, keep at least 1.0m away, limit activity and deny all visits. (2) Home Isolation Medical Observation objects should use only tableware and hygiene products, and all contacts should be burned at high temperature or soaked in 84 disinfectants and washed with running water. (3) Persons subject to isolated medical observation must wear a medical surgical mask when they come into contact with their families, and other families must wear a medical surgical mask when entering the space where isolated people live. (4) Home isolation Breastfeeding mothers who are subject to medical observations. (5) Breastfeeding mothers who are subject of home isolation medical observation can continue to breastfeed their babies, but must correctly choose and wear medical masks and maintain hand hygiene when breastfeeding. Wash hands with soap and running water before breastfeeding, or wash hands with alcohol-containing hand disinfectants. (6) If the home isolation medical observer has suspicious symptoms during the observation period, including fever, cough, sore throat, chest tightness, dyspnea, anorexia, fatigue, poor spirits, nausea and vomiting, diarrhea, headache, palpitation, conjunctivitis, quadriplegia.

Isolation management: Measure body temperature every morning and afternoon, ask and record symptoms, and provide personalized health education and psychological counseling.

Family management of home quarantine personnel: Outbreaks of the COVID-19 occur primarily in families, accounting for more than 83%. We find it particularly important to provide personalized health education to the families of home quarantine observers to prevent family infections. General practitioners provide health guidance to the family of home quarantine observers, such as wearing surgical masks, maintaining ventilation in the room, avoiding access to the quarantine observation room and contacting home quarantine observers, separating at least 1m away, paying attention to hand hygiene (if you come in contact with items from the isolation room, you should, in principle, disinfect and then clean), doing not share the toilet and tableware, guiding mental health, improving the ability of families to prevent plague \[4\].

Cancellation of home quarantine: According to the deadline for quarantine observation, the body temperature is measured 1 hour before the expiration of the quarantine period to check the health condition, normal body temperature, no symptoms such as fever or cough. If not, we will issue a “Health Observation Announcement Notice” to announce medical observations. Home isolation medical observations are an important means of preventing and controlling the spread of the epidemic.
2.4 Make the Most of the Medical Consortium’s Two-way Referral System to Face Public Health Emergencies

National and local governments enhance hierarchical diagnostics and treatments, timely triage patients, and avoid “execution” of superior medical resources in major hospitals with medical consortiums and internet diagnostics and treatment consulting. Meanwhile, it provides residents with online consultation services on new coronary pneumonia, fever clinics, and other chronic diseases, provides home isolation and guidance for mild patients, and promptly refers severe patients to designated hospitals. Meanwhile, designated hospitals will guide patients with stable diagnosis and treatment plans to nearby medical institutions for isolation and rehabilitation [3]. As an important step and system innovation in deepening medical reform, the medical consortium is important for adjusting and optimizing the structure of medical resources, promoting the downward shift of the focus of medical and health work and sinking of resources, improving the ability of grass-roots services, promoting the integration of medical resources, and improving the overall medical service system. Effectiveness, better implementation of graded diagnosis and treatment, and meeting the health needs of the people play an important role.

2.5 Help Key Populations Prevent and Control Epidemics

The special population in this article is the type of special that requires long-term medical care for physiological or illness reasons and has more serious consequences than the general population after being infected with a new coronavirus such as the elderly, children and patients with chronic illness. The purpose of caring for a special group is to (1) reduce the crowd of hospitals caused by non-emergency medical services, thereby reducing the risk of infection. (2) By improving the health of such populations and reducing diagnostic pressure. We recommend canceling or replacing medical activities that may attract crowds with online activities (such as health education).

Children: Vaccination of children during an epidemic can be suspended or postponed according to the relevant requirements of the county’s disease prevention and control agency. For newborn visits, feeding guidance, and growth and development assessments, we recommend using video calling as a priority.

Pregnant and Lying Women: Manage pregnant women, distribute folic acid tablets for long-term treatment, and instruct them to make appointments or cross-peak pregnancy tests. Perinatal examinations are recommended by phone or videophone. If on-site service (such as post-operative suture removal) is required, disinfection and isolation measures should be taken to ensure safety.

Chronic Disease Patients: During the epidemic of the COVID-19, be sure to use long-term prescriptions to reduce the frequency of chronic disease patients while ensuring the safety of medications for chronic disease patients. Medical staffs at primary care institutions provide telephone follow-up guidance for patients with chronic illness, timely intervention and return visits for patients who do not meet management criteria, and for patients with acute complications. Need to provide prompt treatment. In special circumstances, the drug may be delivered to your door.

Patients with Mental Illness: Follow up by phone with people in this group to improve medication compliance, detect side effects of medications and poor illness management in time, and ensure medication supply is needed.

Vulnerable groups living alone: We can provide the necessary home health services for people with restricted mobility [1].

3. Discussion

3.1 Information Management of Home Quarantine Personnel to Improve Work Efficiency

The management of home quarantine medical observers should focus on the use of informatization and improve work efficiency. For example, the Tianjin Municipal Health Commission organized the pilot application of “smart voice outbound” technical services in primary medical and health institutions in various districts, established accounts with general practitioner teams, and served residents in the districts through rapid and batch telephone follow-up and SMS notification. Assist in carrying out health follow-up, disease screening and health education for key populations. After launching the intelligent voice outbound call system, you only need to set up the call task, perform a one-key voice call, and check the results after the task is over. The data statistics are very clear and clear at a glance. It has greatly liberated the grassroots health manpower and devoted more time to serve patients and fighting the epidemic [4].

3.2 Innovative Epidemic Prevention and Control Model, Accurate Prevention and Control

The experience of medical staff in the Community Health Service Center of Wenhui Street, Xicheng District, Hangzhou City in adopting the “8541” trilogy is worth learning. In other words, “8 visits, 5 home monitoring services, 4 visits”. The “File” work initiative standard-
izes and refines the work process with three links: visit, home monitoring, and quarantine. “Eight Ones” refers to surgical masks, home care observation notifications, 1-call systems, 3 + 1 WeChat groups, thermometers, health education materials, and Chinese herbal medicine packages for antisense. Medical Pack Garbage Bags; “Five Services” refers to the five aspects of health services, including medical monitoring, health counseling, drug delivery, psychological counseling, and personnel transfers. “Four confirmations” refers to confirmation of identity, time, temperature, and symptoms. Significant improvements in work efficiency have enabled physicians to more accurately assist people in need, allowing grassroots frontline preventive and managers to have more energy in the areas most needed to prevent and manage epidemics.

3.3 Improve Traditional Medical Models and Develop Step-by-step Diagnostics and Treatments for Medical Consortiums

According to a study conducted by Liu Qiaoyan et al., one of the reasons for the lack of two-way referrals between medical consortia is that it is difficult to change the medical habits of patients and they still tend to go to large hospitals. Recent studies have shown that patients have changed their tendency to seek treatment during the epidemic. During this period, more patients prefer to seek treatment in the community. Therefore, from this perspective, the patient’s tendency to seek treatment can change for a period of time after the epidemic, which facilitates further step-by-step diagnosis and treatment. The main measures taken by the local healthcare community and Beijing Medical Consortium during the Guangdong epidemic are: (1) Organize member units of the medical consortium to provide online diagnostic, therapeutic and protective training related to new coronary pneumonia, and new coronary pneumonia and scientific protection (training) by personnel of the medical consortium unit. Awareness (2) Promote community referral appointments and improve rational allocation of limited medical resources (community patients move up); (3) Emergency infusion patients are transferred to the core Emergency infusion pressure to reduce staff gathering to ease hospitals (emergency infusion down transfer).

Chengdu District has two nearby tertiary A’s to establish a green referral channel so that patients of all kinds can be referred to higher level hospitals in a timely manner during epidemic prevention and management. We have taken the initiative to establish contact with the hospital’s fever clinic. Patients in need of referral will be contacted by a dedicated representative protected according to COVID-19 protection requirements, accompanied by a local physician and sent to a high level hospital for fever. For residents who are not enthusiastic but have a clear history of exposure, special personnel and special vehicles will be sent to the central quarantine point in accordance with COVID-19 protection requirements.

3.4 Transform the Chronic Disease Management Model and Rationally Implement Long-term Prescribing Policies

The impact analysis of Weng Lili and others on promoting long prescriptions on community-related willingness to chronic disease patients indicates that 95.8% of patients will seek the help of a general practitioner as soon as they encounter an emergency. General practitioners are responsible for managing chronic illnesses. I have an important responsibility. Reducing the movement of local residents is one of the important preventive and control measures to effectively control the spread of new coronary pneumonia, and to reduce the number of patient visits, the Shanghai Municipal Health Commission Appropriate extension of prescriptions for patients with chronic diseases in outpatient clinics, outpatient diagnosis for chronic diseases with clear diagnosis, stable condition and long-term use of therapeutic agents during epidemic prevention and management period. And according to the doctor’s assessment of treatment, you can prescribe medication for up to 3 months at a time. The results of the study show that long-term prescribing strategies effectively reduce the number of visits to patients with chronic illness during the epidemic and are beneficial in the prevention and management of the epidemic. However, because it is not relevant, medication knowledge and guidance for patients with chronic illness, side effects may occur. Therefore, general practitioners need to stay in touch with patients with chronic illnesses and provide ongoing service through all non-contact communication channels such as WeChat, email, mobile phones and landlines. Relevant national studies demonstrate that during the period of epidemic prevention and management, general practitioners need to use WeChat groups of patients with chronic illness to organize peer education and achieve good results.

3.5 Strengthen the Service Capabilities of Primary Medical Institutions and Focus on Human Resource Development

The shortage of general practitioners is a bottleneck in the development of contract services for general practitioners. Currently, there are about 30,000 general practitioners in my country. According to international standards, there is one general practitioner for every 3,000
inhabitants, but my country needs about 430,000 general practitioners, which is well below the international standard of about 93%. I will. Today’s general practitioners are primarily backed by former local physicians who are older, of lower overall quality, and have uneven technical skills. Some physicians are primarily engaged in clinical diagnosis and treatment and have little or no contact with public health services. “There is still a big gap with the requirements of a general practitioner with general medical knowledge such as prevention, healthcare, medical care and rehabilitation, and providing comprehensive, continuous, timely and personalized medical and healthcare services. Therefore, there is an urgent need to strengthen the construction of talent teams for general practitioners, increase talent training, and establish fixed general practitioner service teams. On the one hand, talent needs to be secured and absorbed by encouraging general practitioner education, signing orders with districts and counties for targeted free training, setting special employment plans, evaluation and recruitment, etc. There is. On the other hand, in order to improve the human resources development system unique to each region, it is necessary to strengthen re-education such as standardized training for general practitioners [9].

4. Suggestions

4.1 Properly Extend Training Time at Grassroots Practice Bases

The training system in the United States [10] clearly stipulates that the training period for general practitioners is three years. The standardized training system for residents in my country has its origins in the United States [11]. The training period for general practitioners is 3 and 10 years, of which the total training period for general practitioners is 10 months. Enrichment of training as the first line of defense for the prevention and management of major infectious diseases, general practitioners perform the important task of performing community grid management with the municipal sector and “carpet-style” investigations of sudden sources. I will be in charge. Patient burden important responsibilities such as follow-up and follow-up of suspicious patients, early detection, epidemic education, remote management of patients with chronic diseases. Labs at grassroots practice bases to ensure that trainees have a strong ability to prevent and control outbreaks.

4.2 Establish an Effective Positive Incentive Mechanism for General Practitioners

Through the combination of soft remuneration and hard remuneration, soft remuneration includes working environment, work itself, career development opportunities, training opportunities, etc., and hard remuneration includes wages, benefits, etc. Through irregular training, strengthen the communication between community hospitals and higher-level hospitals, select doctors to study in higher-level hospitals, strengthen the continuing medical education of the general practitioner team, and improve the professional skills of general practitioners. Improve the performance appraisal system. The appraisal team will determine the final appraisal result, and the person under appraisal will determine the appraisal result. The two parties discuss the problems that arise in the appraisal result, propose solutions and improvement measures, and establish a performance feedback mechanism to achieve a positive interaction between the two parties and promote the general practitioner services.

It is also possible to link medical insurance with the contracted services of general practitioners, learn from the intervention of the medical security system in Changning District, Shanghai, and realize the reasonable allocation of health resources. Through medical insurance prepayment, the general practice can be fulfilled in accordance with the effective number of contractors, service quality and effects Doctors’ contracted service fees provide incentives for general practitioners’ contracted services [12].

4.3 Highlight the Characteristics and Advantages of Traditional Chinese Medicine in Community Prevention

Combine the Chinese medicine prevention project for new coronary pneumonia with the contracted services of general practitioners. One is to distribute Chinese medicine to residents who are isolated at home on time; the other is to go to communities and rural areas and give free Chinese medicine; Third, the health center has set up a free-drinking Chinese medicine decoction to facilitate the people who come to see the doctor to drink.

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The Application of Block Chain Technology in Medical Management

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ARTICLE INFO

ABSTRACT

With the gradual development of social economy, the social function of medical institutions is becoming more and more important. Strengthening the efficiency and quality of medical management can alleviate the contradiction between doctors and patients and improve the level of medical service. Promoting the sustainable development of medical institutions plays a key role. Under the background of the development of science and technology, the theoretical research and practical application of block chain technology are becoming more and more mature, which can not only store and transmit information effectively, but also strengthen the security of information storage. The effective combination of block chain technology and medical management can create a new operation mode and train of thought for medical management and promote the overall improvement of medical management quality and efficiency. It is very helpful to optimize the social service function in medical field. This paper mainly expounds and probes into the concept characteristics of block chain technology, its concrete application in medical management, application effect control strategy and so on. It aims to further strengthen the depth and breadth of application of block chain technology in medical management, provide strong power support for the improvement of medical management service level and promote the sustainable development of medical and health industry.

Keywords: Blockchain technology, Medical management, Information, Application

1. Introduction

The function of medical institutions in social development is increasing, the business is increasing gradually, and the pressure of medical management is also increasing day by day. It is necessary to optimize and innovate the traditional medical management mode. In order to meet the high demand of medical management in the new period, block chain technology is a decentralized distributed record and storage database.

2. Overview of Blockchain Technology

(1) Concepts and characteristics

Block chain technology is a decentralized distributed bookkeeping technology. By constructing a database, the related data are recorded and stored distributed, and different blocks are effectively combined by using the chain mode. In forming a systematic data structure system, block chain technology has the characteristics of non-tampering, traceability, openness, security, data recording sequence and so on [1]. These characteristics of block chain technology, it strengthens its decentralization and de-trust application core, and simplifies the process of data application. In general, block chain technology is a distributed linked account book, and it is encrypted and

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managed by complicated cryptography to ensure that the account data can not be tampered with, and all the data in the account book can be traced back.

(2) Characteristics of application of blockchain technology in medical management

The block chain can generate large data for each link of medical service management, such as medical record information, hospital internal information, drug use information and so on, and cause non-tampering, and ensure the security of its data information. The main application features are as follows: using the decentralization of block chain, combining cloud storage technology, expanding the storage space of medical information, and realizing seamless connection and sharing of information data; using public-private key consensus mechanism the anonymity and confidentiality of data information in the sharing process, and strengthening the privacy security of medical management information.[2].

3. Problems of Medical Management in the Context of Information Technology

At present, the process of medical information management informatization is not very ideal, which seriously affects the improvement of its comprehensive level of informatization and limits the improvement of the efficiency of medical service industry in China. The main performance is: medical information sharing is difficult to achieve, due to the lack of unified block chain technology application standards and norms, many medical institutions hold a wait-and-see attitude, and in their own interests, there is resistance to information sharing; Lack of perfect medical service management quality supervision mechanism, leading to medical service quality problems, serious doctor-patient contradictions, seriously affect the healthy development of China’s medical industry; The traditional medical insurance claim process is more cumbersome, inefficient, time-consuming, and the compensation results are prone to deviation and other problems, seriously reduce the patient’s medical service experience, but also bring some obstacles to the sharing of medical information.[3].

Application of blockchain technology in medical management

(1) Establishment of electronic health records

Using blockchain technology can establish personalized electronic health files for patients and store them in data sharing centers. When patients seek medical treatment, doctors can quickly and intuitively understand and view the patient’s medical records, disease history and other information, and can use the traceability characteristics of blockchain technology to trace the traces of medical activities received by patients in the past. In the electronic health file, patients can also view their own examination results data, such as doctor diagnosis results, electrocardiogram, imaging and other data information, so as to have a clearer understanding of the condition and health planning.[4,5]. In addition, using the relevant functions of block chain technology, we can also make comprehensive encryption settings for electronic health files, which can not only share data, but also ensure data information security, let patients set their own access rights, and set up custom encryption methods to ensure patient privacy.

(2) Strengthening the whole process of drug supervision

Drugs play a key role in human health. Once drugs are made and sold, they will pose a serious threat to the life and health of patients. Therefore, the block chain technology can be used to track and record the production, sale, management and use of drugs dynamically, and the whole life cycle of drugs can be traced back as the main basis of drug market supervision. When patients buy and use related drugs, they can upload relevant data to the information sharing center and compare with the relevant information of the database constructed by block chain technology, so as to identify and judge the truth and hood of drugs. Can not only protect patient life safety, but also strengthen drug market supervision and effect.[6].

(3) Medical insurance claims

In the traditional medical insurance claim mode, the policyholder needs to go through many links and processes to get the claim payment, in which he has to pay the medical expenses —— obtain the expense list —— the insurance company claims and so on. Because the claim company involves the problem of data information confidentiality in the process of docking with the hospital, the process is often complicated and long, time-consuming and inefficient. Under the background of block chain technology, it can effectively improve the efficiency and experience of medical insurance claims. This is because the block chain technology has the characteristics of non-tampering and traceability, which can record the trace of data change in detail and comprehensively, thus ensuring the security of data information. Based on this, a block chain platform can be constructed to store related information data distributed to enhance the comprehensiveness and security of data information. Moreover, the data information can not be tampered with to prevent the dispute over the related contract in the process of claim settlement, and through the effective fusion of block chain technology and artificial intelligence technology, the related cost information, contract and so on can be intelligently verified, thus
providing the basis for the automatic execution of this process\cite{7,8}. The hospital and the insurance company jointly construct the intelligent contract block chain platform, form the data sharing account book, realize the medical insurance information sharing, simplify the medical insurance claim process, shorten the application time, improve the overall working efficiency of the medical management, and strengthen the medical service experience.

(4) Improving medical records of electronic operations

The block chain technology is used to record and store the surgical records comprehensively, and its non-tampering and traceability characteristics are used to ensure the authenticity of the original data, which is convenient to provide the basis for the investigation of medical malpractice responsibility and to clarify the relevant responsible persons\cite{9,10}.

4. Control Strategy for Application of Blockchain Technology

(1) To sum up experience and improve the promotion effect

The application of block chain technology in medical management started late, practical application experience is less, in the concrete application is still in the groping stage, the operation effect is affected to a certain extent. Moreover, the application of block chain technology poses a great challenge to the previous medical management model, and some medical institutions dare not apply it in depth, which leads to its promotion and application effect is not ideal. And in the domestic and foreign application, it has not formed the unified use standard and the basic standard. Many medical institutions adopt the wait-and-see attitude to it. Based on this, it is necessary to publicize blockchain technology in a wider range, strengthen people’s overall understanding and understanding of it, and enhance their confidence in application. Build a good blockchain academic research atmosphere and environment, build a perfect blockchain medical management knowledge training system, strengthen the learning motivation of medical managers, strengthen the academic interest of experts, and ensure the deep application of blockchain technology in medical management\cite{11,12}.

(2) Expansion of data storage space

The block chain database records and stores the dynamic changes of each data from generation to development, and it also takes up part of the space during the download, transfer, update and use of its data. Especially when the data of all nodes run synchronously, it brings great pressure to the storage capacity of the database. It is very likely that the medical information can not be updated in time because of the insufficient storage capacity, which affects the efficiency of medical management. Based on this, we should pay attention to strengthening the scalability and affordability of block chain database, optimize and perfect its decentralized storage system, expand its storage capacity, and build a decentralized business model in combination with specific conditions. Expand storage space and build a global scale of hard disk storage space\cite{13}.

(3) Enhanced data security

Because block chain technology runs under the background of network information technology, even if it takes diversified encryption measures to ensure the security of block chain data, but because of the open characteristics of network technology, There are still some risks in data security and secret security. Based on this, we should optimize the key mechanism, improve the private key storage mode, strengthen its encryption effect, integrate the dynamic encryption technology and DES algorithm encryption technology, give full play to the functional advantages of complex cryptography. The access rights of users are effectively controlled and block chain data are encrypted to ensure the privacy of medical data\cite{14,15}.

5. Conclusions

To sum up, with the trend of diversification of medical institutions, the quality and efficiency of medical management quality and efficiency of medical management. In combination with the concept and technical characteristics of block chain, we should explore the convergence of block chain technology and medical management, strengthen the effective combination of the two, give full play to the technical advantages of block chain, and carry out deep application in electronic health files, drug supervision, medical insurance claims, surgical information records, etc.

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Clinical Study of Endovascular Treatment of Severe Middle Cerebral Artery Stenosis or Occlusion and Vascular Cognitive Impairment

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ABSTRACT

It is very important to study the factors affecting the incidence, progress and prognosis of patients with vascular dementia. 50 cases of severe middle cerebral artery stenosis or occlusion underwent endovascular treatment (25 cases of mild cognitive dysfunction, 25 cases of moderate cognitive dysfunction) were divided into two groups, where a medical drug treatment group and a control group established with 25 cases in each group. The cognitive function of each group of patients was evaluated before operation, 7 days after operation, 30 days after operation, and 180 days after operation. CTP was used to compare the hemodynamic changes in patients before and after operation. The severe stenosis or occlusion of the middle cerebral artery in patients can be improved, and the intracranial blood supply of patients with poorly compensated medial cranial circulation and hypoperfusion can be restored to a certain extent. Meanwhile, improvement of cognitive function was definitive in some patients with cognitive dysfunction. To guide the formulation of treatment plans for patients with severe middle cerebral artery stenosis or occlusion.

1. Introduction

In recent years, vascular cognitive dysfunction has become the second most common cause of Alzheimer’s disease in China, and the incidence is only lower than Alzheimer's disease. Non-dementia vascular cognitive dysfunction refers to early or mild cognitive impairment caused by cerebrovascular injury, and does not necessarily progress to vascular dementia; if it can be diagnosed and treated early in the VCIND stage, the course of the disease may reverse. Intracranial artery stenosis is closely related to the incidence of cognitive dysfunction, and endovascular treatment can better improve cerebral ischemia. The middle cerebral artery mainly supplies blood to the temporal lobe, parietal lobe and basal nucleus of the brain.

The more severe the stenosis of the middle cerebral artery segment, the higher the degree of cognitive impairment. Insufficient cerebral perfusion may be an important cause of cognitive impairment in patients with cerebral artery stenosis [1]. The mechanism of cognitive dysfunction caused by cerebral hypoperfusion may include: long-term ischemia leading to chronic cerebral ischemia, hypoxia, and anaerobic glycolysis, which induces phosphorous metabolism disorders in the brain hippocampal neuron membrane, producing excessive free radicals and releasing a large amount of excitatory amino acids, leading to intracellular calcium ion overload and other problems, resulting in hippocampus neurotransmitter disorder and neuron loss, causing cognitive impairment; white matter

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lesions are independent risk factors for cognitive dysfunction, long-term chronic ischemia and hypoxia can lead to diffuse demyelination changes in the white matter of the brain and accelerate cognitive decline; cerebral ischemia and hypoxia promote the production of β-amyloid, accelerate the aging and apoptosis of nerve cells, and lead to the decline of cognitive function. In addition, decreased cerebrovascular reserve capacity is also related to declined cognitive function. The pathogenesis of VCI is more complicated and the incidence is high. The incidence of VCI is increasing year by year, and the cost of treatment is relatively high. However, it is currently the only cognitive impairment disease that can be intervened. Recanalization of blood vessels may restore blood supply to brain tissue, and patients with severe stenosis or occlusion of the middle cerebral artery with cognitive impairment may benefit from it.

2. Materials and Methods

2.1 Materials

50 patients with severe stenosis or occlusion of the middle cerebral artery (25 cases with mild cognitive impairment, 25 cases with moderate cognitive impairment) and 50 patients who received internal medications, hospitalized in provincial Grade III Level A hospitals from January 2019 to April 2020 were selected.

2.2 Methods

From January 2019 to April 2020, 25 patients with mild to moderate cognitive impairment with severe stenosis or occlusion of one middle cerebral artery were included. The group receiving endovascular treatment was assigned as treatment group and the group receiving non-endovascular treatment was assigned as control group. CTP inspection was conducted. Inclusion criteria: ① Diagnosed with mild to moderate cognitive dysfunction by Montreal Cognitive Assessment (MOCA) screening (MOCA score ≤2 pts); ② DSA showed severe unilateral middle cerebral artery stenosis or occlusion; ③ Indications for endovascular treatment. Exclusion criteria: ① Have a history of dementia and psychiatric diseases; ② Severe middle cerebral artery stenosis or occlusion causes blood supply area infarction; ③ Severe systemic diseases or neurological deficits such as severe aphasia, unable to cooperate with cognitive function examination; History of alcohol, drug abuse, and psychotic drug abuse.

2.3 Examination Methods and Standards

With the subject’s consent and cooperation, the Montreal Cognitive Assessment (MoCA) was used to evaluate and diagnose the subject’s cognitive function. The assessment was arranged to take place in a quiet room whenever possible, and an experienced and professionally trained neurorehabilitation physician was appointed to conduct the assessment and record the assessment score. MOCA was conducted 1 week before operation, 7 days after operation, 3 months and 6 months after operation on the treatment group and control group during the same period. The MOCA scoring of cognitive function was evaluated by senior neurologists. The total score of MOCA score is 30 points. If the score is less than 26, it is considered that there is cognitive dysfunction. If number of years of education is less than 12 years, 1 point is added to the original score. Meanwhile, ReHo analysis was applied to the preprocessed resting state functional magnetic resonance imaging (MRI) data, and the data analysis was performed with the brain function data processing software developed by Beijing Normal University. Calculation was performed until each voxel of the whole brain reached consistency in time series with its 26 neighboring voxels in the surrounding. In the CTP examination, a CT scanner was used to determine cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT) and peak time (TTP), and then the CBF ratio (rCBF) of the affected side to the healthy side, CBV ratio (rCBV), MTT difference (dMTT) and TTP difference (dTTP) were calculated respectively.

2.4 Statistical Analysis

SPSS statistical analysis software was used. The measurement data are expressed as x±s. The independent sample t-test was used for comparison between the treatment group and control group, and P<0.05 was considered as statistically significant.

3. Results

(1) Comparison of Treatment Efficacy

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<th>Table 1. Comparison of Treatment Efficacy</th>
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<tr>
<td>Group</td>
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**4. Discussions**

At this stage, patients with severe middle cerebral artery stenosis or occlusion have mainly three treatment options: endovascular treatment, surgical treatment and medical drug treatment. In recent years, with the development of endovascular treatment technology, continuous improvement of interventional devices and improvement of operational proficiency, the status of endovascular treatment in the middle cerebral artery is being valued by more and more scholars [2]. However, there are not many studies on the intravascular treatment of the middle cerebral artery. The follow-up time is short, so the patients’ long-term patency is not clear, and the long-term follow-up of hemodynamics is insufficient, especially the cognitive dysfunction is rarely assessed in some patients. Experimental and clinical studies have shown that for mild to moderate middle cerebral artery stenosis, the use of drug therapy and stenting can improve cognitive function; for severe middle cerebral artery stenosis or even occlusion, drug treatment cannot improve the cognitive function. However, interventional therapy can significantly improve intracranial blood supply, thereby improving human cognitive function. After patients with severe middle cerebral artery stenosis or occlusion underwent endovascular treatment, their executive and memory ability may be significantly improved [3].

Vascular cognitive impairment refers to cognitive impairment syndrome, which ranges from mild cognitive impairment to dementia caused by cerebrovascular incidents. In many studies, the detection sensitivity of MOCA for mild vascular cognitive impairment is much higher than that of MMSE, which helps in the early diagnosis of vascular cognitive impairment and prevent vascular dementia in time. Therefore, in this study, we used the Montreal Cognitive Assessment Scale to assess the cognitive function of the subjects. The Montreal Assessment Scale showed that there were significant differences in visual spatial, executive function, language and delayed recall.
between the treatment group and the control group, but the two groups had no significant difference in naming, concentration, abstraction and direction \[^8\]. In this study, central arteriovenous occlusion was assigned as the treatment group and endovascular treatment was performed. The blood flow of the middle cerebral artery was improved, but the patients’ left-side visual spatial, executive, language and memory dysfunction, and concentration were not significantly different from those of the control group. Therefore, in this study, we found that patients with acute central arteriovenous occlusion have been successfully treated and recovered. Although they still have cognitive impairment, their concentration has been improved.

Current research shows that cerebral hemodynamic diseases caused by cerebral artery stenosis can be divided into four stages; stage 0 means that cerebral hemodynamics is completely normal; stage 1 means that as the cerebral perfusion pressure decreases, the body performs differently in level of actions. Cerebrovascular self-regulation dilates cerebral arterioles to reduce vascular resistance, and then reduce the ability of normal CBF by maintaining brain tissue, but at the expense of cerebrovascular reserve and CVR; the second stage is the further reduction of cerebral perfusion pressure. The cerebrovascular bed has reached the maximum expansion state, but the self-regulation ability of the cerebrovascular still cannot maintain the normal CBF of the brain tissue, and it is accompanied by the reduction in CBF and CVR failure. However, at this stage, the normal metabolism of brain tissue can be maintained by increasing oxygen intake. In the third stage, the oxygen uptake could not be maintained, but the decreased cerebral blood flow showed that it could not meet the continuous decrease of cerebral perfusion pressure of normal brain tissue.

Cognitive function is a complex high-level brain function activity, accomplished not through a single brain area or neural structure but through multiple brain functional areas and neural structures \[^5\]. Studies have shown that memory functions involve many cortex and cortical structures. When brain damage occurs, the frontal lobe, temporal lobe, hippocampus, target gyrus, thalamus and midbrain reticular structure may further reduce \[^6\]. Another study showed that the visual spatial function is accomplished by the frontal, temporal, parietal and occipital lobes, thalamus, basal ganglia and cerebellum in the two cerebral hemispheres \[^7\]. The results of this study show that middle cerebral artery infarction can impair visual spatial, executive, language and memory functions, and it supports cortical and subcortical structures to participate in related cognitive activities through specific neural networks or circuits formed. Cerebral artery infarction can cause cognitive impairment in multiple areas \[^8\].

Secondly, there is little difference in the good prognosis of neurological function between the two groups in this study, which may be affected by the sample size. Due to the relatively small sample size, the difference is unclear, but it can be seen that the endovascular treatment group has a higher good prognosis than the non-vascular treatment group. The study found that the speech score of the treatment group was lower compared with the control group, which indicates that patients receiving endovascular treatment and those who have recovered from acute middle cerebral artery occlusion still have language disorders.

5. Conclusions

There is no obvious breakthrough in the treatment of acute middle cerebral artery occlusion and ischemic stroke, and the clinical prognosis of patients after medication is often not ideal. Therefore, in the real world, many neurologists perform endovascular treatment on these patients according to the guidelines, and the results are mixed. Among them, patients with middle cerebral artery occlusion recovered well after endovascular treatment, but their cognitive function was significantly lower than that of the control group. The two groups had significant differences in visual space, executive function, language and delayed recall, but no significant differences in naming, concentration, and direction. Compared with previous studies, the concentration of patients with acute middle cerebral artery occlusion who were successfully treated and recovered has improved. The functional strength of patients with middle cerebral artery occlusion after endovascular treatment is different from that of the control group.

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The Significance of Cytoskeleton System in Tumor Cell Infiltration

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ABSTRACT

Cytoskeleton system is mainly composed of three kinds of fibrils: microtubules, microfilaments and intermediate filaments. They are a complex network of protein filaments in the cytoplasm of eukaryotic cells. They not only act as scaffolds in cells, but also play an important role in maintaining the movement of cells, participating in the material transport and signal transmission in cells. It is found that the whole cytoskeleton system is closely related to tumor invasion and growth. Therefore, this article reviews the overview of the cytoskeleton system and its significance for tumor cell invasion and growth.

1. Overview of the Cytoskeleton System

The cytoskeleton system refers to the protein fiber network framework in eukaryotic cells. It is a three-part system consisting of microtubules (MT), microfilament (MF) and intermediate filaments (IF) composition. The three are highly coordinated and distributed, and are connected with the nucleus, cytoplasmic membrane, and organelles to form a cell morphology skeleton and movement coordination system to maintain the shape of the cell and maintain the function of cell movement, and have important significance for signal transmission. The cytoskeleton system, the genetic system within the cell, and the biofilm system are collectively called the “three intracellular systems”.

2. The Structure and Function of Microtubules and Tumor Cell Infiltration

Microtubules are hollow tubular structures with a diameter of 24-27 nm and an inner diameter of about 15 nm. They are distributed in the cytoplasm and nucleus of many cells. The tube wall is surrounded by 10-13.5 nm protofilaments. The tube length varies from a few microns to a few centimeters. Microtubules can be assembled into single tube, double tube and triple tube, which are found in structures such as cilia, centrioles and spindles, respectively. Microtubules have functions related to cell support, movement and cell division. In addition, it also participates in the transport of intracellular substances. Microtubules constitute the reticular scaffold of cells to maintain cell morphology; participate in cell contraction and pseudopodia movement; participate in the displacement of organelles, especially the division and displacement of chromosomes, which require the help of microtubules. It may also participate in the transportation of substances in the cell, and may play a role in the microcirculation system of transporting macromolecular particles in the cell. The infiltration and metastasis of tumor cells is one of the biological characteristics of malignant tumors. The active mobility of tumor cells is an important factor in infiltration and growth. Microtubules, one of the components of...
the cytoskeleton system, are dispersed in the cytoplasm and move in normal cells. And it plays an important role in the activities of tumor invasion.

In traditional concepts, microtubules determine the shape of cells and play a role in mitosis and organelle transport. However, recent studies by Gomez et al. showed that although the structure of microtubules has an impact on cell morphology, changes in cell shape have a greater impact on the structure of microtubules. They used Drosophila embryonic cells as target cells, treated the cells as nearly elliptical, and used the aspect ratio, eccentricity, and microtubule standard deviation and microtubule deviation as indicators to compare cell shape changes and microtubule structure changes. They first knocked out the embryonic cell’s blastocyst contraction factor, so that the cells could not perform blastocyst contraction, and found that the aspect ratio of the embryonic cell during the same period dropped by 43%, the eccentricity dropped by 10%, and the microtubule standard deviation increased by 45%. Microtubule deviation increased by 141%. They also transfected the Gal4 promoter into the cells to promote apoptosis and change the morphology of the cells. They found that the aspect ratio of the cells decreased by 26%, the eccentricity decreased by 4%, and the standard deviation of microtubules increased by 14%. The above changes are the changes that occur only after the cell morphology changes, not the changes brought about by gene mutations. Subsequently, they completely destroyed the intracellular microtubule structure by making the cell express the microtubule shearing protein spastin, and found that the morphology of the cell did change (P=0.02), but the degree is lighter (the aspect ratio is reduced by 25%, and the eccentricity is reduced by 1%).

The structure of microtubules in the cell is constantly undergoing the conversion of depolymerization and repolymerization. This process is called microtubule dynamics. Depolymerization is the transformation of microtubules from growth to shortening, and repolymerization is the transformation from shortening to growth. Previous studies believe that microtubules are constantly damaged during the aging process and are more prone to disaggregation. Recent studies have shown that depolymerization is easy to occur at the positive end of the microtubules that are initially formed. However, once the positive end begins to extend, the ability to disaggregate rapidly declines. The process of depolymerization and repolymerization of microtubules is affected by microtubule-associated proteins (MAPs) and motor proteins. MAPs include microtubule structure-related proteins, microtubule positive end tracer proteins and so on. Microtubule structure related proteins include MAP1, MAP2, MAP4 family and Tau protein. They are called structurally related proteins because they do not exist.

The enzyme activity is only bound to the surface of the microtubules, thereby enhancing the stability of the microtubules. The positive end of the microtubule tracer protein specifically acts on the growth end of the microtubule and regulates the growth of the microtubule. Early research believed that microtubule structure-related proteins are mainly distributed in the nervous system and perform physical functions. MAP1 is divided into three subtypes: MAP1A, MAP1B, and MAP1S. MAP1A and MAP1B are mainly distributed in neurons and play a role in guiding the formation of axons; MAP1S is widely expressed in cells and regulates cell division and autophagy. MAP2 is the highest content of neurotubule-associated protein. It exists in the entire neuron in the early developmental stage of the neuron. Then MAP2 in the axon disappears and only exists in the dendrites. MAP4 is distributed in a variety of cells and also plays a role in stabilizing the structure of microtubules. Tau protein can promote the polar formation of neurons.

With the elongation of axons, the abnormality of Tau protein can lead to many kinds of neurodegeneration Lesions. In recent years, it has been discovered that in addition to affecting microtubule motility, microtubule structure-related proteins may also be related to the occurrence and development of tumors. Tessema et al. screened the methylation level of gene promoters on 117 frozen case specimens of non-small cell lung cancer, 5 human bronchial epithelial cell lines, 5 human small airway epithelial cell lines, and 23 non-small cell lines. It was found that the CpG island of the promoter of the MAP1B gene has a significantly higher methylation ratio in tumor tissues, and the methylation ratio is higher in the case of COPD combined 68%, not 37%, and the tumor gene map project. The information in the database matches. However, there is no difference in the expression level of MAP1B in tumor tissues and non-tumor tissues. Therefore, the expression of MAP1B is related to tumor transformation, but the specific mechanism remains to be further studied. Bauer et al. collected pathological specimens of breast cancer patients who underwent surgical treatment after paclitaxel adjuvant chemotherapy, extracted total RNA from 14 cases and performed gene chip analysis, and found that in the cases of complete pathological remission, the expression of MAP2 gene was higher than that of incomplete remission. The number of cases is 4 times higher. They collected MAP2 mRNA in 5 breast cancer cell lines and found that the expression of MAP2 was highly correlated with paclitaxel sensitivity in vitro (correlation coefficient R2>0.99). Later, they used two groups of breast cancer
cell lines MCF-7 and MDA-MB-468 to overexpress MAP2, and the two groups of cell lines were treated with paclitaxel and found that the number of cells decreased by 53.7% and 46.4%, respectively. In addition, in 47 needle biopsy cases, patients with high MAP2 expression had a higher percentage of complete remission. Therefore, MAP2 can play a synergistic effect with paclitaxel and can be used as a biomarker of paclitaxel sensitivity. This may be related to the effect of both MAP2 and paclitaxel on microtubules, which promotes cell cycle arrest in G2/M phase. Yang et al. used Western blot and real-time quantitative polymerase chain reaction to detect the Tau protein in three prostate cancer cell lines, and found that two of them had expression. Subsequently, they cultured the two cell lines into docetaxel-resistant cell lines and found that the expression of Tau increased, and the expression of Tau was positively correlated with the PI3K/Akt/mTOR pathway. In addition, silencing the wild-type and drug-resistant strains of Tau inhibited the growth of tumor cells and increased the sensitivity to docetaxel. Therefore, Tau can play an antagonistic effect with paclitaxel and can be used as a biomarker of docetaxel sensitivity, which may be related to the competition between Tau and docetaxel for the binding site of microtubules. The above results all suggest that microtubules are related to the occurrence, development and treatment of tumors, but further research is still needed to reveal the relationship between the two.

3. The Structure and Function of Microfilaments and Tumor Cell Infiltration

Microfilaments are solid filamentous structures with a diameter of 5-7nm, which are distributed in the cytoplasm and nucleus of most cells, but myofilaments in the cytoplasm of the cells are the most developed. Long and short filaments are connected to each other and surround all organelles. Microfilaments can exist in the form of monofilaments, or form a network, or they can exist in bundles. Actin is the main component of microfilaments, and it exists in two forms in the body: actin monomer (G-actin, also known as globular actin) and fibrous muscle assembled from actin monomers F-actin. Among them, the actin monomer is a globular protein with a molecular weight of about 43kDa, which is divided into three types: α, β, and γ according to the isoelectric point [1]. The complex intracellular cytoskeleton network composed of microfilaments and their related regulatory proteins participate in most of the biological behaviors in life. In malignantly transformed cells, cells often show the destruction of cytoskeleton and abnormal aggregation of microfilaments. The infiltration and metastasis of tumor cells are related to the changes in the expression of microfilaments and related proteins. The abnormal aggregation of microfilaments can enhance the mobility of tumor cells.

In order to clarify the functions of various actins, researchers have established different types of knockout mice. Kumar et al. tried to establish α-cardiomyocyte type knockout mouse models. The mice died during embryonic or perinatal period, and the myocardial fibers were severely disordered, suggesting that α-cardiomyocytes are closely related to the formation of cardiomyocytes. Crawford et al. established the α-skeletal muscle type gene knockout mouse model and found that their skeletal muscle function is very weak, and they all died on the 9th day after birth, suggesting that the α-skeletal muscle type is necessary for muscle contraction. Schildmeyer et al. established an α-smooth muscle type gene knockout mouse model and found that the expression of α-skeletal muscle type was increased compensatorily, although the cardiovascular system of mice developed normally. However, the vasoconstriction ability becomes weaker, the blood pressure is lower than that of normal mice, and the blood flow rate becomes slower, suggesting that α-smooth muscle type regulates vasoconstriction, which is closely related to arterial tension and the activity of myofibroblasts. Kumar et al. overexpressed the γ-smooth muscle type gene in α-cardiomyocyte type knockout mice, and some mice survived, but when they became adults, symptoms such as cardiac insufficiency and myocardial hypertrophy appeared, suggesting that γ-smooth muscle type could be partially replaced. The role of α-cardiomyocytes in cardiomyocytes. Shawlot et al. tried to establish a β-cytoplasmic sub-equivalent gene mouse model, all of which died of non-specific exhaustion early in life, suggesting that the β-cytoplasmic type is necessary for cell survival. Belyantseva et al. established a γ-cytoplasmic knockout mouse model and found that these mice are thinner than wild-type or heterozygous types in the early developmental stage, and some can survive to adulthood and are fertile, but a large part of them are due to development. Delayed death, and some random deaths in adulthood, suggesting that γ-cytoplasmic type is related to growth and development. In the cell, actin is divided into two forms: monomericactin (G-actin) white monomer and filamentous actin (F-actin) polymer. F-actin is a long-chain fiber composed of multiple G actins, and two F-actins are combined in anti-parallel to form a spiral chain, which exerts physiological functions. The nucleotide binding site of each molecule of G-actin binds to one molecule of ATP and connects with one Mg²⁺ or Ca²⁺ to form an actin ATP-divalent cation complex. When G-actin is combined with F-actin, ATP is hydrolyzed to ADP, which provides energy for the process.

Actin is widespread in eukaryotic cells. In most cells,
Cells or melanoma T1C1 cells. Simiczjew et al. [2] overexpressed β-cytoplasmic and γ-cytoplasmic type in human colorectal adenocarcinoma LS174T cell line and found that the ratio of F-actin to G-actin increased, indicating the degree of actin polymerization increased, and observed under a phase-contrast fluorescence microscope, the vesicles of the cell membrane grow actively. The above results all suggest that the increase of actin content can enhance the exercise ability of tumor cells.

Cell movement is closely related to the formation of cell membrane protrusions. Moving cells will protrude two kinds of pseudopods at the front, namely filopodia and lamellipods. The formation of pseudopodia relies on the formation of actin microfilament skeletons under the cell membrane. In lamellipodia, G-actin aggregates into F-actin, and F-actin forms a cross branch network; in filament pseudopodia, F-actin forms a parallel bundle structure. Cell movement is relying on pseudopodia to crawl. The formation of pseudopodia is mediated by Rho family GTPase. The formation of lamellipodia is mediated by Rac1 protein, and the formation of filopodia is mediated by Cdc42 protein. Chen et al. analyzed the expression of Rac1 in 150 cases of lung cancer tissues and 30 cases of adjacent lung tissues, and the expression of Cdc42 in 110 cases of lung cancer tissues and 30 cases of adjacent lung tissues. The results showed that Rac1 was expressed in 94/150 (62.67%) of lung cancer tissues, and almost not expressed in lung tissues adjacent to cancer; Cdc42 was expressed in 80/110 (72.73%) of lung cancer tissues, and almost expressed in lung tissues adjacent to cancer.

Almost not expressing. The expression levels of Rac1 and Cdc42 are significantly positively correlated with lymph node metastasis, TMN staging and pathological differentiation. The five-year survival rate of Rac1-negative patients is 32.14%, and the five-year survival rate of positive patients is 17.02%; the five-year survival rate of Cdc42-negative patients is 36.67%. The positive rate is 13.75%, indicating that the occurrence, development and prognosis of Rac1 and Cdc42 tumors are closely related. In cell experiments, they used scratch experiments and invasion tests to confirm that the expression levels of Rac1 and Cdc42 are positively correlated with the motility of cells. After being stimulated by epidermal growth factor, Rac1.

Darby grows more lamellipodia than Rac1 silenced cells, and this process is effected through the Rac1-Pak1 pathway; Cdc42 expresses more filopodia than Cdc42 silenced cells. When the expression levels of Rac1 and Cdc42 are down-regulated, tumor cells are less resistant to anti-tumor drugs such as nedaplatin and curcumin.

Increased sensitivity. The above results all indicate that the expression level and distribution of actin filaments affect the migration and invasion of tumor cells, and the regulatory factors related to filaments may be a new target for tumor treatment.

4. The Structure and Function of Intermediate Filaments and Tumor Cell Infiltration

Intermediate filament (IF) exists in the cell cytoplasm and is a tubular structure with a diameter of about 8-11 nm. Because its diameter is between microfilament and microtubule, it is called intermediate filament. The composition of the intermediate filament is more complicated than that of microtubules and microfilaments. Intermediate filaments are composed of intermediate filament proteins. According to different biochemical characteristics such as immunological and electrophoretic properties, intermediate filament proteins can be divided into five types: cytokeratin, flexible paper-like protein, intermyolin, and glial fibril fibrils. Acidic protein, nerve filament [2]. The expression of intermediate filament protein in tumor cells is closely related to the degree of differentiation of tumor cells.

5. The Importance of the Cytoskeleton System to the Infiltration and Growth of Tumor Cells

Human malignant tumor cells, especially tumor cells that are located around the tumor and infiltrate the surrounding tissues, have pseudopod-like cytoplasmic protrusions, well-developed microfilament meshes inside, and increased filamentous actin aggregates of microfilaments. It is easy to interact with myosin and cause contraction, which is of great significance to the movement and invasive growth of cancer cells. In the process of tumor cell movement, microfilaments are the most important structural skeleton that constitutes the lamellar pseudopodia of the motor cells, and the adhesion bands and adhesion plaques are the physical connections between the extracellular matrix and the stress fibers formed by the microfilaments in the cells. The coordinated aggregation of multiple microfilaments can generate a prominent force on the cell surface, drive the extension of the plasma membrane at the front edge of the cell to form pseudopodia, and promote the movement of tumor cells [3]. In breast cancer, the
actin-related protein ARP2 and WAVE2 are co-expressed, which affects the structure of microfilaments, increases pseudopodia, and enhances cell motility. It is closely related to the invasiveness of breast infiltrating ductal carcinoma cells, so it can be used as one of the prognostic indicators of invasive breast cancer. After the fascin is inhibited, it can block the production of filopodia, reduce the nuclear movement and deformability of tumor cells, thereby inhibiting the migration and invasion of tumor cells. Zhang Hongying, Yang Guanghua et al. studied the relationship between the cytoskeleton and three different human rhabdomyosarcoma cell lines with different metastatic potential and found that the number of microfilaments and microtubule skeletons in rhabdomyosarcoma cells is reduced and dysplasia. The potential is negatively correlated (P<0.05); the frequency of actin bodies is positively correlated with the metastatic potential of rhabdomyosarcoma (P<0.05); there is no obvious abnormality in the structure of intermediate filaments in rhabdomyosarcoma, and there is no difference in fluorescence intensity between the two significance (P>0.05), it is concluded that the abnormality of the cytoskeleton of different degrees may be related to the different infiltration and metastasis potential of rhabdomyosarcoma, and it may become one of the indicators for judging the malignancy and prognosis of rhabdomyosarcoma. Lu Rui, Ke Yang and other studies on human gastric cancer cell line BGC-823 showed that the microfilament skeleton assembly state in tumor cells is negatively correlated with the ability of infiltration and metastasis.

6. Summary

The current research has a relatively systematic and clear understanding of the cytoskeleton system. The growth and infiltration of tumor cells is a multi-stage process and is closely related to the complete microtubule system in the cell. We still lack effective treatments to inhibit the infiltration and growth of tumor cells. The close connection between the cytoskeleton system and the infiltration and growth of tumor cells can provide new ideas for the treatment of tumors.

Funding

(1) The second batch of Chinese medicine clinical research bases in China
   Number: 2018
   Source: State Administration of Traditional Chinese Medicine

(2) The prevention and treatment of tumor complications and the leading team of Chinese medicine research and development technology
   Number: 2020TD04
   Source: Shanxi Provincial Health Commission

(3) Exploring the effect of Huangqi Liuyi Decoction on the 5-FU treatment of colon cancer model rats based on the immune regulation mechanism
   Number: 2019PY-015
   Source: Shanxi University of Traditional Chinese Medicine

(4) A clinical study on the treatment of colorectal cancer with liver metastasis of Qi deficiency syndrome by the method of invigorating the middle and replenishing qi combined with chemotherapy—a randomized, parallel controlled, multi-center clinical study
   Number: 201903D321225
   Source: Shanxi Provincial Department of Science and Technology

References

The Clinical Characteristics and Incidence of Pulmonary Tuberculosis of 7632 HIV Patients in Yunnan Province from 2005 to 2017

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ABSTRACT

Objective: To analyze the age and gender distribution characteristics of 7,632 HIV/AIDS patients at the onset of HIV infection-related high-risk intravenous drug abuse and sexual contact in Yunnan province. Methods: Data were collected from the database of Chinese Medicine Treatment of AIDS Pilot Project in Yunnan province. Gender, age and demographics of HIV/AIDS patients were analyzed. Results: The patients were almost in relatively high educational background. The number of male intravenous drug users (12.90%) was more than female, and the earliest average age was 10-14 years. The percentages of men in 10-19 years and 35-59 years were more than that of women. No obvious difference was found in heterosexual sexual contact in both men (48.11%) and women (51.89%), and the earliest ages was 15-19 years in males and 10-14 years in females. The percentage of males at 10-34 years old was less than that of females, just opposite to the age of 35-85 years. Homosexual contact was more in males (92.73%) than that in females (7.27%). The earliest homosexual sexual contact associated with HIV infection was 15-19 years in males and 25-29 years in females. Among 128 AIDS patients with pulmonary tuberculosis infection, intravenous drug abuse accounted for the highest proportion (76.56%) of the three high-risk behaviors related to HIV infection. Conclusions: Reducing risk behaviors and preventing intravenous drug abuse could be effective in preventing AIDS. Compared with other high-risk behaviors, patients with intravenous drug use and AIDS are at greater risk of contracting tuberculosis.

Keywords: Yunnan province HIV/AIDS High-risk behavior Age Gender

1. Introduction

Yunnan Province is an AIDS epidemic area in China[1]. Yunnan is located near the Myanmar, Laos and Vietnam borders, where the AIDS epidemic currently spreads rapidly and is very serious. It is a major point of drug entry from the “Golden Triangle” and is used for major drug trafficking routes. Yunnan has numerous minority popu-
lations with unique cultures and health habits. Moreover, their cultural health quality and self-protection awareness are relatively poor, leading to intravenous drug abuse and sexual contact as main routes of HIV infection. The percentage of patients with HIV infection-related high-risk intravenous drug abuse and sexual contact varies with age and gender. Needle or syringe sharing is common among people who inject drugs, which leads to HIV and other blood-borne diseases transmission. Men who have sex with men (MSM) and heterosexuals are the populations with the fastest growing HIV infection rates in China, which indicates that reducing unhealthy sexual activity should be put forward as a priority. Pulmonary tuberculosis is a common chronic infectious disease and a common complication of AIDS patients. Studies have found that tuberculosis in the world is related to AIDS, and it is still on a gradual upward trend. For AIDS patients with pulmonary tuberculosis, dual infection will cause the disease to develop rapidly and worsen, and then lead to death.

In the present study, we analysed the age and gender distribution characteristics of 7,632 HIV/AIDS patients at the onset of HIV infection-related high-risk intravenous drug abuse and sexual contact in Yunnan Province between September 2005 and October 2017, and the incidence of tuberculosis in different high-risk behaviors, providing a scientific basis for future specifically planned safety education and management programs, for improvement of the prevention and control measures of AIDS, and for the reduction of the associated morbidity and mortality.

2. Materials and Methods

Data source

Data were obtained from a special version of the ClinResearch data management system created for the Chinese Medicine Treatment of AIDS Pilot Project; ClinResearch is an Electronic Data Capture (EDC) software system hosted and developed by the Academy of Chinese Medical Sciences. This system records patients’ basic information before treatment, including gender, age, ethnicity, marital status, educational background, route of infection, HIV antibody diagnostic report, and age at onset of infection-related high-risk behaviour, and dynamically records patients’ treatment situation.

Study design

Data cleaning and quality verification were conducted on the ClinResearch database in Yunnan Province. From September 2005 to October 2017, all effective cases who consulted or were treated at any of the 44 treatment points in the nine prefectures and cities in Yunnan Province (including Dehong Prefecture, Honghe Prefecture, Dali Prefecture, Wenshan Prefecture, Chuxiong Prefecture, Kunming City, Lincang City, Pu’er City, Baoshan City, Yuxi City, Zhaotong City and Qujing City) and were infected via intravenous drug abuse or sexual contact were collected. Cases with incomplete information, two or more duplicate records, lack of detection results and missing route of infection data were excluded. Additionally, patients’ gender and age at the onset of HIV infection-related high-risk intravenous drug abuse or sexual contact (heterosexual or homosexual) were recorded, and statistical analysis was conducted on 5-year age groups. There were no patients younger than 9 years or older than 85 years in this study.

Ethics approval

The content and plan of this study were reviewed and approved by the Ethics Committee of Yunnan Academy of Traditional Chinese Medicine.

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of Yunnan Academy of Traditional Chinese Medicine. Written informed consent was obtained from individual or guardian participants.

Statistical analysis

SPSS 22.0 was used for data analysis. Qualitative data were described as either the number of cases or percentage, while quantitative data were expressed as the mean ± standard deviation. The differences in age among patients with different routes of infection were analysed by the analysis of variance. Analysis of differences in nationality, marital status and educational background among patients with different routes of infection was performed by the chi-square test or the Fisher exact probability test. Among patients of both genders with the same route of infection, the differences in age were analysed using the chi-square test or the Fisher exact probability test.

3. Results

Demographic Characteristics of Patients with HIV Infection-related Intravenous Drug Abuse or Sexual Contact

Gender

Among the 7,632 patients with HIV/AIDS, males accounted for 61.98% and females for 38.02% (Table 1).
Age

There were statistically significant differences in age among males with HIV infection-related high-risk intravenous drug abuse, heterosexual contact, and homosexual contact \((P<0.001)\). Among them, the mean age at the onset of HIV infection-related high-risk homosexual behaviour was the youngest (33.40 years), followed by the mean age at the onset of intravenous drug abuse (35.77 years), and lastly the mean age at the onset of heterosexual behaviour (39.33 years) (Table 1).

No significant difference in age was found among females with HIV infection-related high-risk intravenous drug abuse, heterosexual contact, and homosexual contact \((P>0.05)\) (Table 1).

Ethnicity

Among the three types of HIV infection-related high-risk behaviour, the number of Han patients was obviously larger than the number of minority patients among both males and females \((P<0.05, P<0.001,\) respectively) (Table 1).

Marital status

The differences in marital status among males with HIV infection-related intravenous drug abuse, heterosexual contact and homosexual contact were statistically significant \((P<0.001)\). Among them, the proportion of married males with HIV infection-related high-risk intravenous drug abuse and heterosexual contact accounted for 45.6% and 67.6%, respectively, while the proportion of unmarried males with HIV infection-related high-risk homosexual contact was the highest, accounting for 64.7% (Table 1).

Additionally, a statistically significant difference was found in marital status among females with HIV infection-related high-risk intravenous drug abuse, heterosexual contact and homosexual contact \((P<0.001)\). Among all three types of high-risk behaviour, the proportion of married females was the highest, accounting for 48.2%, 74.9% and 75.0%, respectively (Table 1).

Educational background

Males with HIV infection-related intravenous drug abuse, heterosexual contact and homosexual contact presented significant differences in educational background \((P<0.001)\). Among them, the proportion of males with middle school education with HIV infection-related high-risk intravenous drug abuse and heterosexual contact accounted for 45.6% and 67.6%, respectively, while the proportion of unmarried males with HIV infection-related high-risk homosexual contact was the highest, accounting for 64.7% (Table 1).

### Table 1. Demographic characteristics of HIV/AIDS patients at the onset of HIV infection-related intravenous drug abuse or sexual contact in Yunnan province between 2005 and 2014.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male (n=4730)</th>
<th>Female (n=2902)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intravenous drug abuse</td>
<td>Heterosexual contact</td>
</tr>
<tr>
<td>Age*</td>
<td>35.77±5.85</td>
<td>39.33±11.92</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Han</td>
<td>1733(75.1)</td>
<td>1700(71.7)</td>
</tr>
<tr>
<td>Minority</td>
<td>576(24.9)</td>
<td>670(28.3)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>1052(45.6)</td>
<td>1602(67.6)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>945(40.9)</td>
<td>510(21.5)</td>
</tr>
<tr>
<td>Divorced</td>
<td>291(12.6)</td>
<td>180(7.6)</td>
</tr>
<tr>
<td>Widowed</td>
<td>21(0.9)</td>
<td>78(3.3)</td>
</tr>
<tr>
<td>Educational background</td>
<td></td>
<td></td>
</tr>
<tr>
<td>College or higher</td>
<td>27(1.2)</td>
<td>239(10.1)</td>
</tr>
<tr>
<td>High school</td>
<td>299(12.9)</td>
<td>349(14.7)</td>
</tr>
<tr>
<td>Middle school</td>
<td>1287(55.7)</td>
<td>1020(43.0)</td>
</tr>
<tr>
<td>Primary school</td>
<td>432(18.7)</td>
<td>509(21.5)</td>
</tr>
<tr>
<td>Pre-school</td>
<td>5(0.2)</td>
<td>4(0.2)</td>
</tr>
<tr>
<td>Illiteracy</td>
<td>259(11.2)</td>
<td>249(10.5)</td>
</tr>
</tbody>
</table>

*is the age at the onset of infection-related high-risk behaviors in the patient.

b Fisher exact probability.

DOI: https://doi.org/10.30564/jams.v4i2.3449
accounted for 55.7% and 43.0%, respectively; the proportion of males with technical school education, junior college education or higher with HIV infection-related high-risk homosexual contact was the highest, accounting for 39.2% (Table 1).

Similarly, there were statistically significant differences in educational background among females with HIV infection-related high-risk intravenous drug abuse, heterosexual contact, and homosexual contact ($P<0.001$). Among them, the proportion of females with middle school education with HIV infection-related high-risk intravenous drug abuse and heterosexual contact accounted for 63.2% and 42.6%, respectively; in addition, the proportion of females with high school education with HIV infection-related high-risk homosexual contact was the highest, accounting for 50.0% (Table 1).

**Route of infection**

Among the 7,632 HIV/AIDS patients, 2,651 (34.74%) patients were infected via intravenous drug abuse, 4,926 (64.54%) by heterosexual contact and 55 (0.72%) by homosexual contact (Table 2).

**Age and gender distribution characteristics of patients at the onset of HIV infection-related high-risk intravenous drug abuse**

Among the 2,651 HIV/AIDS patients infected via intravenous drug abuse, the number of males (2,309; 87.10%) with HIV infection-related high-risk intravenous drug abuse was 6.8 times more than the number of females (342; 12.90%). The age of males and females at the onset of high-risk HIV infection-related intravenous drug abuse ranged from 10 to 14 years. The number of males presented a gradually increasing trend from 15-19 years of age that peaked at 30-39 years of age, followed by a gradual decline in the older age groups; no reports on high-risk HIV infection-related intravenous drug abuse were found after 59 years of age. In females, the number of cases showed a gradually increasing trend from 20-24 years of age that peaked at 30-39 years of age, followed by a gradual decline in the older age groups; no reports on high-risk HIV infection-related intravenous drug abuse were found after 59 years of age (Table 2, Figure 1).

The distribution of males and females in each age group: in the 10-19 years age group, the proportion of

### Table 2. Age and gender distribution characteristics of HIV/AIDS patients at the onset of high-risk intravenous drug abuse and sexual contact.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Intravenous drug abuse (n=2651)</th>
<th>Heterosexual contact(n=4926)</th>
<th>Homosexual contact(n=55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male(n,%)</td>
<td>Female(n,%)</td>
<td>X²</td>
<td>P</td>
</tr>
<tr>
<td>10-14</td>
<td>9(0.4)</td>
<td>1(0.3)</td>
<td>0.000</td>
</tr>
<tr>
<td>15-19</td>
<td>5(0.2)</td>
<td>0(0.0)</td>
<td>0.999</td>
</tr>
<tr>
<td>20-24</td>
<td>47(2.0)</td>
<td>1(0.4)</td>
<td>1.941</td>
</tr>
<tr>
<td>25-29</td>
<td>259(11.2)</td>
<td>60(17.5)</td>
<td>11.265</td>
</tr>
<tr>
<td>30-34</td>
<td>687(29.8)</td>
<td>104(20.9)</td>
<td>11.265</td>
</tr>
<tr>
<td>35-39</td>
<td>730(31.6)</td>
<td>98(20.7)</td>
<td>1.216</td>
</tr>
<tr>
<td>40-44</td>
<td>397(17.2)</td>
<td>51(14.9)</td>
<td>1.104</td>
</tr>
<tr>
<td>45-49</td>
<td>143(6.2)</td>
<td>15(4.4)</td>
<td>1.736</td>
</tr>
<tr>
<td>50-54</td>
<td>23(1.0)</td>
<td>1(0.3)</td>
<td>0.953</td>
</tr>
<tr>
<td>55-59</td>
<td>9(0.4)</td>
<td>1(0.3)</td>
<td>0.000</td>
</tr>
<tr>
<td>60-64</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>-</td>
</tr>
<tr>
<td>65-69</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>-</td>
</tr>
<tr>
<td>70-74</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>-</td>
</tr>
<tr>
<td>75-79</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>-</td>
</tr>
<tr>
<td>80-85</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>2309(100.0)</td>
<td>342(100.0)</td>
<td>-</td>
</tr>
</tbody>
</table>

* Fisher exact probability.
males was slightly higher than that of females \( (P>0.05) \); among the 20-34 years age groups, the proportion of males in each 5-year age group was lower than that of females, especially the proportion of males in the 25-29 years age group \( (P=0.001) \); in the 35-59 years age groups, the proportion of males in each 5-year age group was slightly higher than that of females \( (P>0.05) \) (Table 2).

**Age and gender distribution characteristics of patients at the onset of HIV infection-related high-risk heterosexual contact**

Among the 4,926 HIV/AIDS patients infected via heterosexual contact, there was no obvious difference between the number of males (2,370; 48.11%) and females (2,556; 51.89%) with HIV infection-related high-risk heterosexual contact. The youngest age of males at the onset of high-risk HIV infection-related heterosexual contact was 15-19 years; the number of males was the largest in the 30-39 years age group, followed by a gradual decline in the later age groups; no high-risk heterosexual contact was reported after 85 years of age. The youngest age of females at the onset of high-risk HIV infection-related heterosexual contact was 10-14 years; the number of females was the largest in the 25-34 years age group, followed by a gradual decline in the later age groups; no high-risk heterosexual contact was reported after 85 years of age (Table 2, Figure 2).

The distribution of males and females in each age group: in the 10-34 years age groups, the proportion of males in each age group was lower than that of females, especially, in the 10-14, 15-19, 20-24, and 25-29 years age groups \( (P=0.001 \) or \( P=0.026 \) or \( P<0.001) \); in the 35-85 years age groups, the proportion of males in each age group was higher than that of females, especially, in the 35-39, 40-44, 60-64, 65-69, 70-74 and 75-79 years age groups \( (P<0.001 \) or \( P=0.011) \) (Table 2).

**Age and gender distribution characteristics of patients at the onset of HIV infection-related high-risk homosexual contact**

Among the 55 HIV/AIDS patients infected via homosexual contact, the number of males (51; 92.73%) with HIV infection-related high-risk homosexual contact was 12.8 times more than the number of females (4; 7.27%). The youngest age of males at the onset of high-risk HIV infection-related homosexual contact was 15-19 years; the number of males was the largest in the 20-24 years age group, and high-risk HIV infection-related homosexual contact was not reported after 59 years of age. Additionally, the youngest age of females at the onset of high-risk HIV infection-related homosexual contact was 25-29 years (Table 2, Figure 3).

The distribution of males and females in each age group: due to the small number of female patients, those with homosexual contact were distributed between the 25-29 years age group \( (n=2) \), 35-39 years age group \( (n=1) \) and 40-44 years age group \( (n=1) \). In addition, there was no significant difference in the proportion of males and females in each age group \( (P > 0.05) \) (Table 2).

**Analysis of pulmonary tuberculosis in HIV patients with different high-risk behaviors**

Among the 7,632 HIV/AIDS patients, 128 cases were complicated with pulmonary tuberculosis, and the percentage of intravenous drug abuse was the highest (76.56%), followed by heterosexual contact (21.88%) and homosexual contact was the least (1.56%) (Table 3).

**Demographic characteristics of HIV/AIDS patients in Yunnan Province at the onset of HIV infection-related high-risk intravenous drug abuse and sexual behaviour**

Heterosexual contact was responsible for infection in the largest proportion of patients (64.54%), followed by intravenous drug abuse (34.74%), and homosexual contact (0.72%). Among the 7,632 HIV/AIDS patients, males were more prevalent than females, with a ratio of 1.6:1. The mean age at the onset of HIV infection-related high-risk homosexual contact in males was the lowest (33.40 years), while that of heterosexual contact was the highest (39.33 years), as compared to the other two types of high-risk behaviour. No statistical significance was detected in the mean age of females at the onset of the three types of HIV infection-related high-risk behaviour \( (P>0.05) \). Among the three types of high-risk behaviour, Han patients were significantly more prevalent than minority patients among both males and females \( (P<0.05 \) or \( P<0.001) \). The proportions of married males with HIV infection-related high-risk intravenous drug abuse and heterosexual contact were the highest (45.6% and 67.6%, respectively), and the proportion of unmarried males with homosexual contact was the highest (64.7%). The proportions of married females with three types of HIV infection-related high-risk behaviour were similar. As compared to the other two types of HIV infection-related high-risk behaviour, there was a higher proportion of both males and females with homosexual contact who had a high educational background, perhaps due to having more disposable income to spend on sex \([10-11]\).

In this study, the males with high-risk homosexual
Figure 1. Graphical representation of the number of different age brackets of patients with HIV infection-related intravenous drug abuse. Male was colored in black, and female was colored in grey. The data of $n$, $P$ and $\chi^2$ were shown in Table 2.

Figure 2. Graphical representation of the number of different age brackets of patients with HIV infection-related HIV infection-related high-risk heterosexual contact. Male was colored in black, and female was colored in grey. The data of $n$, $P$ and $\chi^2$ were shown in Table 2.

Figure 3. Graphical representation of the number of different age brackets of patients with HIV infection-related HIV infection-related high-risk homosexual contact. Male was colored in black, and female was colored in grey. The data of $n$, $P$ and $\chi^2$ were shown in Table 2.
contact were young and unmarried, with advanced educational degrees, as compared to those with other high-risk behaviour, perhaps because an open attitude towards sex, an active sex life and multiple sexual partners are common among younger MSM. The high frequency of changing sexual partners, unsafe sexual behaviour, and frequent mobility are also contributing causes.[12-15]

**Age distribution characteristics of HIV/AIDS patients in Yunnan Province at the onset of HIV infection-related high-risk intravenous drug abuse**

Since the HIV antibody was first detected in injecting drug users (IDUs) of Yunnan Province in 1989 [16], intravenous drug abuse has been the main cause of the spread of AIDS in that region. Although the annual incidence of HIV/AIDS in China has been stable in recent years, the patterns of transmission have evolved over time [17,18]. In Yunnan, although HIV infection in IDUs has fallen [19], it is still one of the leading causes of HIV infection. Among the 2,651 HIV/AIDS patients infected via intravenous drug abuse in this study, the age of both males and females at the onset of HIV infection-related high-risk intravenous drug abuse was 10-14 years. Our finding is not unique since a high level of risky injecting behaviour has been observed among younger IDUs in India, Canada and the United States [20-25]. The age group with the most males and females was 30-39 years; however, these people often spread HIV to the general population through sexual contact [24-25]. HIV infection-related high-risk intravenous drug abuse was not reported in males and females after 59 years of age, which could be due to the increasing HIV/AIDS mortality among IDUs with increasing age [26].

Males with HIV infection-related high-risk intravenous drug abuse were 6.8 times more prevalent than females, which may be associated with greater social pressure and more temptation in males [27]. After using drugs, females face greater social pressure, disapproval and rejection than males [28]. In addition, females prefer less addictive drugs; thus, intravenous drug abuse occurs more commonly in males. In the 10-19 years age groups, the percentage of males was higher than that of females, suggesting an apparent tendency of intravenous drug abuse in younger males [29], which may be related to more curiosity for new things in male adolescents [30]. The percentage of males with intravenous drug abuse in each 5-year age group between 35-59 years was higher than the percentage of females with intravenous drug abuse, perhaps due to greater social and life pressures, tension, anxiety, depression and other negative emotions [31]. Between 20-34 years of age, the percentage of males with intravenous drug abuse in each age group was lower than the percentage of females with intravenous drug abuse, perhaps due to less educational opportunities, lower cultural health quality and a lack of survival skills in the females in this age group [32].

**Age distribution characteristics of HIV/AIDS patients in Yunnan Province at the onset of HIV infection-related high-risk heterosexual contact**

Unsafe sexual behaviour, such as early sexual intercourse, unprotected sex and multiple sexual partners, are closely correlated with unintended pregnancy, sexually transmitted diseases, HIV infection and other diseases that harm reproductive health [33,34]. Our study demonstrated that among the 4,926 HIV/AIDS patients infected via heterosexual contact. Meanwhile, a previous study showed that from 1985 to 2014, HIV-1 transmission in China is increasing faster among those with homosexual contact [7], which seems different from the results of our investigation. This may be due to the unique Yunnan culture, where education and concepts of people lag behind, comparatively. That is, homosexual contact may not be accepted by the majority of people. So heterosexual contact and intravenous drug abuse play more important roles in HIV transmission. In our study, the youngest age of males at the onset of high-risk HIV infection-related heterosexual contact was 15-19 years, and the number of males infected via heterosexual contact was the highest at 30-39 years of age. Among females, the youngest age at the onset of high-risk HIV infection-related heterosexual contact was 10-14 years, and the number of females infected via heterosexual contact was the highest at 25-34 years of age, which may be related to the physiological characteristics of female puberty, lack of reproductive health and AIDS education, and the inclination for unprotected sex [35-37]. Younger adolescents were more likely to report that they wished they had waited to have sexual intercourse, but for many, sexual behaviour at an early age can take a serious toll on both psychological and physiological well-being [35]. After 85 years of age, no HIV infection-related high-risk heterosexual contact was reported in both males and females, which may be associated with a gradually increasing hypoactive sexual desire [38].

There was no obvious difference between males and females with HIV infection-related high-risk heterosexual contact. Between 10-34 years of age, the percentage of males with heterosexual contact in each 5-year age group was lower than the percentage of females with heterosexual contact, which may be associated with earlier sexual development in females, lower sexual protection awareness and lack of effective AIDS prevention measures in young females [39,40]. In addition, the percentage of males...
in each age group between 35-85 years was higher than the percentage of females.

**Age distribution characteristics of HIV/AIDS patients in Yunnan Province at the onset of HIV infection-related high-risk homosexual contact**

Among the 55 HIV/AIDS patients infected via homosexual contact, there were 12.8 times more males than females, which may be associated with the inclination of unprotected sex, a higher anal insertion rate and a higher rate of being penetrated in homosexual males. In addition, the youngest age of males at the onset of HIV infection-related high-risk homosexual contact was 15-19 years, and the number of males was the highest in the 20-24 years age group, resulting, potentially, from frequent sexual behaviour, multiple sexual partners, easy drunken sexual intercourse and vulnerability to sexual abuse in young male homosexuals. Moreover, HIV infection-related high-risk homosexual contact was not reported in males after 59 years of age. The age of females at the onset of HIV infection-related high-risk homosexual contact was 25-29 years.

**The incidence of pulmonary tuberculosis of 7632 HIV-related high-risk intravenous drug abuse and sexual contact patients in Yunnan Province**

AIDS and pulmonary tuberculosis are common clinical infectious diseases, and the co-infection of the two poses great challenges to the prevention and control of infectious diseases. Due to the immunodeficiency of AIDS patients and the influence of inflammatory factors, the probability of infection with exogenous Mycobacterium tuberculosis is significantly higher than that of non-AIDS patients. It was found in this study that among 128 AIDS patients with pulmonary tuberculosis infection, intravenous drug abuse accounted for the highest proportion of the three high-risk behaviors related to HIV infection, which may be related to the increased risk of tuberculosis of drug users due to the closed and group use of drugs.

**3. Discussion**

From September 2005 to October 2017, among the 7,632 HIV/AIDS patients surveyed in Yunnan Province, the proportion infected via heterosexual contact was the largest, while the proportion infected via homosexual contact was the smallest. Additionally, the proportion of males was larger than that of females. As compared to patients with other high-risk behaviours, the males with HIV infection-related high-risk homosexual contact were younger, unmarried, and more likely to have an advanced educational degree. Han patients were more prevalent than minority patients in both males and females with the three types of high-risk behaviours. HIV infection-related intravenous drug abuse at a lower age was more common among males than females. No obvious difference existed between the number of males and females with HIV infection-related heterosexual contact, which occurred earlier in females than males. The number of males with HIV infection-related high-risk homosexual contact was more than the females. Furthermore, the latest age at the onset of the three types of HIV infection-related high-risk behaviour, in both males and females, was found in the heterosexual contact group. There were no reports of HIV infection-related high-risk heterosexual contact after 85 years of age. Despite wide antiretroviral scale-up during the past two decades resulting in declining new infections and mortality globally, HIV-associated tuberculosis remains as a major public health concern. Tuberculosis is the leading HIV-associated opportunistic infection and the main cause of death globally and, particularly, in resource-limited settings. In our research, Among AIDS patients with pulmonary tuberculosis infection, intravenous drug abuse accounted for the highest proportion of the three high-risk behaviors related to HIV infection. The route of HIV infection is related to bad behavior and outcome, because intravenous drug use is not only related to HIV infection, and drug addiction can reduce the patient’s compliance with anti-tuberculosis treatment and affect the effect of anti-tuberculosis treatment. For AIDS patients, getting rid of drug addiction or reducing high-risk behaviors such as the use of unclean needles, and BCG vaccination can help reduce the risk of combined TB double infection.

**Limitations**

First, we included only the demographic, age and gender distribution characteristics of HIV/AIDS patients with high-risk intravenous drug abuse and sexual contact from the database of Chinese Medicine Treatment of AIDS Pilot Project of Yunnan Province (patients treated in 44 treatment points in nine prefectures and cities in Yunnan Province from September 2005 to October 2017), which reflects the distribution of patients but not morbidity. Second, a few AIDS patients infected via homosexual contact may have claimed to be infected by heterosexual contact due to social rejection, discrimination and shame related to homosexuality.

It is known that the subtypes of HIV are transmitted via different infectious routes. Along the drug route, subtypes B and C can be transmitted. Subtype CRF01_AE...
was introduced into Yunnan through sexual activities with people from bordering countries. Moreover, circulating recombinant form (CRF) 01 AE has been found in infected men who have sex with men. Yunnan can be a ‘transfer station’ of HIV multidirectional transmission between China and its neighbouring countries. Therefore, it is important to study the subtypes of HIV in Yunnan Province.

Given our technique and sample restrictions, this part has not been investigated in the current study.

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**Conflicts of Interest/Competing Interests**

Not applicable.

**Ethics Approval**

The content and plan of this study were reviewed and approved by the Ethics Committee of Yunnan Academy of Traditional Chinese Medicine.

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of Yunnan Academy of Traditional Chinese Medicine. Written informed consent was obtained from individual or guardian participants.

**Consent to Participate**

Informed consent was obtained from all individual participants included in the study.

**Consent for Publication**

Patients signed informed consent regarding publishing their data and photographs.

**Availability of Data and Material**

The data sets supporting the results of this article are included within the article and its additional files.

**Code Availability**

Not applicable.

**Authors’ Contributions**

Qin Li performed the data analyses and wrote the manuscript, and was a major contributor in writing the manuscript;

Weibo Wen contributed to the conception of the study.

Li Wang performed the experiment;

Fang Ye participated in fund us photography;

Juexuan Wang helped perform the analysis with constructive discussions.

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