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Effect of Physical Intervention on Preventing Upper Limb Venous Thrombosis of Patients with PICC: A Systematic Review and Meta-Analysis of Randomized Trials

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Abstract

Objective: To evaluate the effect of physical methods on the prevention of venous thrombosis in patients with peripherally inserted central catheter (PICC). **Methods:** Randomized controlled trials meeting the inclusion and exclusion criteria were retrieved from the following databases: Cochrane library, Pubmed, EMbase, Web of science, Ovid, CBM, CNKI, Wanfang, VIP, then Review Manage (RevMan) 5.3 software was used for data analysis. **Results:** A total of 24 RCTs including 3496 patients were analyzed in the study. The results of meta-analysis showed that various forms of upper limb movements could effectively decrease the incidence of venous thrombosis in the patients with PICC [RR = 0.23, 95% CI (0.16, 0.33), P < 0.01], upper limb movements could effectively improve the mean blood flow velocity of basilic vein [MD = 1.65, 95% CI (1.19, 2.11), P < 0.01]. **Conclusion**: Upper limb movements can effectively decrease the incidence of phlebothrombosis and improve the mean flow velocity of basilic vein in the patients undergoing PICC. Other forms of physical prevention methods need to be further studied due to insufficient sample size.

Keywords

PICC, Upper Limb Movements, Venous Thrombosis, Blood Flow Velocity, Meta-Analysis

1. Introduction

Peripherally inserted central catheter (PICC) is a kind of central venous catheter punctured by peripheral veins, such as the basilic vein, cubital median vein, ce-

phalic vein [1]. While providing convenient and effective venous access for patients, PICC can also cause some complications, such as shedding, blockage, phlebitis, venous thrombosis, etc., among which venous thrombosis is the most serious, if the thrombus falls off, it will lead to fatal pulmonary embolism [2] [3]. How to prevent venous thrombosis is an urgent problem to be solved in clinical practice. The damage of blood vessel wall, slow blood flow and high blood coagulation state are the three main factors causing venous thrombosis. The endovascular intima was directly damaged during catheterization of PICC. At the same time, as a foreign body floating in the blood vessel for a long time, it can generate mechanical stimulation and affect normal hemodynamics, easily forming eddy-current and producing microthrombus. In addition, after the placement of PICC, patients will also suffer from pain, weakness, bed rest, worry and other factors, as well as physical reactions such as nausea and vomiting after chemotherapy, resulting in decreased voluntary activities, blood stasis, slow blood flow and easy formation of thrombosis. Other predisposing factors include the stimulation of blood vessels by drugs. Tumor cells damage the hypercoagulability of vascular endothelial cells by directly invading blood vessels or secreting vascular penetrating factors, and directly activate the coagulation system and the activation of a variety of coagulant substances to promote thrombosis. The prevention of thrombosis is mainly to use physical or drug methods to prevent the formation of three factors. Current guidelines [4] do not recommend drug treatment for venous thrombosis patients with PICC, therefore, it is urgent for us to find safe, simple and effective physical intervention measures to prevent venous thrombosis. Physical interventions refer to the methods that use physical principles such as motion, light, electricity, radiation, pressure and machinery to achieve the purpose of prevention. At present there are few studies about physical intervention measures for venous thrombosis in foreign countries, although there are more and more studies about the topic in China, the research standards and methods are different. The aim of this study was to evaluate the effect of various physical intervention methods for PICC venous thrombosis, in order to provide evidence and direction for future studies.

2. Materials and Methods

2.1. Inclusion Criteria

1) Study design: randomized controlled trials (RCTs) about physical prevention of upper limb venous thrombosis in patients with PICC. 2) There was no significant difference in the general data between the two groups in age or gender ($P > 0.05$). 3) Patients: aged at least 18 years old; completed PICC for the first time; tolerate color ultrasound examination. 4) Interventions: the experimental group received physical prevention during PICC indentation, the control group didn't receive intervention during PICC indwelling or received another form of physical prophylaxis. Specific physical mode: every day, the medical staff conducted guidance and supervision on the movement mode of the affected limb in differ-

ent modes and frequencies. Regular exercise: the nurse only carries on the health education of the physical exercise mode to the patient, but does not supervise the implementation. 5) Outcome indicators: the main outcome indicators in this study were the incidence of venous thrombosis and the mean velocity of blood flow. Venous thrombosis and venous velocity were determined by color Doppler examination. Normal vein ultrasound showed thin, smooth vein wall, no echo in the lumen, the probe pressure lumen can be compressed, the color blood flow filling good. In the case of venous thrombosis, the inner diameter of the lumen increases, the wall thickens, there are low or moderate echoes around the catheter, and color Doppler flow signals are filled with defective or no color flow signals at all. Venous velocity was compared with that of the patient at steady state.

2.2. Exclusion Criteria

1) Multiple deep vein catheterization (>2 times); 2) use drug treatment for intervention.

2.3. Search Strategy

1) Searchable database: This study systematically searched relevant published original literatures in Chinese and English databases, including Pubmed, Web of sciene, EMbase, Ovid, Cochrane library, CBM, CNKI, Wanfang, VIP. The retrieval time is from their inception until August 2019. 2) Search terms: "arm exercise/make a fist/arm training/upper limb movement/limb function exercise", "PICC/peripherally inserted central catheter", "phlebothrombosis/venous thrombosis/thrombus of vein/venous embolism/deep vein thrombosis", "prevention and control".

2.4. Study Selection and Quality Evaluation

1) Study screening and data extraction: According to the consistent inclusion and exclusion criteria, the data was selected and extracted by two researchers independently, the two researchers have learned knowledge about evidence-based medicine, and received standardized training. Extract contents including basic information (author, year of publication, country, baseline situation, etc.), research design, sample size, interventions, outcome indicators, etc. Any disagreement was resolved by discussion with a third reviewer. 2) The two researchers independently evaluated the included studies according to Cochrane Assessment Manual 5.1.0 Assessment Criteria. The evaluation items included: random sequence generation, allocation hiding, blind method, data integrity of outcome indicators, selective reporting of findings and other sources of bias. A: fully meet the above criteria, the risk of a variety of bias is low; B: partially meet the above criteria, bias risk is moderate; C: completely dissatisfied with the above criteria, bias risk is higher. Both A and B studies were included in this study.

2.5. Statistical Analysis

Meta-analysis was performed using the RevMan5.3 software, provided by Coch-

rane collaboration network. Weighted mean difference (*MD*) was used for measurement data, such as the venous blood flow velocity. Relative Risk (*RR*) was used for enumeration data. 95% Confidence interval (*CI*) was calculated for all analyses. Firstly, Chi-square (X^2) was used to confirm whether there was heterogeneity among the studies. If there was no significant statistical heterogeneity ($P > 0.1$, $I^2 < 50\%$), the fixed effect model was adopted. If there was heterogeneity ($P < 0.1$, $I^2 \geq 50\%$), the random effect model was used to combine with the effect size. Descriptive analysis was adopted if heterogeneity was too obvious and can't be judged.

3. Results

3.1. Study Selection and Study Characteristics

A total of 579 trials were initially retrieved, 334 are Chinese and 245 are English. After screening, 24 literatures were finally included, and the specific steps of inclusion were shown in Figure 1. Three literatures [5] [6] [7] divided the intervention group into two groups (experimental group 1 and experimental group 2). The two groups had different exercise frequency, in order to make the data clearer and the consistency of intervention measures and outcome indicators, the two groups were divided into two RCTs. The basic features of the included articles are shown in Table 1.

3.2. Methodological Quality Evaluation of the Included Literature

The methodological quality of the included 24 trials was all of grade B. The baseline levels of the subjects were compared. Two of the studies [7] [8] made an intentionality analysis of the missing patients, and the specific evaluation indicators and results were shown in Table 2.

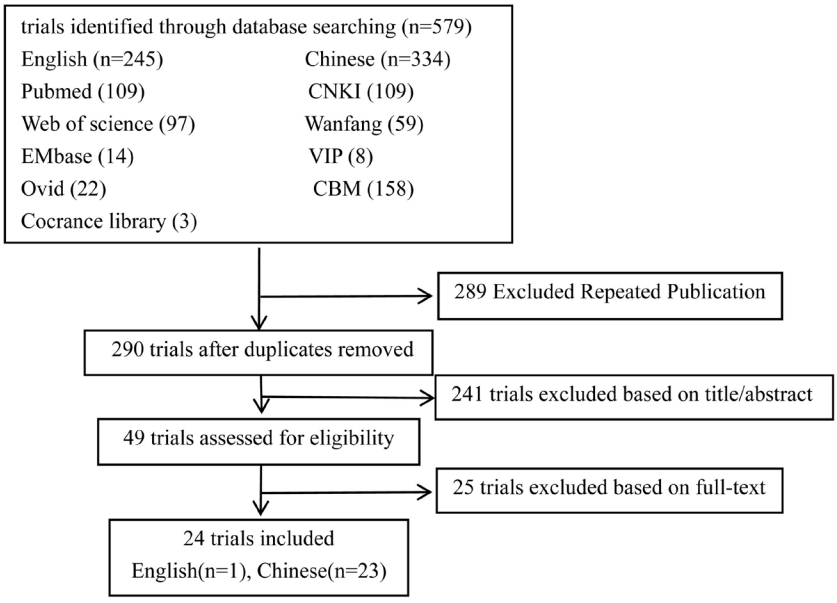

Figure 1. Flow diagram of studies identified in the systematic review.

Table 1. Characteristics of the included studies.

Included studies	Year	Nation	Objective	Sample size E/C	Interventions		Outcome indicator
					E	C	
Chen, B. [5]	2016	China	Tumour	40/40	Electronic grip	R	B + D
Zhang, M. [6]	2015	China	Hepatobiliary Surgery	20/20	Electronic grip	R	B + D
Zhou, Y. [7]	2015	China	Tumour	32/38	Elastic ball handgrip exercise	R	DVT + A
Rabinstein [8]	2019	USA	Neurocritical patients	31/31	Sequential compression devices	without pressure treatment	DVT
Hao, R. N. [9]	2016	China	Gastrointestinal tumor	83/83	Arm exercise	R	DVT
Li, L. [10]	2018	China	Hematological tumor	159/159	Active fist-making	R	DVT + A
Li, Q. [11]	2017	China	Tumor chemotherapy	113/110	Video combined with drawing exercise	R	DVT
Lun, X. P. [12]	2014	China	Tumor	43/43	Upper limb exercise	R	DVT
Ren, Y. C. [13]	2018	China	Tumour	52/52	Handgrip exercise	R	DVT + A
Wang, M. [14]	2016	China	Cerebrovascular disease	42/42	Active fist clenching	R	DVT + A
Wang, Y. P. [15]	2018	China	Tumour	42/42	Ball grip exercise	R	DVT
Wang, Z. Y. [16]	2017	China	Hematological tumor	87/88	Quantitative physical intervention	R	DVT
Wu, T. T. [17]	2017	China	Leukemia	76/84	Functional exercise	R	DVT
Xie, F. M. [18]	2019	China	Hematological tumor	87/88	Music grip strength training	R	DVT
Yin, R. H. [19]	2017	China	Tumour	60/60	Quantitative PICC functional exercise	R	DVT
Zhang, J. W. [20]	2019	China	Elderly patients	75/75	Fisting activity	R	DVT + A
Zhu, W. Y. [21]	2019	China	Tumor	42/42	Different ball holding motion patterns	R	DVT + A
Zou, X. M. [22]	2014	China	Breast cancer	80/80	Grip training	R	DVT
Xia, R. [23]	2010	China	SENILE tumor	60/60	Electric acupoint stimulation	R	DVT + A
Zhang, J. [24]	2019	China	tumor	137/137	Microwave hyperthermia combining with limb movement	R	DVT
Lin, L. Z. [25]	2018	China	tumor	150/150	Microwave physiotherapy	R	D
Wang, J. M. [26]	2013	China	cancer	45/45	Static and static pulse system	R	DVT
Zhu, B. Z. [27]	2019	China	tumor	86/85	Compression with sphygmomanometer	R	DVT
Jin, L. [28]	2015	China	tumor	100/100	Air wave pressure therapeutic apparatus	R	DVT

Note: A: blood flow velocity in axillary vein; B: the mean flow velocity of basilic vein; D: Blood vessel diameter; DVT: Incidence of deep vein thrombosis; R: regular exercise; C: control group; E: experimental group.

Table 2. Assessment of risk of bias.

Included studies	Random sequence generation	Allocation concealment	Blinding of participants	Blinding of outcome assessment	Incomplete outcome data	Other bias	Quality grade
Chen, B. [5]	low	unclear	unclear	low	low	low	B
Zhang, M. [6]	unclear	unclear	unclear	low	low	low	B
Zhou, Y. [7]	low	unclear	unclear	unclear	low	low	B
Rabinstein [8]	low	low	high	low	high	low	B
Hao, R. N. [9]	low	unclear		unclear	low	low	B
Li, L. [10]	low	unclear	unclear	unclear	low	low	B
Li, Q. [11]	unclear	unclear	unclear	unclear	low	low	B
Lun, X. P. [12]	low	unclear	unclear	unclear	low	low	B
Ren, Y. C. [13]	low	unclear	unclear	unclear	low	low	B
Wang, M. [14]	unclear	unclear	unclear	unclear	low	low	B
Wang, Y. P. [15]	unclear	unclear	high	unclear	low	low	B
Wang, Z. Y. [16]	low	unclear	unclear	unclear	low	low	B
Wu, T. T. [17]	unclear	unclear	unclear	unclear	low	low	B
Xie, F. M. [18]	low	unclear	unclear	unclear	low	low	B
Yin, R. H. [19]	unclear	unclear	unclear	unclear	low	low	B
Zhang, J. W. [20]	low	unclear	unclear	unclear	low	low	B
Zhu, W. Y. [21]	low	unclear	unclear	unclear	low	low	B
Zou, X. M. [22]	low	unclear	unclear	unclear	low	low	B
Xia, R. [23]	unclear	unclear	unclear	unclear	low	low	B
Zhang, J. [24]	low	unclear	unclear	unclear	low	low	B
Lin, L. Z. [25]	low	unclear	unclear	unclear	low	low	B
Wang, J. M. [26]	unclear	unclear	high	unclear	low	low	B
Zhu, B. Z. [27]	low	unclear	high	unclear	low	low	B
Jin, L. [28]	low	unclear	unclear	unclear	low	low	B

3.3. Results of Meta-Analysis

3.3.1. Effect of Upper Limb Exercise on Venous Thrombosis

15 literatures [5] [9]-[22] evaluated the effect of clenched fist exercise on venous thrombosis, and the combined results showed that there was no heterogeneity between the studies (I^2 = 0%, P = 1.00), the fixed effect model was used. The results showed that the incidence of phlebothrombosis in the upper limb exercise group was lower than that of the conventional exercise group, and the difference was statistically significant [RR = 0.23, 95% CI (0.16, 0.33), P < 0.01], as shown in **Figure 2**.

3.3.2. Effect of Exercise on the Venous Blood Flow Velocity of Basilic Vein

2 studies [5] [6] reported the effect of exercise on the venous blood flow velocity

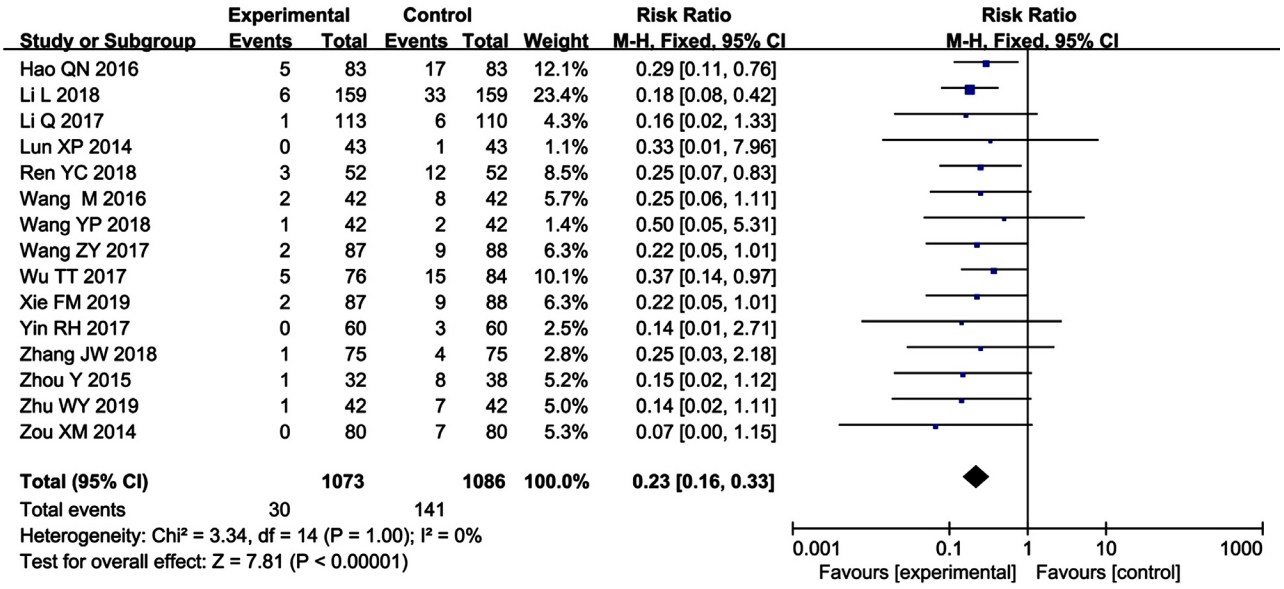

Figure 2. Effect of clenched fist exercise on thrombus.

of basilic vein of patients with PICC. The combined results showed that there was no heterogeneity between the studies (I^2 = 0%, P = 0.70), there is no heterogeneity. By using the fixed effects model, the results showed that the venous blood flow velocity of basilic vein in the exercise group movement is faster than that of the routine group, the difference was statistically significant [RR = 1.65, 95% CI (1.19, 2.11), P < 0.01], as shown in **Figure 3**.

3.3.3. Effect of Exercise on Blood Flow Velocity in Axillary Vein

6 studies [5] [10] [13] [14] [20] [21] evaluated the effect of exercise on the blood flow velocity of axillary vein in patients with PICC. 3 studies [5] [20] [21] were excluded for inconsistent thrombotic measurement time, but heterogeneity still existed (I^2 = 92%, P < 0.001), as shown in **Figure 4**. Descriptive analysis showed that all the 6 studies indicated that the velocity of axillary vein after exercise was faster than that of conventional exercise, and the difference was statistically significant (P < 0.05).

3.3.4. Effect of Bioelectrical Stimulation on Thrombosis

1 literature [23] evaluated the effect of electrical stimulation on vein of patients with PICC catheterization. It was concluded that the difference of axillary vein blood flow velocity and the incidence of deep venous thrombosis between the experimental group and the control group was statistically significant.

3.3.5. Effect of Microwave on Vein Thrombosis in Patients with PICC Catheterization

2 literatures [24] [25] evaluated the effect of microwave on vein thrombosis in patients with PICC catheterization. One literature [24] showed that the incidence of venous thrombosis in microwave group was lower than that in control group, and the difference was statistically significant (P < 0.05). One literature

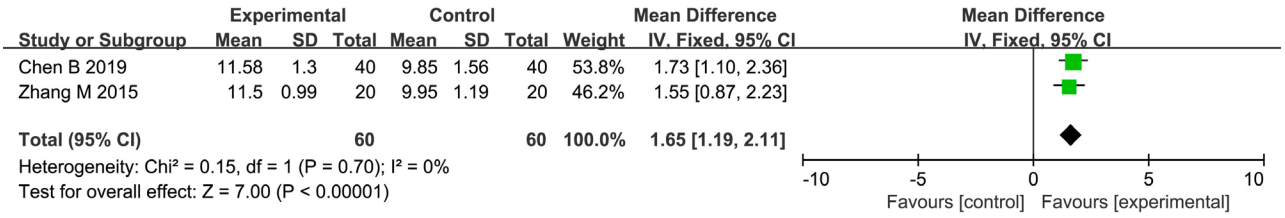

Figure 3. Effect of exercise on the venous blood flow velocity of basilic vein.

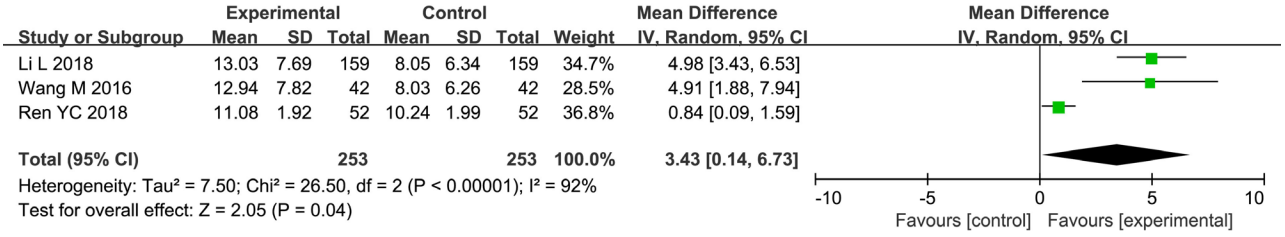

Figure 4. Effect of exercise on blood flow velocity in axillary vein.

[25] evaluated the effect of microwave on the diameter of basilic vein in patients with PICC catheterization. The result showed that the inner diameter of cardinal vein in microwave group was significantly dilated with higher success rate at a time than that of control group and the difference was statistically significant ($P < 0.05$). Due to the different outcome indexes of the two literatures, meta-integration could not be carried out, and only descriptive analysis was conducted.

3.3.6. Effect of Pressure on Venous Thrombosis in Patients with PICC

3 domestic studies [26] [27] [28] evaluated the effect of pressure on venous thrombosis in patients with PICC. Heterogeneity showed that fixed effect model should be selected to analyze ($I^2 = 0\%$, $P = 0.96$). The results showed that the incidence of thrombosis in the pressure group was lower than that of the conventional exercise group, and the difference was statistically significant [$RR = 0.22$, 95% CI (0.09, 0.55), $P < 0.01$], as shown in **Figure 5**.

3.3.7. Sensitivity Analysis and Publication Bias

Funnel plot analysis was performed on 15 literatures [5] [9]-[22]. Through sensitivity analysis about the above combined results, the meta-analysis results had no change, suggested that the results of this study are stable. Scatter plots show a symmetrical distribution. The test results of 15 studies are symmetrical without publication bias, as shown in **Figure 6**.

3.3.8. Effect of Catheter Material on Venous Thrombosis in PICC Patients

In this paper, 14 [5] [6] [7] [9] [11] [13] [15] [17] [20] [23] [24] [25] [26] [27] studies mentioned that the PICC catheters used are all Brands, and 9 [10] [12] [14] [15] [16] [19] [21] [22] [28] studies did not describe the brands, this is consistent with Li Xin [29]. Related studies [30] [31] pointed out that the incidence of complications of domestic PICC in patients with PICC catheterization was

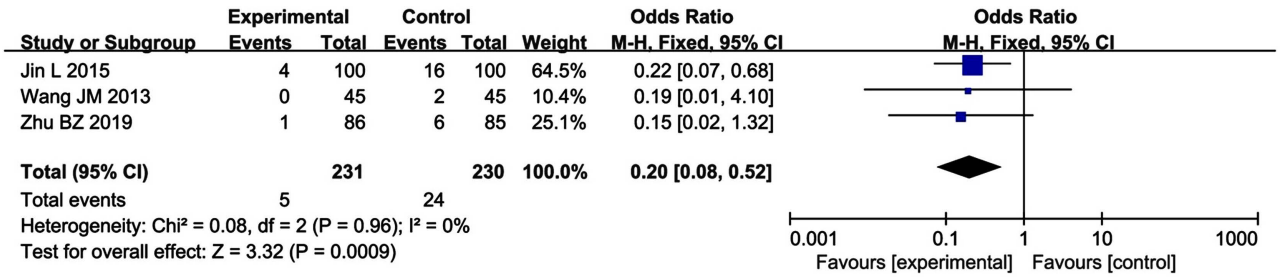

Figure 5. Effect of pressure on thrombus in patients with PICC.

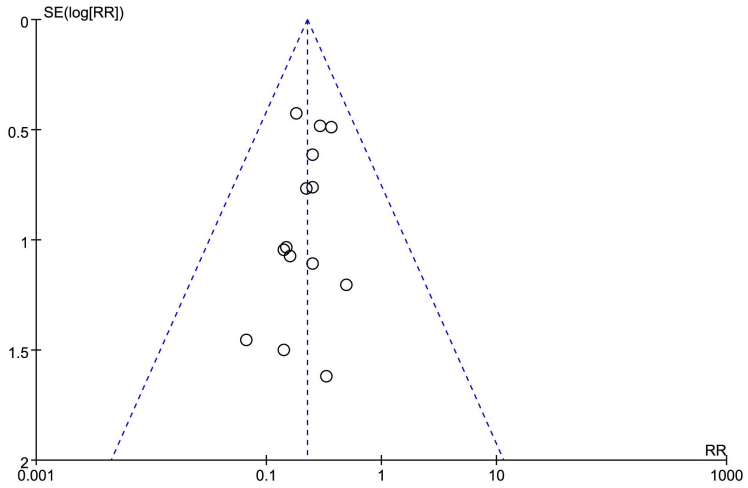

Figure 6. Publication bias of effect of clenched fist exercise on thrombus.

20% (6/30) and 10.3% (7/68), which was the same as foreign brands (8% - 50%) [32]. Meanwhile, Seckold, T. pointed out that there was no difference in the total incidence of complications of catheters in terms of materials, but the types of complications were different. The incidence of complications of PICC catheters of different brands is shown in **Table 3**.

4. Discussion

4.1. Effect of Physical Exercise on the Incidence of Thrombosis in Patients with PICC

There are many ways to prevent thrombus in patients with PICC. The results of meta-analysis show that all kinds of upper limb movements can reduce the incidence of thrombus in patients with PICC and accelerate the blood flow speed of venous catheterization. Although there are different forms of upper limb movements, they can through the movement of upper limbs to accelerate the blood flow speed of the limb on the side of catheterization, promote the venous and lymphatic circulation, so as to reduce the incidence of thrombus. Some studies have confirmed that [33] [34], compliance of exercise is positively related to the incidence of thrombus in patients with PICC, patients with good compliance have better exercise effect than patients with regular exercise, and the incidence of thrombus is low. It may be due to the lack of supervision of the patients in the

Table 3. The incidence of complications of PICC of different brands.

Brand	Complication incidence	Normal cases	Total cases	Total complication rate
Kangxin [30]	7	61	68	10.3%
Domestic brands [31]	6	24	30	20%
Foreign brands [32]	-	-	3767	8% - 50%

Note: "-" means no relevant data.

routine exercise group, few of them aware the importance of exercise, fear of the adverse consequences of the activity on the body, resulting in the failure of the exercise intensity and frequency to reach the standard, therefore, the blood flow of the limb on the catheter side is slow, which is easy to cause thrombosis. This study failed to confirm that bioelectric stimulation and microwave can reduce the incidence of venous thrombosis in patients with PICC, which may be related to the fact that this study is less involved in related studies, the implementation process of bioelectricity is relatively cumbersome, and the cost is high, the number of patients received in clinical is less or the intervention time is short, etc., and it is expected that large sample and high-quality randomized controlled trials to be further explored.

The relationship between pressure and venous thrombosis is uncertain. Domestic studies [26] [27] [28] have confirmed that intermittent pressure can reduce the incidence of venous thrombosis. This study found that the upper limb movements can effectively improve the average blood flow velocity of the vein and slow down the formation of thrombus. Therefore, effective education should be provided to patients with PICC, reasonable limb movements and physical therapy should be guided to reduce their fear of exercises and physical pain, and improve patients' compliance and initiative of exercise. However, the incidence of PICC catheterization in foreign countries is low, only one study included, so the sample size is small, and in the course of the study, the treatment group was forced to suspend the experiment due to the high incidence of thrombus, and the integrity of the outcome data was affected. Therefore, more high-quality RCTs and multilingual literatures are needed to evaluate the effect of intermittent pressure on thrombosis.

PICC with different brands, types and materials have been used in China. According to the survey [29], more than 94.6% of PICC catheters in the market are imported brands such as BARD, USA, BD, ARROW, MEDCOMP, B BRAUN, while the utilization rates of domestic brands are low, such as BAIDUOAN and EILANG, which may be related to the late start of PICC in China. With the improvement of catheterization and maintenance technology of domestic PICC, and the gradual improvement of the brand, its complications are also decreasing, and the price is half of that of imported brands [30], so it may be more suitable for patients in China. However, there are fewer literature about the related complications of domestic brands, more high-quality RCTs studies are needed for

the comparing between domestic and foreign brands, to promote the development of intravenous therapy specialty in China.

4.2. Limitations of This Study

Most RCTs included are Chinese, only one [8] is English, and this study only retrieves the published Chinese and English trials, some trials may not be included. There are great differences in the sample and methodology of the included studies, and the intervention schemes are not consistent, which may have a certain impact on the results of the study. Some studies did not illustrate the generation of random sequence and blinding of allocation, which may also affect the results of Meta analysis. In addition, the quality of the studies included in this study is low, there is a certain risk of bias, and the effect of exercise needs to be further studied and practiced. It is suggested that future research should standardize the interventions. For example, the exercise of mode, method, frequency, intensity, measurement time and standard of outcome indicators, etc., should be refined and specific economic conditions and willingness of the patients should to be taken into consideration, so as to improve the consistency of the prevention. In the future, we can expand the sample size and carry out high-quality randomized controlled trials to validate the effect of standardized physical prevention on venous thrombosis of patients with PICC, and provide more comprehensive theoretical support for clinical nursing staff.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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The Development and Functional Regulation of the Fetal Adrenal Cortex

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Abstract

As an important endocrine organ of the human, the adrenal cortex is responsible for the synthesis of various steroids. It is important to maintain pregnancy, promote fetal growth and nervous system development. Moreover, it is also important for the growth and development of the fetus after birth. Abnormal functional development of the adrenal cortex will induce the abnormal synthesis of steroid hormones and trigger the occurrence of related diseases. In this review, we discuss the morphological and functional development of fetal adrenal cortex and its regulatory factors in humans and rodents.

Keywords

The Fetal Adrenal Cortex, Steroid Hormones, Growth Factor, Transcription Factor, Placental Hormone

1. Introduction

It is known that the adrenal glands include outer cortex and inner medulla, which come from different embryonic development layers respectively. Medulla cells come from nerve ectoderm and cortex comes from mesoderm tissue, which has different physiological functions. As an important endocrine organ of the human body, the adrenal gland is responsible for the synthesis of various steroid hormones. It is of great significance to maintain pregnancy and promote fetal growth and nervous system development. Moreover, it is also important for the growth and development of the fetus after birth. Therefore, the normal development of the adrenal gland during the fetal period is the key to determine the maturation and destiny of the fetus [1] [2] [3] [4]. Abnormal development of the adrenal cortex can lead to metabolic disorders of electrolytes and carbohydrates, leading to circulatory failure, hypoglycemic coma and death.

2. The Development of Adrenal Glands

2.1. The Development of Adrenal Cortical Morphology

The adrenal glands are a pair of organs located adjacent to the superior pole of each kidney. The left adrenal gland is half-moon and the right adrenal gland is triangular. The adrenal cortex is thicker and is located in the outer layer of the adrenal cortex, which accounts for about 80% of the adrenal gland. It can be divided into three parts: the zona glomerulosa (zG), the zona fasciculata (zF) and the zona reticularis (zR). Cortical hormones secreted by the adrenal cortex are classified into three categories, namely, salt corticosteroids, glucocorticoids and sex hormones [5]. All kinds of cortical hormones are secreted by epithelial cells of different layers of adrenal cortex: and the globular band cells secrete saline corticosteroids, which are mainly aldosterone; the middle zona fasciculata produce glucocorticoids, which are mainly cortisol; and the innermost zona reticularis contains a network of cells that synthesize sex steroid hormones such as dehydroandrosterone and estradiol, also secrete a small amount of glucocorticoids [6] [7].

2.1.1. Human

The landmark study of human adrenal cortex development began in the 1960s [8]. The urinary genital ridge and the body cavity between the epithelial membrane epithelium is gathered themselves together at about the fourth week of gestation, and then the proliferation, migration, formed in the kidney area in the cells, called adrenal blastema, which is the first to recognize the adrenal gland. By the eighth week of gestation, the adrenal rudiment has been formed, with the fetal zone and the definitive zone [9]. At around the ninth week of gestation, the adrenal blastemais are completely enclosed by the adrenal capsule, which are composed of specialized mesenchymal cells migrating from the area of Bowman's capsule. At the same time, an extensive network of sinusoidal capillaries develops between the cords of the fetal zone. This vasculature predominates in the central portion of the fetal zone and persists throughout fetal life. Consequently, the adrenal cortex is one of the most highly vascularized organs in the primate fetus. Abundant vascularization is likely required to facilitate access of hormonal products to the circulation. The adrenal cortex is further differentiated, and the fetal zone is dominant in the 16th - 20th weeks of gestation. It is composed of a large number of eosinophilic cells and is typical of the morphology of steroid secretory cells. The outside of the fetal zone is the definitive zone, which is composed of a narrow band of small tightly packed basophilic cells that exhibit structural characteristics typical of cells in a proliferative state. With the development of pregnancy, the lipid droplets in the permanent cells gradually increased and began to have the characteristics of steroid secretory cells, with the characteristics of adult adrenocortical globules at 30 weeks [10] [11]. In the midgestation, the transition zone may have the capacity to synthesize cortisol and thus be analogous to cells of the zona fasciculata of the adult adrenal [1]. By

the 30th week of gestation, the transitional zone began to take on the appearance of the zona fasciculata [8]. After birth, the fetal band begins to atrophy and degenerates into the reticularis zona, While the fetal cell apoptosis, the definitive zone and the transitional zone proliferate and thicken, however, until the age of 10 to 20, the adrenal cortex is gradually mature and has the characteristics of mature adrenal cells.

To sum up, the morphological development of adrenal gland can be divided into five main stages [8]: 1) 3 - 4 weeks of gestation, condensation of the celomic epithelium; 2) 4 - 6 weeks of gestation, the proliferation and migration of coelomic epithelium; 3) Between 8 - 10 weeks of gestation, the fetal adrenal cortical cells differentiated into the fetal zone and the definitive zone; 4) In the first 3 months after birth, the fetal band degeneration disappeared; 5) From 10 to 20 years old, the adult adrenal band is established and mature. It can be seen that the development of adrenal glands begins in the embryo and continues into adulthood.

2.1.2. Rodent

The development of adrenal cortical cells in rodents is different from that of human beings, but the basic development process and genes that play an important role in development are basically consistent [12]. The development of the adrenal cortex of rodents also begins with the migration of the epithelial cells of the urinary reproductive ridge, which occurs in the 9th day of embryo formation. In embryo 10.5 days, adrenal cortical cell differentiation [13]. Mouse embryos 12 - 14 days, rat embryos 15 days, neural crest cells begin to migrate into the fetal band, establish adrenal medullary [14] [15] [16]. There is a gender difference in fetal band degeneration in mice, male degeneration occurs in adolescence, and females occur in pre-pregnancy [12].

There is a slight difference between adrenal growth in rats and mice. In the late 1950s, Roos [17] suggested that the rat's adrenal glands had appeared in 18 days, and histology is similar to adult adrenal glands. This phenomenon has been reported in other laboratories as well [16]. Also not found in the development of adrenal gland of rats with fetus, and the results suggest that exist in the rat adrenal gland and zoning, called Undifferentiated belt (Undifferentiated Zone), the belt does not express steroid enzyme, preliminary considered by the differentiation of the centrality of migration, and maintain the adrenal cortex function, may be the stem cells [18]. It has been reported that the undifferentiated zone cells and surrounding cells are stem cells/derived cells of the rat adrenal cortex, maintaining the functional zoning of the adrenal cortex. There is the Sonic hedgehog (Shh), an important factor in embryonic development and adult stem cell maintenance, exists in zU of the rat adrenal gland and the Shh containing cells seem to migrate bidirectionally [19].

2.2. The Functional Development of the Adrenal Gland

The adrenal cortex produces a series of steroid hormones (as shown in **Figure 1**)

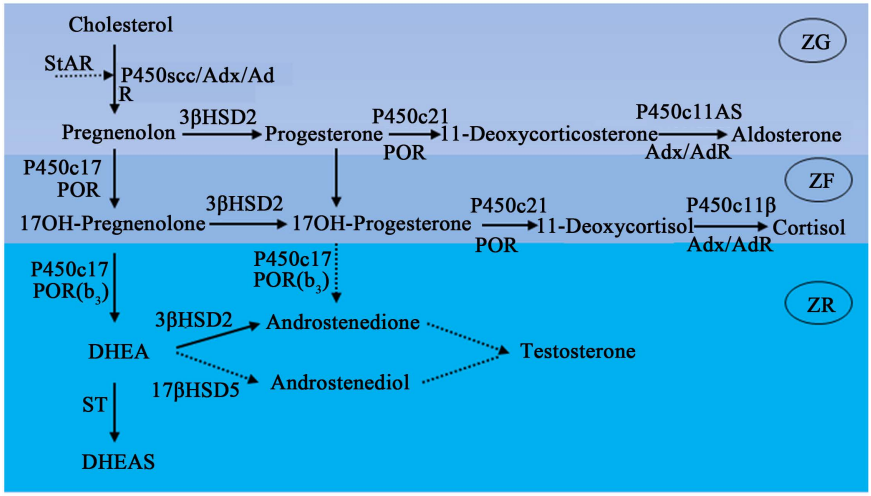

Figure 1. Adrenal steroid hormone synthesis in the human [20]: zona, ZF, zona fasciculata, ZR: mesh belt, Adx: adrenocortical iron oxide reduction protein, AdR: adrenocortical iron oxide reduction reductase, protein FOR: P450 oxidoreductase, ST: sulfuryl transferase.

[20], any changes in the expression of these steroidal synthase will lead to abnormal synthesis of adrenal steroid and related diseases [20] [21]. Dehydroepiandrosterone (DHEA) and cortisol are the main steroids produced by the adrenal glands of early pregnancy [1]. Cholesterol is a precursor of the synthesis of steroid hormone, cholesterol is absorbed by the body, the first is the synthesis of steroid hormones in acute regulatory proteins (steroidogenic acute regulation protein, StAR) to the mitochondrial membrane, Cholesterol P450 side chain cleavage enzyme (cytochrome P450, P450scc) is converted to progesterone. Pregnenolone is 17α-hydroxylase (17α-hydroxylase/17,20-lyase, P450c17) hydroxyl into 17α-hydroxy pregnenolone (17α-OH-pregnenolone, 17αOH Preg), by 3-hydroxy beta steroid dehydrogenase (3 beta-hydroxysteroid dehydrogenase, 3 beta HSD) into 17 alpha hydroxy progesterone, is 17,20-lyase cracking, come in further formation of dehydroepiandrosterone sulfate (DHEAS). Or, progesterone is metabolized by 3 beta-hsd, and then 17 alpha-hydroxylated progesterone. In the end, 17 alpha-hydroxyprogesterone was metabolized by 21-hydroxygenase (steroid 21-hydroxylase, P450c21), 11 beta-hydroxylase (steroid 11 beta-hydroxylase, P450c11). It can be seen that the related enzymes involved in the synthesis of adrenal steroid hormones include: StAR, P450scc, P450c17, 3 beta-HSD, P450c21 and P450c11. For rats, because the adrenal glands do not express P450c17, the progesterone can be converted to progesterone directly from 3 beta-hsd to progesterone, which is catalyzed by P450c21 and P450c11 to produce corticosterone. In both humans and animals, P450scc and StAR are both speed limit enzymes, regulating the speed of steroid synthesis [22].

2.2.1. Human

The human fetal adrenal cortex has steroidogenic capabilities early in gestation.

This is first seen at 6 - 8 weeks when the cells in the adrenal blastema differentiate and acquire steroidogenic characteristics [10], In about 8 - 10 weeks of gestation, the fetal adrenal cortex produce a large number of precursor of estriol--DHEA-S, and in the 12th week of pregnancy, there is a significant increase in the concentration of estrogen in the maternal blood circulation [23]. The study confirmed that the fetal adrenal glands were able to express the enzymes needed to synthesize cortisol during the 7 - 10 weeks of gestation [24]. Observations of infants with congenital adrenal hyperplasia (CAH)suggest that the fetal adrenal cortex produces cortisol in about 8 - 9 weeks of gestation under the regulation of adrenocorticotropic hormone (adrenocorticotrophic hormone, ACTH) [25], and negative feedback inhibit the excessive secretion of pituitary ACTH. Sexual differentiation of external genital organs was established during the seventh to twentieth week of pregnancy, fetal adrenal cortisol secretion in early pregnancy to prevent gender differentiation in androgen sensitive period of excessive androgen, high levels of cortisol inhibiting fetal HPA axis, remain in relatively low DHEA-S, particularly in females [25]. Subsequently, the expression of 33β-HSD2 is decreased, so is the secretion of cortisol, the inhibition of the HPA axis is also decreased, and the secretion of dhea-s is increased [26]. With the progress of the pregnancy, fetal paraventricular nucleus (paraventricular nucleus, PVN) strengthen the central drive of HPA axis, fetal adrenal cortex and HPA axis get rapid development [27] [28] [29] [30]. The late pregnancy HPA axis can respond to various physiological stresses in the uterus, and the reactivity of HPA axis to stress will increase continuously, therefore, fetal basal glucocorticoid level is gradually increased to promote the maturation of tissue morphology and function.

The early fetal adrenal cortex secretes a very small amount of salt corticosteroids, which increases during the last three months of gestation. In this period, the fetal blood of the human and rhesus monkey is 80% aldosterone from the fetal adrenal gland [31] [32]. At the 18th to 20th week of gestation, the metabolic pathways of corticosteroids in fetal adrenal glands are concentrated in the definitive zone, and their activity is very low [32].

2.2.2. Rat

Literature reported [17], the fetal rat adrenal gland growth and tissue development began at the 16th week of gestation, in this period, the expressions of P450scc, P450C11 and P450c21 were detected, as well as the secretion of cortisone [16] [33], it continued to increase from day 17 to day 18, followed by a significant decrease, and continued until the first day after birth, which was similar to previous reports [34] [35]. It is speculated that the decrease of corticosterone secretion after birth indicates the beginning of the stress non-responsive period (SNRP) [36]. This change is intended to stabilizethe body of glucocorticoid levels, ensure the normal development of neurons in brain regions sensitive to glucocorticoid. The expression of P450aldo and the secretion of aldosterone are detected in the 20th day of gestation [16] [33] and peaked at birth [34] [37].

3. Regulatory Factors of Adrenal Development

The adrenal gland develops autonomously 20 weeks ago. After 20 weeks, the fetal adrenal gland is regulated by the hypothalamus and pituitary gland, but the fetal adrenal gland does not have the circadian rhythm. Fetal adrenal gland development is mainly regulated by the pituitary anterior lobe adrenocortical hormone ACTH in addition to the growth factor (bFGF and EGF, IGF, and promote element, statin and TGF beta), intracellular receptor (SF-1, DAX-1), and the effect of placental hormone.

3.1. The Fetal Pituitary and ACTH

It is expected that the extraordinary growth and steroidogenic activity of the fetal adrenal cortex are dependent on an intact fetal pituitary gland as it produces ACTH, the primary tropic regulator of the adrenal cortex. If there is absence of ACTH stimulation in anencephalic fetuses, the adrenal cortical development is obstructed. The experimental results showed that the fetal adrenal glands of the non-cerebral rhesus monkey were atrophied rapidly, and the maternal plasma estrogen decreased sharply [38] [39]. On the contrary, it can increase the concentration of fetal cortisol and maternal estrogen [38]. It can be seen that ACTH plays an important role in the growth and function of adrenal gland.

Although ACTH is the mainly regulation factor for fetal adrenal development, the early in gestation fetal zone growth and function are independent of ACTH. Before 10 - 15 weeks of gestation, adrenal development in anencephalic fetuses is normal, but thereafter the fetal zone fails to develop and does not exhibit characteristic growth and steroidogenic activity [40], indicating that early in gestation fetal zone growth and function are independent of ACTH. On the contrary, the definitive zone appears normal in anencephalic fetuses despite the absence of ACTH stimulation [41], suggesting that its growth is not dependent on ACTH at any stage in gestation, although its functional maturation appears to be regulated by ACTH [42]. In mid and late gestation, the reaction of fetal adrenal gland to ACTH is enhanced, which is caused by ACTH itself [43] [44] and other factors [45], especially the insulin-like growth factor I and II (insulin like growth factor I and II, IGFI and IGFII). ACTH is combined with the specific receptors on the surface of the adrenal cortex cells, the black leather receptor 2 (melanocortin receptor 2, MC2R) [46] [47], increases intracellular cAMP, also increases free cholesterol content and mitochondrial transport increases, thus promotes the synthesis of steroid hormones [48] [49].

3.2. Growth Factor Regulation

Research suggests that ACTH may not be a direct regulator of fetal adrenal glands. ACTH concentration in fetal circulation during middle and late gestation may be decreased, but the fetal band grows rapidly and produces a large amount of steroid hormones [50]. Although adrenal glands continue to be exposed to ACTH after birth, the fetal band atrophy and degenerate rapidly.

Therefore, other factors, which may be unique to the intrauterine environment, play an important role in regulating the growth and development of the adrenal cortex.

3.2.1. Insulin-Like Growth Factors I and II (IGF-I and IGF-II)

IGF-I and IGF-II affect proliferation and differentiation in a wide variety of cell types and can act as autocrine, paracrine, or endocrine factors. There are two IGF receptors, designated type I and type II, have been identified. The type I receptor is structurally related to the insulin receptor and binds both IGF-I and IGF-II with high affinity and insulin with lower affinity. The type II receptor binds IGF-II with high affinity but will not bind IGF-I or insulin. Many fetal tissue express of IGF-I and IGF-II in the middle of the pregnancy, and the expression of IGF-II dominate in the womb [51] [52], fetal adrenal IGF-II expression is only after liver [53].

IGF-I and IGF-II can promote fetal adrenal cortical cell proliferation [53] through IGF-I receptor mediated [54]. IGF-I promote fetal sheep adrenal cortical cell proliferation, and by enhancing responsiveness of adrenal to ACTH and the activity of key enzymes of steroid hormones increase ACTH induced the synthesis of steroid hormones [55]. IGF-I can increase the expression of MC2R adrenal gland cells, promote the production of steroid hormone [56]. The mechanism may be related to the activation of the signaling pathways of adenosine/protein kinase A (PKA) and phospholipase C/protein kinase C (PKC) in the cell inner ring [57]. IGF-II also can affect the function of human fetal adrenal cortical cells differentiation [58], also reinforce induced fetus with ACTH cell secretion of cortisol and DHEA-S and steroid hormone synthetase P450scc, P450c17 and the expression of 3 beta HSD [45] [59].

3.2.2. Epidermal Growth Factor (EGF)

The fetal adrenal fetal band and the permanent cell surface express EGF receptor, which can promote the cultured cell division of the fetal adrenal fetal band and the definitive band in the mid trimester of pregnancy [60]. EGF treatment in vivo increased the amount of 3bHSD protein in definitive and transitional zone cells of fetal rhesus monkeys [61]. EGF can promote stimulate fetal sheep fetal adrenal gland secretion, secretion of pituitary ACTH and cortisol secretion of CRH hypothalamus [62], therefore, in addition to its potential direct effect on adrenal cortical cell proliferation, EGF also may modulate adrenal growth and functional maturation indirectly by affecting the hypothalamic-pituitary-adrenal axis.

3.2.3. Basic Fibroblast Growth Factor (bFGF)

BFGF can promote the proliferation of mesoderm and neuroectoderm cells, and has the function of promoting angiogenesis and neuronutrition. BFGF can promote the division of adrenal cortical cells in the Y-1 mouse [63]. BFGF can promote the proliferation of fetal adrenal zone and permanent zone, and the permanent zone is more sensitive to the mitogenic effect of bFGF [60]. At the

same time, also to participate in rats after unilateral adrenal resection of compensatory growth of bilateral adrenal gland [64]. It has been shown that the increase of ACTH can be increased by 2 - 3 times with ACTH in the primary culture of human fetal adrenal cells [1]. Guasti, L. research that specific deletion of the Fgfr2 IIIb isoform impairs adrenal development, causing reduced adrenal growth and impaired expression of SF1 and steroidogenic enzymes [65]. Therefore, bFGF may be an important mediator of ACTH action in human fetal adrenal development.

3.2.4. Transforming Growth Factor *β* (TGF*β*)

TGFb is the prototypical peptide of a large family of growth factor proteins. Studies have shown that TGFb is involved in the regulation of fetal adrenal development, which may be related to the atrophy of the fetal band after birth, also may stimulate fetal cells differentiation for cortisol secretion [1], the effects on the fetus with specificity, inhibit the growth and the transformation of its birth. There are TGF beta-specific receptors in mammals, and TGF beta has a significant effect on the function of steroidal secretory cells. TGF beta binds to a specific site regulated by ACTH of human fetal adrenal cortex cells [66], inhibiting the proliferation of human adrenal fetuses and perinatal cells [67], basis and ACTH and cortisol, DHEA induced by cAMP-S secretion, inhibit expression of P450c17 [68], However, it has no effect on the expression of ACTH receptor and P450scc [69]. Therefore, TGF beta may inhibit the proliferation of fetal adrenal gland and steroid hormone synthesis by interacting with the specific ACTH binding site of the cell surface.

3.3. Transcription Factors

There are six important transcription factors which play a key role in the function of the adrenal cortex development: steroid synthesis of transcription factor (steroidogenic factor 1, SF1), DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia congenita, X-linked-1, DAX1), CBP/P300 transcription contain glutamic acid/aspartic acid C-end structure domain 2 (CBP/P300-interacting transactivator with Glu/Asp-rich-terminal domain 2 C, CITED2), Pre-B-cell transcription factor (pre-B-cell transcription factor 1, PBX1), Wilms Tumor factors (Wilms Tumor 1, WT1) and GATA binding protein (GATA). Mutations in these transcription factors will lead to the development, growth and/or dysfunction of the adrenal cortex [70] [71] [72].

3.3.1. SF-1

SF-1 is also known as the nuclear receptor family of five and group A (nuclear receptor subfamily 5, group A, member 1, NR5A1), don't find the endogenous ligand in the cell nuclear receptor, is a solitary nuclear receptor, is the first found to adjust the cytochrome P450 steroid synthetase transcription factor [73]. Subsequently, it has been confirmed in other laboratories that sf-1 regulates the expression of various steroid genes and the synthesis of steroid hormones in the

hypothalamic-pituitary-steroid hormone synthesis organs [74] [75]. Sf-1 is expressed in early fetal adrenal development, both in the fetal zone and in the final differentiation zone [76]. Sf-1 knockout mice would die 12 hours after birth due to the absence of adrenal and gonadal glands [77]. Similar studies have shown that the human adrenal glands do not form without the expression of sf-1 [78].

Due to the lack of sf-1 in adrenal gonads, apoptosis of urinary reproductive crest cells and abnormal adrenal development can be caused [79]. These studies suggest that sf-1 regulates apoptosis and proliferation and affects the development of adrenal glands. Particularly to stress is that exist in SF-1 promoter regions increase fetal adrenal hadron (fetal adrenal enhancer, FAdE) sequence, can regulate the expression of SF-1 through to affect the growth of fetal adrenal gland, and the regulation of adult adrenal gland have obvious different [80].

The promoter of all steroid hormone synthase has the binding site of sf-1, which regulates the expression of these enzymes, thus promoting the synthesis of steroid hormones [81] [82]. SF-1 is interaction with other transcription factors exist, Val and Swain [71] has speculated, adrenal tissue development in SF-1 activation model: first of all, WT1 induces SF-1 expression in adrenal glands primordium, CITED2 collaborative participation, then after PBX1 role in SF, FadE-1 promoter element to further promote, in the end, through self-adjusting for SF-1 expression. More importantly, SF-1 can promote expression of DAX1, while DAX1 can inhibit the activity of SF-1 and regulate the activity of adrenal steroid hormone through the negative feedback regulation pathway [82] [83] POD-1 inhibits SF-1 expression in adrenal cells [84].

The mutations and regulation of SF-1 are associated with the human gonadal gland and abnormal adrenal gland. Male SF-1 heterozygous mutation will lead to adrenal hypoplasia, and male and female sex reversal, while females heterozygous mutations, will delay the adrenal glands to develop and mature, there is ovarian dysplasia occasionally [81] [82] [85] [86]. These studies suggest that there is a gender difference in the effects of SF-1 on adrenal glands. On the contrary, there are also studies suggesting that the overexpression of SF-1 also causes adrenal cortical carcinoma [87] [88].

3.3.2. DAX-1

Dax-1 is another transcription factor that plays an important role in the regulation of adrenal development. Mutations in the DAX-1 gene are responsible for X-linked adrenal hypoplasia congenita [89] [90]. On day 10.5 of gestation in rodents, dax-1 was expressed in the urinary reproductive crest, followed by the adrenal gonadotropin, and finally in the fetal adrenal gland and ovary [91]. The dax-1 mRNA abundance in adrenal glands is significantly higher in fetuses than in adults [92], which may be more important for fetal adrenal development. Dax-1 deficiency leads to early differentiation of adrenal cortical progenitor cells, leading to progenitor cell failure and adrenal insufficiency [88].

The tissue distribution of DAX-1 expression is similar to that of SF-1 [77] [93], suggesting that these two transcription factors may be a co-regulation fac-

tor of the development and function of steroid hormone secretion. It has been reported that Dax1 promoter has SF-1 binding site [94], and subsequently in vitro experiments have also confirmed that SF-1 can be combined in its promoter region and promoted expression in JEG-3 cells [95]. In addition, in vitro experiments show that DAX1 inhibited the activity of SF-1 by directly binding SF-1 or by recruiting cosuppressor factors [71] [96]. Gummow [83], which give excess sugar cortical hormone in vitro experiments, SF-1 and glucocorticoid receptor (glucocorticoid receptor, GR) combined with Dax1 promoter, activate Dax1, restrain activity of SF-1, reduce the generation of steroid hormones. At the same time, the ACTH stimulus is given to isolate the SF-1 and GR complex, so as to inhibit Dax1 transcriptation and increase the steroidal synthesis of steroidal synthesis by SF-1 promotion [83].

In the mouse model of Sf1 knockout, the expression of Dax1 is not reduced completely, and there may be other channel regulation [97]. In vitro experiments have confirmed that DAX1 can be combined with its own promoter region to predict that the gene has its own regulation [98]. Jordan *et al.* [99] find that Wnt4 can increase the expression of Dax1 in developing ovaries, and block Wnt4 and Dax1 to cause the female and male of the fetus to be reversed. It is also reported that WT1 activated Dax1 promoter activity, which played an important role in the process of gender identification [100].

3.3.3. CITED2

CITED2 is a co-transcriptional activator of the cAMP (cAMP response element binding protein, CREB), which is associated with the development of multiple tissues, including the heart, adrenal gland and nerve tissue [101] [102]. There are adrenal development defects in the Cited2-/-mouse [103]. Val [104] finds that in the study of Cited2 in adrenocortical development, Cited2 is expressed in the epithelial cells of the urogenital crest in the 10th day of the mouse embryo and co-activated Sf1 with Wt1. Research has also confirmed that [71] the synergistic effect of Cited2 and Wt1 causes the fluctuation of Sf1 in the adrenal gland primordium, resulting in the development defect, and the development of adrenal gland is more sensitive to this fluctuation than the gonadal gland.

CITED2 is expressed in the fetal adrenal glands [105]. Studies have shown that sf-1 can be combined in the CITED2 promoter region to promote expression [105]. However, there is no CITED2 mutation in patients with adrenal dysplasia or defect, but there is new gene polymorphism. There is also a link between CITED2 and endothelial chemokines [102]. Adrenal gland has a complex vasculature, Endothelial cell is very important to the migration of adrenal cortex cells [106], CITED2 can be adjusted by angiogenesis factor and bFGF [103], thus speculate that CITED2 plays an important role in contact with adrenal cortical cells and adrenal endothelial cells, its specific mechanism of action is unknown. Studies have shown that in AGP, the expression of CITED2 is necessary for proper proportional differentiation of the adrenal primitive area (AP or fetal area-FZ) [4] [104].

3.3.4. PBX1

PBX1 is a homologous structural protein that regulates adrenal development. Pbx1 was expressed in epithelial cells of the urinary reproductive crest on the 13.5th day of gestation. Female mice with Pbx1 knockout were unable to complete the complete differentiation of the urinary reproductive crest, which resulted in the loss of adrenal gland and subsequent renal dysplasia [107]. The Pbx1 knockout causes the hair and adrenal gland and the adrenal gland to be abnormal, and the hypothesized that the cell proliferation slows more than the cell apoptosis. Lichtenauer *et al*. [108] confirm that Pbx1 in adrenal gland development plays an important role in the embryonic development, not only in adult adrenal function also play an important role in maintain, at the same time, also finds that can increase Pbx1 expression Sf1, both increase Mc2R expression. Some studies have reported [105] that SF-1 has a weak activation effect on the promoter of PBX1, but when SF-1 and DAX1 work together, it promotes enhanced transcription.

3.3.5. WT1

WT1 is a zinc finger protein, which plays an important role in the development of urinary reproductive crest and subsequent adrenal gland [14] [71] [109]. WT1 is the first transcriptional factor needed for adrenal development. It is expressed in the urinary reproductive crest in the 9th day of pregnancy in mice [71], which is combined with Cited2 to stimulate the expression of Sf1 and promote adrenal development [104] [110] [111]. Therefore, it is speculated that human chorionic gonadotropin (HCG) acts as an adrenocortical corticotropin in the fetus and regulates the production of fetal adrenal DHEAS. However, it does not affect the production of fetal adrenal steroid hormone [41] when it is given to the brainless HCG.

3.3.6. GATA Binding Protein (GATA) 4 and GATA6

GATA binding protein (GATA) 4 and GATA6 transcription factors have long been implicated in adrenal development [112] and adult adrenal function [113] [114] [115] [116]. Sergei, G., *et al*. [72] determined that adrenal loss of GATA function is incompatible with adrenocortical development. Specifically, a combined loss of GATA4and GATA6 in the precursor cells results in the loss of SF1expression, decreased adrenocortical proliferation, and adrenal agenesis in both sexes. GATA6 serves as a principal driver of adrenocortical cell maintenance, whereas GATA4 protein acts in an ancillary role, carrying out basic regulatory functions to support the requisite number of steroidogenic cells to assure animal viability in the absence of GATA6.

3.4. Placental Factors

Rapid degeneration of the fetuses at birth after separation from the placenta suggests that certain substances produced by the placenta may play a role in the development and/or maintenance of the placenta. Human placenta produces a variety of hormones (such as HCG, CRH, estrogen, etc.) that may affect the

growth and function of the fetal adrenal gland [3] [117].

3.4.1. HCG

The CG produced by the placenta is a specific hormone characteristic of primates. Human fetal HCG production peaks at the 10th week of pregnancy and then begins to decline. Lanman [118] first proposed that HCG can regulate the development of fetal adrenal glands. Studies have found that [119], babies born to give a week after HCG, urine excretion of DHEA increased significantly, its effects on the fetus zone to promote the synthesis of steroid hormone, and is more sensitive than the full term of HCG in preterm neonates. In vitro experiments also confirmed [120] [121] that the secretion of DHEA-S increased when HCG was given in fetal adrenal cell culture medium of primary culture. Although numerous studies have confirmed the role of HCG in fetal adrenal development in primates, the mechanism of its action is still unclear.

3.4.2. Estrogen

DHEA-S produced by the fetal and maternal adrenal glands provide precursor to the synthesis of placental oestrogen, and the oestrogen produced by the placenta can also promote the secretion of DHEA-S in fetal adrenal glands [122]. Estrogen increases the ACTH-induced DHEA-S secretion in the fetal adrenal cortex and inhibits the secretion of cortisol [59], which does not affect the expression of the enzyme P450scc, P450c17 or 3 β-HSD of synthetic cortisol [123]. Estrogen, the adjustment is the opposite of DHEA-S as a result, in vivo and in vitro experiments [124] [125], estrogen inhibits mid pregnancy baboons adrenal DHEA-S produce, offer estrogen suppresses the generation of fetal adrenal DHEA-S is to maintain the physiological balance between estrogen during pregnancy.

Estrogen can affect the target organs by estrogen receptors (estrogen receptors, ERs). Hirst [123] and other studies have found that monkeys in the middle of a pregnancy, the fetus fetal adrenal don't express ERs, It is speculated that estrogen to the fetus with function is not through the regulation of ERs, and then take the enzyme activity of steroid hormones on the fetus than other gene transcription regulation.

3.4.3. CRH and ACTH

Placental tissue can produce CRH [126] and ACTH [127]. CRH produced placental trophoblastic cells can be induced fit trophoblast cells secrete ACTH [128] [129], but the placenta ACTH secretion to adjust the action of fetal adrenal cortical level is not very clear, because its role in fetal adrenal gland development is small. The CRH secreted by the placenta can enter the fetal body, increasing CRH content in the fetus, affecting the development of fetal adrenal gland through the pituitary-adrenal axis of the fetus [130] [131].

4. Summary

The development and function of fetal adrenal cortex in mammals are unique, and play an important role in regulating the metabolic homeostasis of electro-

lytes and nutrients. The structure and function of the adrenal dysplasia will lead to the production of a variety of diseases, not only has its recent harm, but there is also a long-term adverse effect, so it is of very vital significance to know the structure of fetal adrenal gland development and discuss its function regulation. At present, great progress has been made in the study of functional regulation of adrenal cortex. Transgenic and gene knockout mice play an important role in the study of adrenal cortical development. The development and function of adrenal cortex are regulated by multiple hormones, genes and mechanisms. Although significant progress has been made, studies on its underlying mechanisms, such as the occurrence of significant epigenetic modification of transcription factors and changes in specific gene-specific gender differences, have been relatively superficial. Recently, the primate of placenta on fetal adrenal cortical development regulation provides us a new point of view, through the fetus-placental unit to expand our physical contact between the placenta and fetal adrenal gland, and provides a new research perspective and field for us to study the function of the adrenal gland development.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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The Correlation between sST2 and Atrial Fibrillation and Its Clinical Significance

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Abstract

Objective: To explore the level of soluble growth-stimulated expression gene 2 (sST2) in patients with atrial fibrillation (AF) and its relationship with atrial remodeling. **Methods:** From December 2018 to July 2019, 86 patients were enrolled in the cardiovascular department of Jingzhou Central Hospital in Hubei Province. AF group was 60 patients with AF diagnosed according to the diagnostic criteria of electrophysiology and pacing branch of Chinese Medical Association, while the control group was 26 normal ECG patients with sinus rhythm examined by ECG and/or DCG. Clinical data, NT proBNP and birth rate of all patients were collected chemical indicators. All the patients in the groups were examined by ECG and color Doppler echocardiography. The serum level of ST2 was detected by enzyme-linked immunosorbent assay (ELISA), and the indexes related to atrial remodeling in echocardiography, left atrial diameter (LAD), were collected, compared and analyzed. **Results:** Serum sST2 levels and LAD and RAD values in the AF group were higher than those in the control group ($P < 0.05$ or $P < 0.01$). Serum sST2 levels were higher in the AF group than in the paroxysmal atrial fibrillation group ($P = 0.031$). 1) The sST2 level was positively correlated with LAD and RAD measured by cardiac ultrasound ($r = 0.388$, $P = 0.029$). **Conclusion:** Serum sST2 levels have early recognition value in patients with high-risk atrial fibrillation, which contributes to the stratification of risk of atrial fibrillation and the prediction of disease activity. Elevated serum sST2 levels may be associated with atrial remodeling. sST2 may be an objective biomarker that predicts the risk of emergency admission in patients with atrial fibrillation. Elevated sST2 levels may be associated with atrial fibrillation progression.

Keywords

Atrial Fibrillation, Atrial Remodeling, Soluble Growth Stimulator Gene 2

1. Introduction

At present, the mechanism of atrial fibrillation (AF) has been studied extensively, but its specific pathological mechanism is still unclear. More and more research results support that atrial remodeling caused by AF plays an important role in AF [1]. Recently, it has been pointed out that soluble growth stimulating gene 2 (soluble growth stimulation expressed gene 2, sST2) may be involved in the development of atrial fibrillation. Therefore, this study aims to explore the level of sST2 in patients with atrial fibrillation and its relationship with atrial remodeling. As one of the members of the interleukin-1 receptor superfamily, ST2 mainly exists in soluble ST2 (sST2) and trans model ST2 (ST2L) [2]. At present, it has been found that high concentration of sST2 can competitively bind to IL-33 as a "decoy receptor". By inhibiting the anti-fibrosis effect of ST2L/IL-33 pathway in the heart, it can promote abnormal cardiac hyperplasia and cardiac remodeling [3] [4] [5]. As a biomarker of myocardial fibrosis, sST2 may also be related to AF [6]. Therefore, high concentration of sST2 in patients with AF plays an important role in the risk stratification and disease activity prediction of AF. At present, an imaging index to evaluate atrial remodeling in clinical work is atrial diameter. Therefore, according to the adult echocardiography standard recommended by ASE/EACVI [7] in 2015, this study studies the progress of sST2 and atrial fibrillation and its relationship with atrial remodeling by comparing the correlation between serum sST2 and atrial diameter.

2. Data and Methods

2.1. Subjects

The AF group randomly selected 60 patients who were admitted to the cardiovascular department of Jingzhou Central Hospital of Hubei Province from December 2018 to July 2019, including 34 males and 26 females, aged (65.44 ± 7.73) years. The diagnosis and classification criteria of AF Group [8]: the criteria formulated by the electrophysiology and pacing branch of the Chinese Medical Association: subjective symptoms of palpitation and chest distress and evidence of abnormal ECG and/or DCG: P wave disappeared during AF, F wave replaced, frequency 350 - 600 times/min, the QRS rhythm is absolutely irregular, which shows that the RR interval is uneven, and the QRS wave shape is mostly normal. If it is accompanied by indoor differential conduction or by-pass, it can cause QRS wave width deformity. Exclusion criteria: Patients with acute and chronic bacterial or viral infection, active asthma, chronic obstructive pulmonary disease, acute pulmonary embolism, pregnancy, malignant tumor, tuberculosis, rheumatism and immune system disease, chronic liver and kidney dysfunction, congenital heart disease, cardiomyopathy, valve disease and heart failure. In the control group, 26 normal ECG patients without the diseases listed in the above exclusion criteria were selected, including 13 males and 7 females, aged (60.12 ± 8.02) years. The clinical data and biochemical indexes of all patients were collected. This study was approved by the clinical research ethics committee of the

Second Clinical Medical College of Yangtze University. All the subjects signed informed consent forms.

2.2. Research Methods

All the selected subjects collected about 5 ml of venous blood in the morning of the second day after admission and placed it in the coagulating blood vessels. They were centrifuged at 4˚C at 3000 r/min for 10 min, sealed and sealed after serum separation, and stored in a low-temperature refrigerator at −70˚C for testing. After the centralized collection of blood samples, the serum level of sST2 was detected with the sST2 detection kit (enzyme-linked immunosorbent assay, American C & D Company), and the operation steps were strictly in accordance with the instructions of the kit. At the same time, venous blood was collected and sent to the hospital laboratory to be tested by the professional physician of the laboratory of the hospital for blood routine test, blood biochemistry and other indicators. The left atrium diameter (Left atrial diameter, LAD) and right atrium diameter (Right atrial diameter, RAD) were measured by the specialists in the ultrasound room of our hospital.

2.3. Statistical Analysis

S-W method is used to test the normality of measurement data. The measurement data conforming to the normal distribution is expressed in x ± s, and the analysis of variance is used for the comparison between groups; the measurement data not conforming to the normal distribution is expressed in M (QL, QR), and the comparison between groups is expressed in Kruskal-Wails H test. Spearman correlation analysis was used to test the correlation of measurement data. Taking the grouping of AF as the dependent variable, the items with statistical significance in single factor analysis as the independent variable, and at the level of $\alpha = 0.10$, the logistic regression analysis of multi-classification disorder was carried out. The difference was statistically significant ($P < 0.05$).

3. Results

3.1. Comparison of General Clinical Data of Subjects

There was no significant difference in age, gender, height, weight, diabetes mellitus, coronary heart disease, smoking history and drinking history between paroxysmal AF group and persistent AF group and control group.

3.2. Comparison of Serum NT-ProBNP, LAD and Serum sST2 Levels

The level of NT proBNP in paroxysmal atrial fibrillation group and persistent atrial fibrillation group was significantly higher than that in the control group ($P < 0.05$). Lad in paroxysmal AF group and persistent AF group was significantly higher than that in control group ($P < 0.05$). The level of serum ST2 in persistent atrial fibrillation group was higher than that in paroxysmal atrial fibrillation

group and control group (P < 0.05) (see Table 1).

3.3. Correlation between NT-ProBNP, LAD and sST2 in AF Group

There was a positive correlation between NT proBNP and ST2 (r = 0.314, P < 0.05). It is suggested that both of them have the same function in AF, suggesting that AF is not good after rain (see Table 2). LAD was positively correlated with serum ST2 (r = 0.523, P < 0.05). It is suggested that both of them have the same function in AF, suggesting that AF is not good after rain (see Table 2).

3.4. Serum sST2 Was Associated with Persistent AF (P < 0.05)

The risk of sustained AF increased by 1.447 times for every unit increase of serum sST2. There was no significant correlation between serum sST2 and paroxysmal AF (P > 0.05), but the risk of paroxysmal AF increased by 0.950 times for each additional unit of serum sST2, but it remains to be studied (see Table 3).

Table 1. Comparison of the results of NT-proBNP, LAD and sST2 levels in each group [M, (QL, QR].

Grouping	Number of cases	NT-ProBNP (ng/ml)			LAD (cm)			sST2 (pg/ml)		
		M	P25	P75	M	P25	P75	M	P25	P75
Paroxysmal atrial fibrillation	35	465.0	200.0	900.0	3.9	3.7	4.6	17.7	11.2	24.4
Persistent atrial fibrillation	25	1120.0°	715.5	1693.5	4.5	4.0	5.7	39.9°	37.2	49.3
Control group	25	50.0*$^{\triangle}$	36.5	68.0	3.3*$^{\triangle}$	3.2	3.6	14.3$^{\triangle}$	10.3	19.5

Note: "*" indicates that there is significant difference between the control group and the paroxysmal AF group; "$^{\triangle}$" indicates that there is a significant difference between the control group and the persistent AF group; "$^{\circ}$" indicates that there is significant difference between paroxysmal AF group and persistent AF group. P < 0.05 was statistically significant.

Table 2. Correlation analysis of serum ST2 level with NT proBNP and LAD in AF group.

Research factors	Serum ST2 level (pg/ml)	
	r value	P value
NT-ProBNP (ng/ml)	0.523	<0.05
LAD (cm)	0.314	<0.05

Table 3. Regression analysis of NT-proBNP, LAD and sST2 in serum of AF patients.

Project		Regression coefficient	Standard error of regression coefficient	wald	P	OR	95% CI
Paroxysmal atrial fibrillation	NT-proBNP	0.016	0.007	5.503	0.019	1.016	1.003 - 1.030
	LAD	2.645	1.749	2.286	0.131	14.086	0.457 - 434.335
	sST2	−0.052	0.066	0.618	0.432	0.95	0.835 - 1.080
Persistent atrial fibrillation	NT-proBNP	0.014	0.007	4.292	0.038	1.014	1.001 - 1.028
	LAD	3.472	1.98	3.075	0.08	32.203	0.664 - 1560.776
	sST2	0.37	0.146	6.413	0.011	1.447	1.087 - 1.926

4. Discussion

At present, many studies have determined the predictive effect of serum sST2 level on myocardial infarction and heart failure [9] [10] [11]. In 2018, a study found that the serum concentration of sST2 in patients with AF was significantly higher than that in the healthy control group, and the serum concentration of sST2 in patients with persistent AF was higher than that in patients with paroxysmal AF in the AF subgroup, suggesting that the increased serum concentration of sST2 may play an important pathophysiological role in the development of AF [12]. The results of this study also showed that the correlation between AF and serum sST2 level was higher than that of the control group. Other studies have shown that BNP level in persistent AF is higher than that in paroxy-small AF [13]. BNP level in patients with paroxysmal AF was significantly higher than that in patients with sinus rhythm [14]. The findings of this study are consistent with this. More and more studies have found that BNP is useful in predicting some cardiovascular diseases, but it has limitations, such as age or renal function and other factors may affect the results, and sST2 may play a role in supplementing and replacing these limitations of BNP. Another important finding of this study is that there is a positive correlation between sST2 and LAD. Cardiac hemodynamic overload will lead to atrial relaxation, and atrial dilation is the main sign of left atrial remodeling. Atrial remodeling promotes the occurrence or maintenance of atrial fibrillation. Cardiac hemodynamic overload will lead to atrial relaxation, and atrial dilation is the main sign of left atrial remodeling. Atrial remodeling promotes the occurrence or maintenance of atrial fibrillation [15], and may also stimulate the secretion of sST2 and BNP [16]. The results of this study also showed that the LAD of patients with AF was higher, and there were also statistical differences in the levels of serum sST2, NT-proBNP and LAD among different AF subgroups. These are consistent with the above results. Of course, there are some shortcomings in this study, such as the small number of cases, the lack of detection of serum sST2 level in patients undergoing atrial fibrillation ablation, so the results still need more clinical research to confirm. In conclusion, the increase of serum sST2 level may be involved in the progress of atrial fibrillation and may be related to atrial remodeling, which may be a potential marker of atrial fibrillation. At the same time, the combination of sST2 and NT-proBNP is more helpful to judge the cardiac remodeling after AF. Therefore, serum sST2 can be used as a biomarker for the activity of atrial fibrillation disease, as an independent biomarker for the prediction of other cardiovascular diseases, especially heart failure in patients with atrial fibrillation, and can be used to guide clinical treatment.

5. Summary

In conclusion, we found that the increase of serum ST2 concentration may play an important pathophysiological role in the development of atrial fibrillation, and it is related to atrial remodeling, which suggests that the increased serum

ST2 level can be used as a biomarker of the activity of atrial fibrillation disease, and it can predict the recurrence of persistent atrial fibrillation after the treatment, which is helpful to our atrial fibrillation. The clinical diagnosis and treatment of the disease have important guiding significance.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Combined Detection of Serum Heat Shock Protein-90α and Prostate Specific Antigen for Prostate Cancer Diagnosis

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Abstract

Objective: To explore the relationship between heat shock protein-90α (HSP-90α) and occurrence of prostate cancer, and clinical value of combined detection of serum HSP-90α and prostate specific antigen (PSA) in the diagnosis of prostate cancer. **Method:** A total of 30 patients with prostate cancer, 30 patients with benign prostatic hyperplasia (BPH) and 30 healthy men (control group) were selected from September 2018 to September 2019, then to detect levels of serum HSP-90α, total PSA and free PSA (FPSA) by ELISA, serum testosterone level by radioimmunoassay, prostate cancer tissue was removed by operation, and relative expression of tissue HSP-90α protein by Western blot. **Results:** The levels of serum HSP-90α and total PSA in prostate cancer group were significantly higher than other two groups, and testosterone level was lower than other two groups ($P < 0.05$); there was no difference of serum FPSA level between the three groups ($P > 0.05$). It was found by Pearson test that serum HSP-90α was positively correlated with total PSA level ($r = 0.659$, $P = 0.005$), while negatively correlated with testosterone level ($r = -0.549$, $P = 0.006$). According to TNM stage of prostate cancer, there were 17 cases of stage I - II, 13 cases of stage III - IV, 6 cases of Gleason score 1 - 4, 13 cases of 5 - 7, 11 cases of 8 - 10, tumor diameter range from 0.8 to 6.2 cm, with average of (3.9 ± 1.5) cm. The relative expression of HSP-90α protein in tumor tissue was closely related to TNM stage, Gleason score and tumor diameter ($P < 0.05$). By ROC analysis, it was found that accuracy of combined detection of serum HSP-90α and PSA levels for prostate cancer diagnosis was 0.896, and that of single PSA detection was 0.852. **Conclusion:** Higher expressions of HSP-90α in prostate cancer tissue and serum may be closely related to occurrence and development of prostate cancer, and combined detections of serum HSP-90α and PSA levels are of great significance in improving early diagnosis of prostate cancer.

Keywords

Heat Shock Protein-90*α*, Prostate Specific Antigen, Prostate Cancer

1. Introduction

Prostate cancer is the second most common malignant tumor in men, with a rapid increase in recent years, increasing with age [1]. The diagnosis and TNM staging of Prostate cancer are mainly based on prostate specific antigen (PSA) level, MRI, biopsy of Prostate biopsy, and isotopic bone scan, etc., which are the main basis for the diagnosis and treatment of Prostate cancer clinically [2]. China's PSA screening is not yet complete, at least 35.8% of prostate cancer patients have advanced to advanced prostate cancer when diagnosed [3]. With the in-depth study on the molecular mechanism of tumor development, it has been found that Heat shock protein-90 (HSP-90) may be activated through multiple signaling pathways in the process of prostate cancer carcinogenesis, which is closely related to the occurrence, development, treatment and prognosis of prostate cancer [4] [5]. This study mainly discussed the relationship between HSP-90 and the incidence of prostate cancer, and the clinical value of combined detection of serum HSP-90 and PSA in the diagnosis of prostate cancer.

2. Objects and Methods

2.1. Object Data

A total of 30 patients with pathologically diagnosed prostate cancer, 30 with benign prostatic hyperplasia (BPH) and 30 healthy males (control group) were randomly selected from September 2018 to September 2019. Inclusion criteria: 1) Age 18 - 75; 2) Consistent with the diagnosis of prostate cancer and BPH [6] [7]; 3) Prostate cancer and BPH without surgery, chemoradiotherapy or biological therapy; 4) Obtain informed consent and ethics medicine of our college, the clinical data completed. Exclusion criteria: 1) Kidney tumors, urinary tract infections, and stones; 2) Severe liver and kidney dysfunction and autoimmune diseases.

Patients with prostate cancer ranged in age from 48 to 74 years, with an average age of 56.5 ± 9.7 years, body mass index (BMI) 23.5 - 27.8 kg/m^2, mean (25.5 ± 2.6) kg/m^2. There were 17 cases of stage I - II and 13 cases of stage III - IV in TNM stage. Gleason scores ranged from 1 to 4 in 6, 5 to 7 in 13, and 8 to 10 in 11. Tumor diameter ranged from 0.8 to 6.2cm, with an average (3.9 ± 1.5) cm. Patients with BPH ranged in age from 45 to 73 years, with an average age of (56.3 ± 9.5) years. BMI 23.3 - 27.5 kg/m^2, mean (25.2 ± 2.5) kg/m^2; The control group was 44 - 75 years old, with an average of (56.6 ± 9.9) years old, and a BMI of 23.2 - 27.9 kg/m^2, with an average of (25.6 ± 2.8) kg/m^2. The age and BMI of the three groups were comparable ($P > 0.05$).

2.2. Research Methods

Serum HSP-90, total PSA and free PSA (FPSA) levels were detected by ELISA, serum testosterone levels were detected by radioimmunoassay, prostate cancer tissues were surgically removed, and the relative expression of HSP-90 protein in tumor tissues was detected by Western blot. The normal reference range of serum HSP-90 was 0 - 82 ng/ml. The normal PSA reference range is 0 - 4 ng/ml, the FPSA reference range is 1 - 1.2 ng/ml, and the testosterone reference range is 300 - 1000 ng/dl for men.

2.3. Detection Method

2.3.1. Main Steps of ELISA

Fasting cubical vein 6 ml was collected in edta-k2, mixed upside down for 6 - 8 times, and the upper layer of plasma was absorbed into another EP tube. Adjust the microplate analyzer, open the microplate analyzer software, set the plate layout, including 6 calibration holes, 1 - 2 quality control holes and sample holes; The kit was taken out and balanced at 37˚C for 30 min. The freeze-preserved samples were melted at room temperature and thoroughly mixed, and diluted 20 times (5 l sample + 95 l diluent). Adding samples, adding 50 l calibration products, quality control products and diluted samples to the plate hole continuously and without interruption according to the sequence of hole layout of the enzyme marker, the whole plate was completed within 10 min. Add markers, add 50 l marker liquid to each plate hole, cover the sealing plate film and slightly mix; After incubation, the solid plate was incubated at 37˚C for 60 min. Wash the plate in the plate washer, add 50 l chromogenic agent A and B into the plate hole, cover the sealing plate film and slightly mix; The solid plate was incubated at 37˚C for 20 min. Add 50 l stop fluid to the plate hole, slightly mix; The solid phase plate was placed into the microplate to read OD value within 10 min at 560 nm wavelength. The normal reference range of serum HSP-90 was 0 - 82 ng/ml [8]. The normal PSA reference range is 0 - 4 ng/ml, the FPSA reference range is 1 - 1.2 ng/ml, and the testosterone reference range is 300 - 1000 ng/dl for men [9].

2.3.2. Main Steps of Western Blot

The freshly resected tumor tissue was then ultrasonically crushed, and RIPA lysate was added to fully lyse the cells and release the protein. BCA protein quantitative reagent (Sigma) was used to extract the cell protein and test the concentration and purity. Groups were 30 mu of g protein samples, 8% sds-page electrophoresis separation, the separation zone electric transferred to the PVDF membrane, increase the mouse anti human HSP-90 alpha and internal beta actin antibodies to a resistance (dilute the concentration of 1:2000, the sigma company) stand for the night, PBS washing resistance drops plus rabbit to sustain rat corresponding antibody two (1:500, the sigma company) at room temperature, 4 h incubation, PBS washing, ECL color. Results: Lab Works 4.5 gel imaging software (Invitrogen, USA) was used for semi-quantitative analysis, and the gray

scale ratio of HSP-90 tablets and gam-actin protein electrophoresis strip was used for expression [10].

2.4. Statistical Methods

SPSS20.0 software was used for statistical analysis, the measurement data were expressed as mean ± standard deviation, single factor ANOVA analysis was used for comparison between the three groups, lsd-t test was used for pairwise comparison, t test was used for comparison between the two groups, Pearson test was used for correlation analysis, and the counting data were expressed as cases (%), and the comparison was performed by 2 test. The serum PSA or combined HSP-90 and PSA levels were used as the indicators for the diagnosis of prostate cancer. The accuracy (expressed by the AUC value of the area under the curve), sensitivity and specificity of the diagnosis were analyzed by ROC, and the critical value was obtained. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of Serum HSP-90, PSA and Testosterone Levels between Groups

Serum HSP-90 and total PSA levels in the prostate cancer group were significantly higher than those in the other two groups, and testosterone levels were lower than those in the other two groups ($P < 0.05$). There was no difference in serum FPSA levels among the three groups ($P > 0.05$), as shown in Table 1.

3.2. Correlation Analysis

Pearson's test showed that serum HSP-90 was positively correlated with total PSA, while negatively correlated with testosterone ($P < 0.05$), as shown in Table 2.

Table 1. Comparison of serum HSP-90, PSA and testosterone levels between groups.

Group	Cases	HSP-90α (ng/ml)	TPSA (ng/ml)	FPSA (ng/ml)	Testosterone (ng/dl)
Prostate cancer	30	105.9 ± 23.5	5.9 ± 1.5	1.3 ± 0.6	426.5 ± 65.9
Prostatic hyperplasia	30	38.4 ± 8.9	2.5 ± 0.8	1.2 ± 0.5	756.5 ± 123.9
Control group	30	35.6 ± 7.7	2.3 ± 0.6	1.1 ± 0.3	762.9 ± 154.8
F		32.635	5.326	0.352	65.639
P		0.000	0.002	0.428	0.000

Table 2. Correlation analysis.

	TPSA	FPSA	Testosterone
HSP-90α	R = 0.659, P = 0.005	R = 0.523, P = 0.248	R = −0.549, P = 0.006
Testosterone	R = 0.632, P = 0.421	R = 0.529, P = 0.632	-

3.3. Relationship between HSP-90 Protein Expression in Prostate Cancer Patients and Tumor

The relative expression level of HSP-90 in tumor tissues was closely related to TNM stage, Gleason score and tumor diameter ($P < 0.05$), as shown in Table 3 and Figure 1.

3.4. ROC Analysis

ROC analysis showed that the accuracy of the combined detection of serum HSP-90 and PSA level in the diagnosis of prostate cancer was 0.896, while the accuracy of the separate detection of PSA was 0.852, as shown in Table 4 and Figure 2.

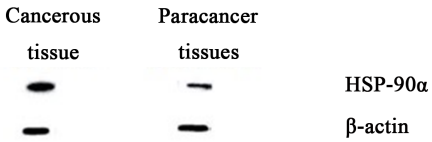

Figure 1. The relative expression level of HSP-90 protein in prostate cancer and adjacent tissues was detected by Western blot (the relative expression level of HSP-90 protein in cancer tissues was significantly higher than that in adjacent normal tissues ($P < 0.05$).

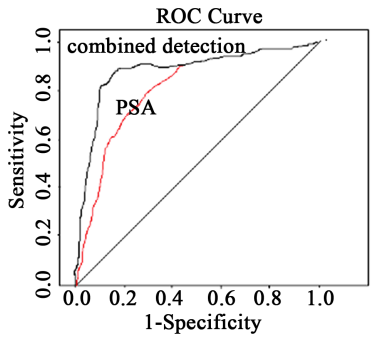

Figure 2. ROC analysis was performed for the diagnosis of prostate cancer by combining serum HSP-90 and PSA levels with a separate PSA test.

Table 3. Relationship between HSP-90 protein expression in prostate cancer patients and tumor.

	Cases	HSP-90α	T or F	P
TNM staging			5.006	0.006
I - II	17	0.36 ± 0.07		
III - IV	13	0.45 ± 0.09		
Gleason score			8.326	0.000
1 - 4	6	0.31 ± 0.05		
5 - 7	13	0.41 ± 0.06		
8 - 10	11	0.49 ± 0.08		
Tumor diameter (cm)			5.231	0.003
<3.9	14	0.35 ± 0.06		
≥3.9	16	0.42 ± 0.08		

Table 4. ROC analysis.

indicators	AUC value	Sensitivity	Specificity	The critical value
PSA	0.852	86.7%	80.5%	3.2 ng/ml
The joint detection	0.896	90.6%	86.5%	PSA = 3.0 ng/ml, HSP-90α = 68.9 ng/ml

4. Discussion

HSP is a group of highly conserved proteins commonly found in eukaryotes, which are mainly involved in protein folding, transport, activation, degradation and protein signaling as molecular chapels [11]. HSP plays an important role in promoting cell survival, maintaining cell autoimmune regulation and other defense mechanisms, and protecting cells from radiation-induced death [12]. HSP is not only expressed in normal cells, but also in some invasive tumors or metastatic cancers. In colorectal cancer, gastric cancer, cervical cancer tumor tissue detection [13] [14], tumor tissue HSP-90 alpha mRNA and protein expression of relative quantity obviously higher than that of precancerous normal tissue, cell differentiation and TNM staging of the tumor, the level, closely related to lymphatic metastasis, tumor malignant degree is higher, HSP-90 alpha positive expression rate and quantitative expression level is also higher. It is suggested that the up-regulation of HSP-90 expression is closely related to the degree of malignancy of various malignant tumors.

This study mainly discussed the pathogenesis of HSP-90 in prostate cancer. It was found that serum HSP-90 and total PSA levels in prostate cancer group were significantly higher than those in the other two groups, while testosterone levels were lower than those in the other two groups ($P < 0.05$). There was no difference in serum FPSA levels among the three groups ($P > 0.05$). It is suggested that the incidence of prostate cancer may be related to the increased release of HSP-90 and PSA, and the decreased secretion of testosterone. Tumor cells affected by the changes of external environment in the body, such as inflammatory factor, the inductive effect protein combined with molecular chaperone and release heat shock factor (heat shock factor, (HSF) expression of HSP-90 alpha expression is regulated by the HSF, HSF forming complexes with HSP-90 alpha in inactivation state, stress complex dissociation, HSF into the nucleus, start the heat shock protein gene and protein transcription process [15] [16]. HSP-90 can prevent protein aggregation, promote the folding of the initial polypeptide chain, and participate in the refolding of damaged proteins and the isolation of degraded damaged proteins [17]. HSP-90 can also reduce cell apoptosis and regulate immune response, thus increasing cell viability [18].

Pearson test found that serum HSP-90 was positively correlated with total PSA level, while negatively correlated with testosterone level ($P < 0.05$). Furthermore, the relative expression of HSP-90 protein in tumor tissues was closely related to TNM stage, Gleason score and tumor diameter ($P < 0.05$). It is suggested that the malignancy of prostate cancer is closely related to the increased

expression of HSP-90 protein. The main function of HSP-90 is to maintain the stability of cell proteins, improve the tolerance of cells to stress response, and make cells maintain normal physiological functions. The content of HSP-90 in tumor cells is 2 - 10 times that of normal cells, and its substrate proteins include a variety of key signaling molecules for tumor growth and metastasis, and HSP-90 is present in a high activity state in a variety of malignant tumor cells [19] [20]. ROC analysis showed that the accuracy of the combined detection of serum HSP-90 and PSA level in the diagnosis of prostate cancer was 0.896, while the accuracy of the separate detection of PSA was 0.852.

PSA as a screening and diagnosis of prostate diseases has a good sensitivity, but the detection level is susceptible to a variety of factors, and the accuracy needs to be improved [21]. High expression of HSP-90 in prostate cancer may be due to rapid growth of cancer tissues, nutrient deficiency and chronic hypoxia, which stimulates increased transcription synthesis of HSP-90 in prostate cancer cells, and sustained high induction of expression. At the same time, the self-protection ability of cancer tissues is enhanced to improve the survival ability of cancer cells [22] [23] [24].

5. Conclusion

In conclusion, the increased expression of HSP-90 in prostate cancer tissues and serum may be closely related to the occurrence and development of the tumor, and the combined detection of serum HSP-90 and PSA levels is of great significance to improve the early diagnosis of prostate cancer. Due to the limited sample size, this study did not dynamically analyze the changes of serum HSP-90 serum level in prostate cancer patients before and after treatment, and failed to elaborate the relationship between HSP-90 serum, PSA and testosterone changes; and cell pathway HSP-90 serum affects the occurrence and development of prostate cancer.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Efficacy of "Acceptance and Commitment Therapy" Psychological Nursing in a Stroke Patient

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Abstract

Objective: To summarize 1 case of ACT (Acceptance and Commitment Therapy, as "ACT") psychological nursing clinical experience. **Method:** ACT Application for psychological nursing, provides detailed program guidance for psychological care. **Results:** Sleep quality, psychological flexibility and self-management ability of the patient have been improved. **Conclusion**: It's the first time to apply ACT to the psychological nursing for a stroke patient, which has achieved good results; it provides a detailed clinical experience as guidance for the development of psychological nursing; it also provides a clinical basis for the follow-up exploration of psychological nursing technology model.

Keywords

Stroke, Psychological Nursing, Acceptance and Commitment Therapy, Sleep Quality, Psychological Flexibility, Self-Management Ability

1. Introduction

According to the World Health Organization, stroke is the second leading cause of death and the third leading cause of disability worldwide [1]. In recent years, due to its high mortality and disability, serious burden has been taken for patients, their families and society, but its negative emotions, such as anxiety, depression, emotional imbalance, anger tendency and so on, are easy to ignore. Patients can't accept the status quo fully, make changes or endure efforts to achieve goals and get values, that is mental flexibility [2] declining. It leads to a series of problems to affect the prognosis for patients, like insomnia, refuse rehabilitation

training and so on. Although psychological nursing develops widely and deeply in China, the clinical nurses still have many problems in the mental nursing knowledge, ability and the initial implementation of psychological care; they meet difficulties. Therefore clinical adverse events are common because of psychological neglect, and there isn't a detailed and professional clinical case for psychological nursing [3] [4] [5] [6]. The nurse in our hospital, who has double degrees in nursing and psychology, accepted the face-to-face training of Steven C. Haye (the founder of Acceptance and Commitment Therapy, ACT). She has explored the combination of psychological treatment methods and clinical nursing experience after the observation on stroke unit patients, used ACT to psychological care for middle-aged stroke patients while cooperating with doctors for active treatment, to compare the results of psychological assessment before and after, that is, to use their own before-after study experimental methods to verify whether this method is effective, and the effect is significant. Therefore, select a representative nursing case to explain the operation process of psychological nursing in more detail below.

2. Materials and Methods

2.1. Clinical Materials

A patient, male, 58 years old, high school diploma, veteran, stroke duration of more than 10 months, has suffered from stroke in January 2018 without other disease histories. He was transferred from the emergency department to our department (Stoke Unit) because of fainting suddenly on November 24, 2018. His CT scan showed multiple lacunar cerebral infarctions in the brain and he was diagnosed as acute ischemic stroke. After active treatment, his condition was stable on December 2, 2018, without conscious disorder and cognitive impairment, but gait instability and right limb movement limited was happened. The right side of his body was in the first III stages (early joint movement)for The Brunnstrom Stroke Recovery Grade, the right upper limb can do cooperative movement at will, grasp and pinch hook shape, but the fingers can't extend; the right lower limb had cooperative flexion in hip, knee and ankle in sitting and standing position. The score of Carr-shepherd Motor Function Evaluation is 17, he had moderate dyskinesia. The doctor suggested him do rehabilitation training 2 hours every day, infusion therapy with drugs for stroke such as butylphthalide, and routine nursing including monitoring vital signs and intravenous infusion as advised by the doctor. When the nurse made ward visiting on December 3, 2018, she found that the patient was conscious and in poor spirits and communicated with the patient and his family actively. Patient complained that he felt anxiety, fear and insomnia; his family said he became silent, avoided talking to others, and refused relatives and friends to visit him.

2.2. Method of Psychological Nursing

At present, the main method of professional psychological intervention is Cog-

nitive Behavioral Therapy (for short as CBT), that is, through psychological counseling, interviews, sand tables, pictures and other ways to change the cognition to change the behavior of patients, but the system reviews show many kinds of results for stroke patients who have mood disorders, and there is no conclusive evidence to show that it works; its psychological support for patients is also limited, and the period, 12 times psychological interventions as a cycle generally, is too long; it's limited to cognitive changes that make individual cognitive burden heavier possibly, even influence the effects of intervention [7] [8] [9]. "Acceptance and Commitment Therapy" (for short as ACT) has been used in this case, a new method of psychological intervention, which based on science and Relational Theoretical Framework and proposed in 1999 by Steven c. Hayes, an American psychologist [10]. The ACT theory includes 6 parts: flexible attention to the present moment, acceptance, cognitive defusion, self-as-context, committed action, valuing [11]. Relevant research results show that, it not only improves physical function, but also improves a range of mental health problems [12]. The difference between CBT and ACT is that ACT encourages people to accept the facts not try to change, and psychological flexibility improved as the final aim of ACT. Many domestic researches [13]-[18], using ACT to relieve mental health problems for cancer patients, COPD patients, diabetics, postpartum depression infertility and their families, show that it can improve them psychological flexibility, thus improve them sleep quality and self-management ability. Many studies at home and abroad have shown that, to some extent, psychological flexibility reflects mental health level [19]. But it doesn't be used in stoke patients, and there isn't a case described in more detail to guide clinical using, thus this case dose it.

2.3. Psychological Assessment

2.3.1. Self-Management Behavior Rating Scale for Stroke Patients

Self-management is an act [19], which improves patients' health through managing some behaviors during the treatment of their illnesses, in order to reduce the influences of illness on social function, emotions and relationships. The scale [20] has 50 entries in 7 dimensions, the total score is 50 to 255, the score higher, the self-management behavior ability better; the Cronbach's α is 0.83.

2.3.2. Pittsburgh Sleep Quality Index, PSQI

The scale [21] is applicable to the evaluation of sleep quality for patients with sleep disorders and mental disorders, as well as the evaluation of sleep quality for general people; the total score is 0 to 21 points, the score higher, the sleep quality worse; the Cronbach's α is 0.845.

2.3.3. Acceptance and Action Questionnaire-II, AAQ-II

The questionnaire is used to measure the degree of "empirical avoidance", there are 7 items in the scale, with points from 1 (never) to 7 (always), the score higher, the degree deeper of empirical avoidance; the Cronbach's α is 0.84 [22] [23] [24]. "Empirical avoidance" [25] is an act that people attempts to change the

form, frequency, or sensitivity of their internal experiences (such as thoughts, emotions, and somatosensory sensations) in their minds, even if doing it can lead to actions that are inconsistent with their personal values or goals (such as giving up pursuing a long-term goal in order to avoid anxiety).

2.3.4. Cognitive Fusion Questionnaire, CFQ

"Cognitive Fusion" is the tendency of people's behavior to be excessively controlled by language rules and thought content, which will enable individuals to automatically extract the literal meaning of thought events, and thus unable to guide their behavior with the direct experience of the "here and now" [26]. There are 9 items in the scale [27], with points from 1 (never) to 7 (always), the score higher, the degree deeper of cognitive fusion; the Cronbach's α is 0.89.

3. Psychological Nursing Processes

3.1. Interview

Uses ACT for analysis; results are listed in **Figure 1**.

3.2. Application Details

3.2.1. Cognitive Defusion

Soft music is used to guide the patient to recall the terrible inner events and compare it or them to "the monster on the bus". The patient is the bus driver and there are many passengers on the bus. At this time, he complained of "monster" as "permanent hemiplegia".

3.2.2. Acceptance

He was guided to imagine the route of the bus, with each passenger having his or her own station to get on or off. It was impossible to predict which passengers

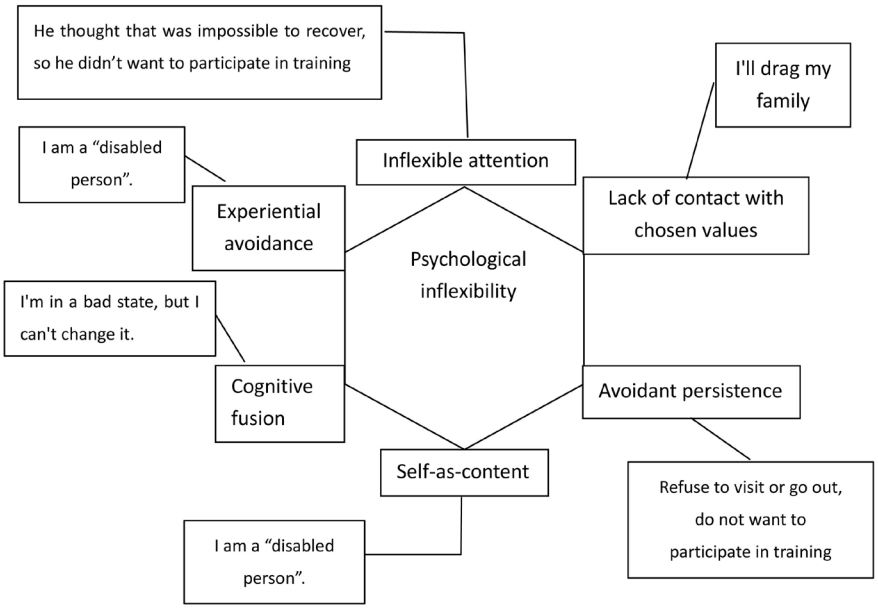

Figure 1. Results of interview.

would get on. There may be hidden "monsters" in it, but anyway the car will arrive at the terminal.

3.2.3. The Present Moment

Guide him to imagine the scene in the car, "monster" suddenly appeared, what will the driver do? He replied that there will be panic in the car and delay the journey, and as the driver, he should ensure the passengers' safety, find ways to subdue the "monster", reduce the loss, maximize the optimization results, and ensure passengers to the station safely, and to the terminal finally.

3.2.4. Self-as-Context

Guide him to imagine that if there were other bus drivers saw such a bus appear at the terminal, how would others feel? Others admire or ridicule? "Admire". He answered.

3.2.5. Valuing

Ask him: what will happen if you had abandoned the car? The bus is like your body. It can be your life. Some things happen unexpectedly, but after the occurrence, you can choose the result. You can abandon the car, or face it and try to solve.

3.2.6. Committed Action

Guide him to talk in detail about how he would address this situation if he were the driver. What specific goals are achieved? According to "each passenger will have a safe arrival station" and "to the destination", guide him to make rehabilitation plans and design simple and convenient rehabilitation actions. Such as picking fruit from large to small, he still can implement after discharge. Determine the goal of phased rehabilitation, as well as the ultimate goal. The nurse and his family should be based on trust and guidance, supplemented by scientific and technological tools for telephone video follow-up and exchange program progress, rather than absolute review, to avoid causing psychological pressure for patients.

3.3. Results

Measurement results of these scales before and after ACT psychological nursing are listed in Table 1.

Table 1. Results of ACT psychological nursing.

	2018-12-3	2018-12-15	2019-1-15
PSQI	16	10	9
Self-management	142.59	182.59	205.17
AAQ-II	30	22	15
CFQ	59	39	23

4. Discussion

The psychological load of stroke patients in rehabilitation period is heavy, especially for the elderly patients with impaired limb function, the rehabilitation period is a long and slow process, so should pay more attention to psychological nursing. Due to psychological rigidity, most patients are unwilling to accept rehabilitation training, self-denial, self-abandonment and other negative emotions, resulting in poor sleep quality and self-management ability, thus affecting the quality of life and even causing secondary stroke [28]. In this case, ACT was applied for psychological nursing for stroke patients at the first time with innovative methods, clear operating regulations, and detailed procedures recorded and displayed to provide guidance for the implementation of clinical psychological nursing. ACT psychological nursing greatly improved patients' sleep quality and psychological flexibility, improved self-management ability, and improved quality of life, making patients pay attention to the moment, take the initiative to set action goals, and in the behavior to achieve the goal of perseverance, so as to find self-value. There are also many domestic research [29] [30] [31] [32] [33] is applied to the patient's psychological nursing, such as cancer patients and their families, patients with COPD, postpartum depression and mental health problems such as infertility crowd, to improve diabetes self-management ability, mental flexibility. And ACT psychological nursing intervention cycle is short, without trauma.

This study shows that ACT Application can significantly improve patients' self-management ability, which is consistent with the findings of other scholars. However, at present, there are few clinical nurses with professional psychological background, and due to the heavy clinical work, they tend to ignore the further study of psychological knowledge, and relatively lack of professional nursing psychology education, so the implementation of professional ACT psychological nursing is facing certain difficulties, so it is urgent to train a batch of psychological nursing specialist nurses. Some studies have shown that no statistical significance about ACT was used by psychologists and trained ordinary medical workers for patients [34]. This suggests that we can train more medical workers to master ACT intervention technology, train professional psychological nursing teams, and make clinical psychological nursing more standardized.

5. Summary

To sum up, patients have heavy psychological burden in the rehabilitation period of stroke, especially with impaired limb function. The application of ACT can improve patients' psychological flexibility, thus improving the quality of sleep and self-management ability. ACT also can be applied to the psychological nursing of patients with other diseases skillfully, to provide reference for the standardized development of psychological nursing in China.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Exploration and Research on Animal Model of Brain Schistosomiasis

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Abstract

Objective: A rabbit model of schistosoma granuloma was established by injecting *Schistosoma japonicum* eggs via carotid artery after opening blood-brain barrier with mannitol. **Methods:** Rabbits in the experimental group were injected with 1 ml of *Schistosoma japonicum* egg suspension after injecting mannitol into carotid for 20 min to establish a rabbit model; The rabbits in the negative control group were injected with mannitol through carotid artery for 20 min, followed by 1 ml normal saline injection; rabbits in the positive control group were injected with 1 ml suspension of *Schistosoma japonicum* eggs after coning cranial. The clinical manifestations of three groups of animals were observed after surgery, and brain tissue was taken for pathological examination 30 days later. **Results:** Brain histopathological examination: one rabbit in the experimental group showed inflammatory changes and no granuloma; no granuloma and inflammatory changes were observed in the negative control group; in the positive control group, granulomatous changes were observed in two rabbits and fibrillary glia astrocytosis in eight rabbits. **Conclusion:** Further studies are needed to establish an animal model by injecting eggs into the carotid artery and the method of injecting schistosoma egg suspension into brain tissue after skull drilling has a high success rate.

Keywords

Schistosoma japonicum, Granuloma of Worm Eggs, Animal Models, Mannitol

1. Introduction

Brain schistosomiasis was first reported by Yamagiwa in 1889, and came to widespread attention after the US invaded the Philippines in 1944 [1]. The eti-

ology is mainly due to the granuloma formed by schistosoma eggs deposited in brain tissue, which causes headache, dizziness, blurred vision, incoordination, epilepsy and other neuropsychiatric symptoms [2]. The establishment of schistosoma animal model is very important for the study of schistosoma. Unfortunately, no animal model of the disease was reported for several decades after the discovery of the disease, and it was not until 1970 that the primate experiment of the disease was reported by John A. *et al.* [3]. Domestic scholars have successfully established the animal model of schistosoma granuloma in rabbits by injecting egg suspension into the brain tissue [4]. Some scholars opened the blood-brain barrier by radiotherapy and injected schistosoma eggs through the carotid artery; imaging studies showed successful modeling [5]. However, these methods have shortcomings. Injection after skull drilling directly destroys the blood-brain barrier, and radiotherapy also has a certain destructive effect, and can reduce animal immunity. To reduce these disadvantages, in this study, the animal model of brain schistosoma granuloma was further explored by injecting the egg suspension after opening the blood-brain barrier by injecting mannitol into carotid artery.

2. Materials and Methods

2.1. Experimental Animals

Thirty New Zealand white rabbits (weighing approximately 2 kg, of either sex) were purchased from China Three Gorges University. They were kept in a 25˚C animal house and fed standard feed and clean water.

2.2. Egg Suspension and Injection Materials

Live eggs of *Schistosoma japonicum* were provided by Jiangsu Institute of Schistosomiasis Control. After three times of washing with sterile normal saline, 5000 eggs/ml suspension was prepared for experimental injection. There were some 1 ml and 10 ml syringes, indwelling trocars (22 G).

2.3. Animal Grouping and Processing

Experimental group: ten rabbits were injected 1 ml suspension of eggs. Positive control group: ten rabbits were injected 1 ml suspension of eggs through their skulls. Negative control group: ten rabbits were injected 1 ml suspension of eggs through carotid artery.

2.4. Eggs Injection

Experimental group: The rabbits were supine and fixed on the disinfection table, then their skin of the neck was ready for surgery. They were put under intravenous anesthesia with 3% sodium pentobarbital (1 ml/kg). Their neck skin was disinfected by tincture of iodine and covered with towel. The right common carotid artery was separated along the right side of the trachea. The indwelling trocar was inserted in a cephalic direction, then 20% mannitol was injected with

a 10 ml syringe from the other end of the trocar. Each rabbit was injected with 4 ml/Kg in 30 seconds, then close the trocar. After 15 min, 1 ml syringe was used to inject 1 ml of prepared egg suspension through trocar, and 1 ml sterile normal saline was injected to wash the trocar, then close the trocar. After 5 min, the needle was pulled out and the puncture point was quickly compressed. After the bleeding stops, their skin was sewn up. The rabbits were returned to the animal room when the anesthetic effect disappeared.

Negative control group: The animals were injected with 1 ml sterile saline instead of the egg suspension, and the rest of the steps were the same as the experimental group.

Positive control group: The rabbits were fixed in the prone position on the disinfection table, and their skin of head was ready for surgery. They were anesthetized by intravenous injection of 3% pentobarbital sodium (1 ml/kg) at the ear margin, then their skin of head was sterilized with iodine and then covered with towels. Incision was made along the midsagittal line of the top of the head, about 3 cm long. A hole was drilled by hand drill 0.5 cm behind the anterior fontanelle of the skull and 0.5 cm below the midline. The bleeding part of the skull was sealed with bone wax. A 1 ml syringe was punctured into the dura mater at a depth of 0.5 cm vertically and then 1 ml egg suspension was injected slowly. The skull was filled with gel foam and the scalp was sutured. The rabbits were returned to the animal room after the anesthetic effect had disappeared.

2.5. Brain Histopathology

Leica pathology biopsy machine, Olympus microscope and HE dye were all provided by the pathology department of Jingzhou First People's Hospital. Thirty days after the operation, the three groups of experimental animals were killed by intravenous injection of air through the ear margin. Their brain tissue was removed intact and preserved in 4% formalin solution. The brain tissue was fixed and dehydrated, embedded in paraffin block. The paraffin blocks were cut into thin slices which were then stained with HE dye. Two pathologists independently observed the existence of pathological changes such as egg granuloma, fibroglial cell healing and inflammation under the light microscope.

3. Results

3.1. Clinical Manifestations

After the operation, five rabbits of the experimental group showed anorexia and burnout, among which three showed unilateral limb movement disorder. The three rabbits were relieved two days later without epilepsy symptoms. In the positive control group, there were six animals with seizures, which were relieved after one week. No obvious abnormality was observed in the negative control group.

3.2. Pathological Examination

None of the three groups of experimental animals died. Pathological examina-

tion of the negative control group showed no abnormality (**Figure 1(a)**). In the experimental group, inflammation was observed in the brain tissue of one rabbit (**Figure 1(b)**) and the other nine rabbits had no obvious abnormality (**Figure 1(c)**). In the positive control group, schistosoma egg antigen was interwoven with collagen fibers in the brain histopathological sections of 2 rabbits, with a few lymphocytes, neutrophils and eosinophils around (**Figure 1(d)** and **Figure 1(e)**) and fibrillary glia was observed in the other 8 rabbits (**Figure 1(f)**).

4. Discussion

Schistosomiasis is one of the most prevalent parasitic diseases [6]. About 240 million people worldwide are infected with schistosoma and 700 million are at potential risk [7]. Schistosomiasis mainly includes schistosomiasis mansoni, schistosomiasis japonicum and schistosomiasis haematobium. Schistosoma mansoni and *Schistosoma japonicum* migrate to the distal mesenteric vein to lay eggs which pass through the intestinal wall and are eventually excreted in feces, while *Schistosoma haematobium* mainly migrates to the bladder vein [8]. The main pathogenesis of schistosomiasis is inflammatory granulomas caused by egg deposition in the liver and intestines of mammals [9]. Schistosoma eggs can also be deposited in brain, spinal cord, kidney, lung, breast, uterus, cervix, skin, bone marrow and other tissues [8]. Schistosomiasis of the central nervous system occurs as a result of abnormal adult migration or eggs deposition into the central nervous system [10]. *Schistosoma mansoni* and *Schistosoma haematobium* infections are more likely to cause spinal cord injuries, while schistosoma

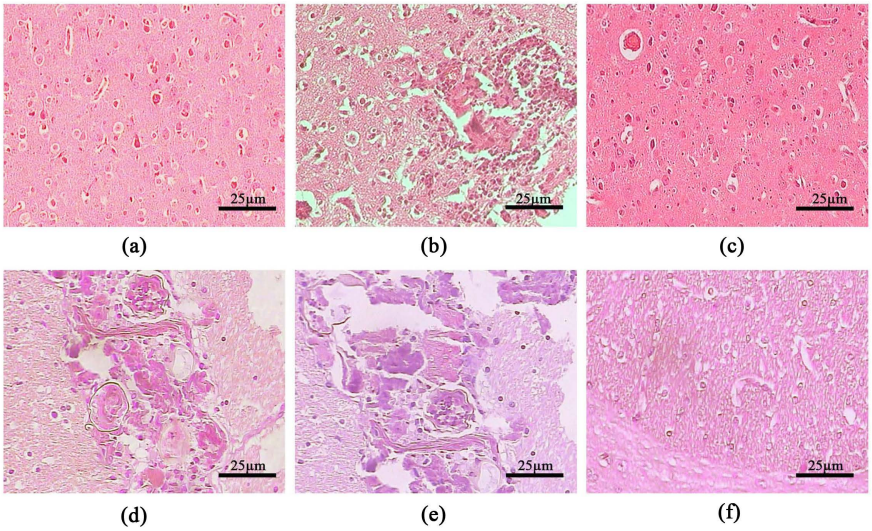

Figure 1. The pathological findings of the three groups of animals (H & E stain, 200×). Pathological examination of negative control group showed no abnormality (a); In the experimental group, inflammation was observed in only one brain tissue (b) and the other nine rabbits had no obvious abnormality (c); In the positive control group, schistosoma egg antigen was interwoven with collagen fibers in the brain histopathological sections of 2 rabbits, with a few lymphocytes, neutrophils and eosinophils around ((d), (e)) and fibrillary glia was observed in the other 8 rabbits (f).

japonicum infection mainly causes brain damages [11]. There are two possible pathogenesis mechanisms of schistosomiasis in the central nervous system. First, schistosoma eggs pass through the blood circulation, from mesenteric capillaries to cerebral vessels, and then deposit, resulting in granuloma (also known as indirect deposition hypothesis). Second, adult schistosoma worms can lay eggs directly in the cerebrovascular system and cause granuloma (also known as direct deposition hypothesis) [2] [12].

JOHN A. was the first researcher to report an animal model of cerebral schistosomiasis [3]. In the 1970s, he experimented with schistosomiasis in black long-tailed monkeys, mainly by injecting hermaphrodite adults or eggs through the carotid artery and jugular vein. Granulomatous changes were found in brain tissue in only one monkey, which was injected twice with large numbers of schistosoma eggs through the carotid artery. The injection of massive schistosoma eggs into the carotid artery is likely to cause cerebral microvascular embolism, which can be proved by the observation of cerebral hemorrhage and schistosoma egg embolus by pathological examination. Therefore, his method has high animal mortality and low modeling success. Aloe *et al.* reported in 1996 that cercariae suspension was used to infect mice by contacting their skin, and then the mice were sacrificed in different periods [13]. Liver and brain tissues were taken at autopsy for pathological examination. Schistosoma eggs and granulomas were observed in the brain tissues of mice 15 - 20 weeks after infection of cercariae. In a preliminary study published in 2014, José Roberto Lambertucci reported for the first time that changes of schistosoma egg granulomas were observed in brain tissue of mice with neurological symptoms at 4 and 8 months after cercaria infection [14]. Luciana M Silva said no granulomatous changes were found in the brain tissue of mice infected with cercariae [15]. Therefore, whether this animal model can be used in schistosomiasis induced granuloma is still controversial. In 2011, wang peng, a domestic scholar, reported for the first time that the animal model of schistosoma egg granuloma was successfully established by injecting schistosoma eggs into the brain after drilling the skullsg of rabbits [4]. Subsequently, Xu Jia thinned the skulls of rabbits and injected the suspension of schistosoma eggs, which has also been successful [16]. According to Wang Peng's method, Ge Yuxi injected live schistosoma eggs to build models and conducted imaging studies on the animals [17].

Although the granuloma of schistosoma eggs can be observed after injecting schistosoma eggs directly into brain tissue, this method cannot fully reflect the pathological evolution and immunological changes of schistosomiasis in the natural state. The design of our experiment is closer to the pathogenesis of schistosomiasis in the brain under natural conditions, in which the schistosoma eggs deposit in brain tissue through the blood-brain barrier and cause granulomatous changes. Ge Yuxi used radiotherapy to open the blood-brain barrier of rabbits, and then injected schistosoma eggs into the carotid artery. Because of the blood-brain barrier, his method did not detect the granuloma of schistosoma eggs on pathological examination of brain tissue [5]. It has been found that

mannitol can improve the permeability of the blood-brain barrier [18] [19]. Therefore, in this study, the blood-brain barrier was rapidly opened by injecting mannitol into carotid artery in advance, and then schistosoma egg suspension was injected into the carotid artery in the hope that schistosoma eggs could more easily reach the brain through the blood-brain barrier. Compared with the drilling method, the destruction of the blood-brain barrier is reduced, and the influence on immunity is reduced compared with the radiotherapy. The trocar puncture injection can better ensure the puncture success rate and the suspension of injected eggs will not flow out of the puncture point due to the blood pressure. The brain tissues of all experimental animals were pathologically examined 30 days after the injection of eggs into the carotid artery. No abnormal changes were observed in the brain tissues of the negative control group animals. Inflammatory changes were found in one of the ten rabbits of the experimental group. However, no changes of granuloma and fibrillary glia were found in the experimental group, which were found in the positive control group. The reasons are as follows: 1) Schistosoma eggs do not enter the brain through the blood-brain barrier, but only some antigens secreted by the eggs reach the brain, thus forming inflammatory changes. 2) The number of eggs injected may be relatively low, most of which are directly collected in the cerebral veins through the cerebral arteriovenous pathway, and then reach other tissues and organs through the blood circulation. 3) The observation time after injection of the eggs may be short, and the eggs may be located in the capillaries of brain tissue.

5. Conclusion

This experimental method has made some achievements, but still has some shortcomings, and should be improved. Compared with the success rate, the injection of schistosomiasis eggs after drilling into the skull was higher than that by carotid artery injection, and the pathological changes were typical. The formation mechanism of cerebral schistosomiasis by injection of schistosoma eggs into carotid artery is close to that of natural state. However, the method still needs further research and improvement.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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IgG4-Related Pancreatitis: A Case Report and Review of Literature

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Abstract

Background: Immunoglobulin G4-related pancreatitis (IgG4-RAIP) is a relatively rare chronic inflammatory disease of the pancreas. The cause of this disease is still unclear. Due to the lack of specific clinical symptoms and imaging findings, it is often misdiagnosed as pancreatic cancer. Clinicians are paying more and more attention to it. **Case report:** We are reporting an IgG4-RAIP case, in which the patient had been misdiagnosed as pancreatic cancer and prepared for surgical treatment but finally was diagnosed as IgG4-RAIP after pancreatic biopsy and serum immune test. And the patient was recovered after the treatment of glucocorticoids. **Discussion:** In order to make more patients get correct diagnosis and reasonable treatment, when we encounter undiagnosed cases of pancreatitis or pancreatic cancer in clinical work, it is very important to conduct pancreatic biopsy and serum immune blood test before operation for these patients. **Conclusion:** Pancreatic biopsy and serum immune blood test can effectively reduce the occurrence of misdiagnosis of IgG4-RAIP.

Keywords

IgG4, IgG4-RAIP, Pancreatitis, Pancreatic Cancer, Glucocorticoid

1. Introduction

The concept of autoimmune pancreatitis (AIP) was first put forward by Yoshida *et al.* in 1995 [1]. At present, it is internationally recognized that the disease is divided into two types, namely type 1 and type 2, in which type 1 is more common, or called immunoglobulin G4-related pancreas (IgG4-RAIP), type 2 is rel-

atively rare [2]. A study by Japanese scholar Okazaki K *et al.* showed that type 1 is characterized by elevated serum IgG4 and is often associated with extrapancreatic tissue lesions, especially in 50- to 70-year-old males [3]. Another global epidemiological survey showed that the average age of the first diagnosis of IgG4-RAIP was 61.4 years [4]. Type 2 pancreatitis, also known as idiopathic central ductal pancreatitis, is mainly characterized by granulocyte epithelial damage in pancreatic tissue; serum IgG4 is generally not elevated, rarely involving extra-pancreatic tissue; the age of onset is usually lower than type 1; this type of clinical is rare. The etiology and pathogenesis of IgG4-RAIP have not been fully elucidated [5] [6]. At present, most scholars believe that the disease is a disease with the participation of multiple factors and mechanisms, in which the immune mechanism is dominant, that is, pancreatic diseases mediated by autoimmune mechanisms, mainly cytoplasmic and lymphocyte infiltration, are often accompanied by pancreatic fibrosis and pancreatic dysfunction. It is a rare chronic inflammatory disease of the pancreas [7]. The onset of the disease is usually concealed; painless obstructive jaundice is the first symptom for most patients, accounting for about 75% [8]; some patients may be accompanied by mid-upper abdominal discomfort or mild to moderate pain [9]. Imaging examination is often localized or diffuses enlargement of the pancreas as the main manifestation, so it is often misdiagnosed as pancreatic cancer, and the misdiagnosis rate is as high as 56% [10] [11]. The case reported in this article has also been misdiagnosed as pancreatic cancer and even nearly underwent surgical treatment. The purpose of this report is to summarize the standardized diagnosis and treatment of IgG4-RAIP, provide clinical reference, reduce the misdiagnosis rate, reduce unnecessary surgical treatment, and improve the prognosis of patients.

2. Case History

A 74-year-old male patient was admitted to hospital because of "pancreatic mass found for more than one month". The patient was treated with pancreatitis in another hospital 2 years ago because of epigastric pain. After discharge, the pain of epigastrium and xiphoid process appeared repeatedly, and the effect of symptomatic treatment was not good. A month ago, the epigastric pain occurred again, and there was no improvement in symptomatic treatment. The abdominal computed tomography (CT) examination in the outpatient clinic of our hospital showed pancreatic mass and choledochal dilatation. Therefore, he was incorporated into our department with "pancreatic space occupying with choledochal dilatation" and planned to undergo surgical resection of "pancreatic tumor".

3. Examination, Diagnosis, Differential Diagnosis and Treatment

The values of cancer antigen 12-5 (CA12-5), CA19-9, alpha-fetoprotein test (AFP) and carcinoembryonic antigen (CEA) in blood examination were all within the normal range. Plasma D-dimer (308 ng/ml), blood amylase (283.5

U/L) and jaundice index (+) were increased. Color Doppler ultrasonography revealed enlargement of the gallbladder with cholestasis, dilatation of intrahepatic bile duct and pancreatic duct, and abnormal echo in the head of the pancreas (space-occupying lesions to be excluded) (**Figure 1(a)**). Magnetic resonance imaging (MRI) plain scan showed nodular mass in the head of pancreas with slightly decreased T1-weighted imaging (T1WI) signal and slightly increased T2-weighted imaging (T2WI) signal (**Figures 1(b)-(d)**); Magnetic resonance cholangiopancreatography (MRCP) showed mass in the head of pancreas; truncation of the head of common bile duct and pancreas, dilatation of intrahepatic bile duct, common bile duct and pancreatic duct, enlargement of gallbladder (**Figure 1(h)**). Enhanced CT scan of the upper abdomen showed small patches of slightly low-density shadow in the head of the pancreas, unclear boundaries, edge enhancement in the arterial phase, and filling enhancement gradually in the venous phase and delayed phase (**Figures 1(e)-(h)**). Combined with the above imaging and biochemical examination, it was diagnosed as cancer of the head of the pancreas with obstructive jaundice. Percutaneous transhepatic cholangial drainage (PTCD) was performed to relieve the symptoms of biliary obstruction. In order to further confirm whether the diagnosis was correct, pancreatic tumor biopsy was performed under the guidance of CT 4 days later. Cytological hematoxylin-eosin (HE) staining showed that a large number of lymphocytes and some necrotic cells were seen under the microscope, and a few enlarged naked nuclei were seen (**Figure 2(a)** and **Figure 2(b)**). Pathological examination showed that interlobular fibrous tissue hyperplasia with a large number of

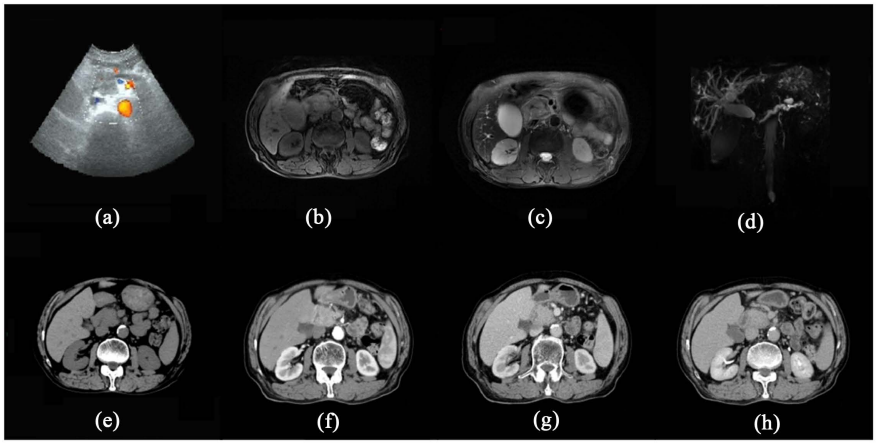

Figure 1. (a) Color doppler ultrasonography showed that slightly hypoechoic shadow could be seen in the pancreatic head area, the boundary was not clear, and there was no obvious blood flow signal in the lesion area. (b)-(d) MRI and MRCP: the pancreatic head area enlarged, the signal intensity of T1WI sequence decreased slightly, the signal intensity of T2WI sequence increased slightly, and MRCP showed dilatation of intrahepatic and extrahepatic bile duct and pancreatic duct. (e)-(h) CT examination: on plain scan, the pancreatic head area was enlarged, nodular isodensity shadow was seen, the boundary was unclear, and the pancreatic duct was dilated; enhanced scan showed slight enhancement of the pancreatic head area in the arterial phase and filling enhancement in the venous phase and delayed phase.

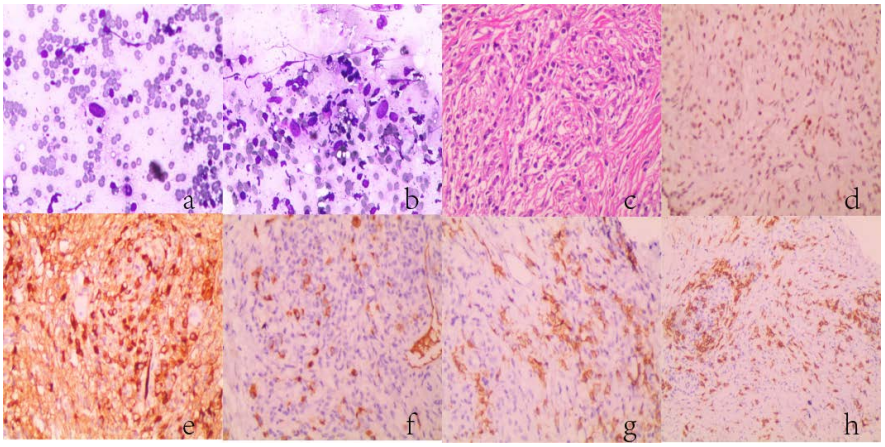

Figure 2. (a) (b) Cytological examination: a large number of lymphocytes and some necrotic cells were seen under the microscope, and a large number of enlarged naked nuclei were seen; (c) (d) Pathological HE staining: microscopically, diffuse interlobular fibrous tissue hyperplasia with a large number of chronic inflammatory cell infiltration, mainly lymphocytes and plasma cells, a small amount of chronic inflammatory cell infiltration could be seen in the lobules, and slight acinar hyperplasia could be seen in some areas; (e) (f) Pathological immunohistochemistry: plasma cells were positive for CD38, CD138, IgG, and partially positive for IgG4, in which IgG4 > 10 HPF, and IgG4/IgG > 40% Bing p53 negative.

chronic inflammatory cell infiltration, mainly lymphocytes and plasma cells, a small amount of chronic inflammatory cells were seen in the lobules, slight acinar hyperplasia could be seen in some areas, and no other special abnormal changes were seen (**Figure 2(c)** and **Figure 2(d)**) [12]. The pathological examination showed that pancreatic interlobular fibrous tissue hyperplasia with a large number of chronic inflammatory cell infiltration, mainly lymphocytes and plasma cells. Because no tumor cells were found in pathological examination, there were mainly chronic inflammatory changes. Combined with the indexes of imaging and biochemical examination, we believe that it may be an autoimmune-related disease. The biopsy specimens were examined by immunohistochemistry again. The results showed that the plasma cells were positive for CD38, CD138 and IgG, and partially positive for IgG4, in which IgG4 > 10/HPF and IgG4/IgG > 40%. Serum immunoglobulin A and immunoglobulin M were normal, but immunoglobulin G was elevated (19.31 g/L) and antinuclear antibody was positive (+). It is consistent with the changes of IgG4-RAIP (**Figures 2(e)-(h)**). The serum IgG4 level increased on the next day (13 g/L). Reviewing this patient's recurrent symptoms of epigastric pain in the past two years, combined with the results of all imaging, biochemical and pathological examinations after admission, we finally diagnosed IgG4-RAIP and sclerosing cholangitis. Therefore, we decided to treat the patient with prednisone, 40 mg/day, oral administration [13].

4. Results and Follow-Up

After 4 weeks of oral glucocorticoid treatment, plain CT scan and enhanced MRI examination showed that the lesion was significantly reduced (**Figure 3**), and the

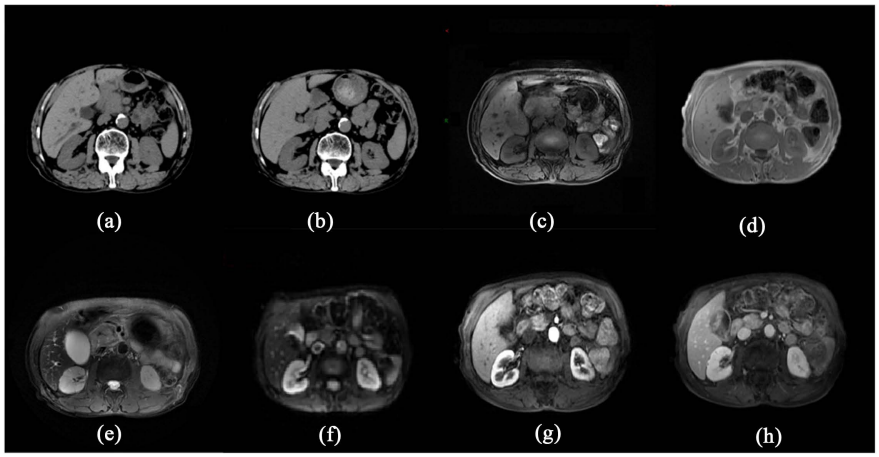

Figure 3. (a) Pre-treatment CT plain scan; (b) Plain scan of CT after 4 weeks of treatment; (c) Pre-treatment T1WI; (d) T1WI after 4 weeks treatment; (e) Pre-treatment T2WI; (f) T2WI after 4 weeks treatment; They showed that the size of the focus was reduced after treatment, the T1WI signal of the focus was higher than that before treatment, and the signal intensity of T2WI was slightly lower than that before treatment; (g) (h) After 4 weeks of treatment, MRI enhancement showed that the lesions showed filling enhancement.

symptoms of epigastric pain were completely disappeared. We will adhere to the long-term follow-up of the patient to observe the medium-and long-term effect.

5. Discussion

In this case, pancreatic mass was found by imaging examination, but eventually was diagnosed as IgG4-RAIP, and the patient had a good effect on glucocorticoid treatment. At present, it is believed that IgG4-RAIP can affect all organs of the human body, not only for a single organ, but also for multiple organs at the same time, and the pancreas is one of the most frequently affected organs [14]. After many efforts, research and comprehensive evaluation, the International Association of Pancreatic Diseases finally released the diagnostic criteria of IgG4-RAIP in 2011, which mainly includes five basic features: 1) Imaging changes of pancreatic parenchyma or pancreatic duct; 2) Serum IgG4 level; 3) Involvement of other organs outside the pancreas; 4) Histopathological findings of pancreas; 5) Sensitivity to glucocorticoid therapy (2). The imaging findings of IgG4-RAIP can be divided into three types: diffuse type, focal type and multifocal type [15]. Among them, diffuse type is the most common, imaging examination showed diffuse enlargement of pancreas; CT showed decreased density of pancreas in the lesion area, MRI showed decreased T1WI signal in the lesion area, T2WI signal increased slightly, the internal structure of pancreatic parenchyma disappeared, and the edge was clear. Usually, the pancreas showed "sausage-like" changes and the fat space around the pancreas was clear [16]. CT or MRI enhanced lesions showed signs of progressive enhancement, that is, slight enhancement in arterial phase, and gradual increase in enhancement in venous phase and delayed phase. About 36% of patients showed characteristic "halo

sign". The localized type usually occurred in the head of pancreas, and the ultrasonography showed hypoechoic lesions in the focus area [17]; CT or MRI showed focal low-density lesions or decreased signal intensity on T1WI, localized enlargement of the pancreas and mild dilatation of the pancreatic duct upstream of the focus. MRCP showed dilatation of intrahepatic bile duct, truncation of the head of common bile duct and pancreas and dilatation of pancreatic duct. This type is easy to be confused with pancreatic cancer [18]. Contrast-enhanced CT showed enlargement of the head of pancreas, dilatation of pancreatic duct, obvious enhancement at the edge of the focus in arterial phase, filling-like delayed enhancement in venous phase and delayed phase (**Figures 1(b)-(e)**); focal hypoechoic area, dilatation of intrahepatic and extrahepatic bile duct and pancreatic duct were vaguely seen in color Doppler ultrasonography (**Figure 1(a)**). It is consistent with the imaging findings of localized IgG4-RAIP reported in the literature. An international multicenter study shows that the sensitivity and specificity of endoscopic retrograde cholangiopancreatography (ERCP) in the diagnosis of IgG4-associated pancreatitis are 71% and 83% respectively [19]. Because our hospital does not have the conditions for ERCP, this test is not performed. In terms of serological examination, the most valuable indicator of IgG4-RAIP is the increase of serum IgG4 [20], and more than 2 times higher than normal serum IgG4 can significantly increase the accuracy of diagnosis, with a sensitivity and specificity of 93% and 99%, respectively. A small number of patients with IgG4-associated pancreatitis may be associated with slightly elevated CA19-9 [21] [22]. In this case, the level of serum IgG4 was 13 g/L, while CA19-9 was normal.

IgG4-RAIP is often accompanied by involvement of other organs outside the pancreas, among which sclerosing cholangitis and retroperitoneal fibrosis are the most common (3). According to the guidelines of the International Association of Pancreatology on autoimmune pancreatitis (2) and studies on the clinicopathological characteristics of IgG4-related diseases (IgG4-RD) [23], histopathological changes of IgG4-related pancreatitis include: 1) there are a large number of lymphocytes, granulocytes and plasmacytes around the pancreatic duct, showing interstitial inflammatory changes or interlobular fibrosis; 2) the number of IgG4-positive plasma cells > 10/HFP, IgG4/IgG4 > 40%; 3) pancreatic parenchyma showed striated fibrosis; 4) occlusive venous inflammation. According to these criteria, in the diagnosis of IgG4-RD, the sensitivity can reach 84.9%, the specificity can reach 100%, and the accuracy can reach 93.8% [24].

IgG4-RAIP and cancer of the head of pancreas have many characteristics in common (10, 11). However, the treatment of each disease is very different. Glucocorticoid is the first choice for the treatment of IgG4-RAIP. A multicenter cohort study by Ebbo M *et al.* shows that more than 90% of patients are sensitive to hormone therapy, and most patients can significantly alleviate their clinical symptoms after 2 weeks of hormone treatment, and imaging and serological tests can be significantly improved [25]. There is no international consensus on the initial dose and maintenance dose of hormone therapy for the disease. At

present, it is recommended to take prednisone orally for 30 mg - 40 mg/days or 0.6 mg/kg/days for 4 weeks, then gradually reduce the dose according to the patient's clinical symptoms, imaging signs and serological examination indicators, and then maintain the drug dose for 5 mg/days for more than half a year. After half a year of maintenance treatment, if the patient's clinical symptoms disappear. The normal size and shape of pancreas by imaging examination and normal IgG4 by serological examination can be used as indications for drug withdrawal [26].

6. Conclusion

IgG4-RAIP is easily misdiagnosed as acute/chronic pancreatitis or pancreatic cancer due to the lack of specific clinical symptoms and special imaging findings, thus delaying the treatment of patients or leading to wrong treatment, and even resulting in unnecessary surgical treatment. Therefore, when we encounter patients with suspected IgG4-RAIP or patients with acute/chronic pancreatitis or pancreatic cancer who cannot be completely diagnosed, we need systematic imaging and serological examinations. For those who still have doubts about serum immunological examination, targeted puncture biopsy is recommended, and when the conditions for biopsy are not available, corticosteroids can be used for diagnostic treatment [11] [27].

Ethical Approval and Consent to Participants

The involved patient has signed the consent form for this case report.

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Authors' Contributions

LCL and JH contributed equally.

Authors contributed to this paper with the design (LCL, JH, and BXR), literature search (LCL and JH), drafting (LCL, JH), revision (JH and BXR), editing (LCL and JH) and final approval (LCL, JH and BXR).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Analysis of Risk Factors Associated with Cognitive Dysfunction in Patients with Atrial Fibrillation

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Abstract

Objective: To discuss the risk factors of cognitive dysfunction in patients with atrial fibrillation. **Methods:** The 150 cases of patients with atrial fibrillation were analyzed in the first affiliated hospital of Nanchang University who were treated in the cardiovascular department, general medicine department and gerontology department from August 2018 to June 2019. We used Mini-Mental State Examination (MMSE) score to evaluate cognitive function of patients with atrial fibrillation. According to the level of education and MMSE score, patients with atrial fibrillation were divided into three groups: normal cognitive function group, mild cognitive impairment group (MCI) and dementia group. And then the demographic data, the previous use of taking drugs, the results of ultrasonic cardiogram (UCG) and laboratory test were analyzed. **Results:** 1) The basic situation of research object: a total of 150 patients with atrial fibrillation were enrolled in the study, and the average age of these patients was 65.05 ± 8.74 years old, which included 78 males (52%) and 72 females (48%). The mean MMSE score was 23.42 ± 4.65. According to MMSE score, 86 cases (57.3%) of cognitive dysfunction occurred in 150 patients with atrial fibrillation, which included 41 cases (27.3%) of mild cognitive impairment and 45 cases (30%) of dementia. 2) The comparison of general clinical data: there were significant differences in age, smoking, level of education, left ventricular ejection fraction, left atrial diameter, D-dimer, fibrinogen, homocysteine, platelet and previous use of taking warfarin, dabigatran, CCB, statins among the three groups ($P < 0.05$). 3) The linear correlational analysis between risk factors of cognitive function and MMSE score in patients with atrial fibrillation: there was a positive correlation between left ventricular ejection fraction and MMSE score, but age, left atrial diameter, homocysteine, low density lipoprotein, platelet, BMI, NT-proBNP, D-dimer

were negatively correlated with MMSE score. 4) The risk factors with statistical significance in ANOVA were analyzed by ordinal and multinomial logistic regression, which showed that age (OR = 1.174, 95% CI: 0.091 - 0.231), the level of education (illiteracy OR = 4.162, 95% CI: −0.032 - 2.955, primary school OR = 2.751, 95% CI: −0.172 - 2.197, junior high school OR = 3.539, 95% CI: −0.048 - 2.577, senior high school and special secondary school OR = 1.332, 95% CI: −1.080 - 1.655), no CCB (OR = 1.174, 95% CI: 0.091 - 0.231), no warfarin (OR = 13.749, 95% CI: 1.480 - 3.762), no dabigatran (OR = 16.395, 95% CI: 1.462 - 4.131), D-dimer (OR = 2.745, 95% CI: −0.611 - 2.631), fibrinogen (OR = 3.228, 95% CI: 0.399 - 1.946) were related to the high occurrence of cognitive dysfunction. **Conclusions:** 1) Patients with atrial fibrillation had a higher risk of cognitive dysfunction (the incidence of 57.4%). 2) There was a positive correlation between left ventricular ejection fraction and MMSE score, but age, left atrial diameter, homocysteine, low density lipoprotein, platelet, BMI, NT-proBNP, and D-dimer were negatively correlated with MMSE score. 3) High level of education, previous use of taking warfarin and dabigatran etexilate were protective factors for cognitive function in patients with atrial fibrillation; but age, previous use of taking CCB, D-dimer and fibrinogen were the risk factors in patients with atrial fibrillation.

Keywords

Atrial Fibrillation, Cognitive Dysfunction, Dementia, Risk Factors

1. Introduction

Atrial fibrillation can cause the symptoms of palpitations and dizziness, and some patients will also bring about cerebral infarction, heart failure and other problems, posing a great threat to human health. We need to improve the strategies for the treatment of atrial fibrillation through more studies on atrial fibrillation and cognitive impairment. Therefore, this study analyzed the risk factors of patients with atrial fibrillation complicated with cognitive dysfunction in order to provide directions for clinical and theoretical research.

2. Materials and Methods

2.1. Research Object

The 150 cases of patients with atrial fibrillation were analyzed in the first affiliated hospital of Nanchang University who were treated in the cardiovascular department, general medicine department and gerontology department from August 2018 to June 2019. Inclusion criteria: 1) the study was for patients who over 18 years old; 2) atrial fibrillation was diagnosed by the 12-lead electrocardiogram and 24 h dynamic electrocardiogram by the diagnosis criteria of P wave disappears, and the spacing off wave is uneven, with different sizes and shapes, frequency up to 300 - 600 times/min; the R-R spacing is absolutely irregular;

there was no abnormal morphology of QRS wave, and patients with atrial fibrillation complicated with indoor differential conduction presented malformed and wide spacing. Exclusion criteria: 1) the patients less than 18 years old; 2) the patients with congenital mental retardation; 3) the patients used drugs that affect cognitive function recently; 4) the patients with hepatic encephalopathy, pulmonary encephalopathy, central nervous system infection and other diseases affecting cognitive function; 5) the patients who can't cooperate to complete the cognitive function assessment for various reasons; 6) the patients with schizophrenia, depression and other mental disorders; 7) the patients with serious brain injuries; 8) the patient with repeated hospitalization.

2.2. Research Method

2.2.1. Investigation Method

Inpatient data of all patients were collected as observational indicators, which included the demographic date, previous use of taking drugs, the results of ultrasonic cardiogram (UCG) and laboratory test. And the cognitive function of patients was evaluated by mini-mental State Examination (MMSE) scale [1]. All patients with atrial fibrillation were divided into three groups according to the level of education and MMSE score, which included normal cognitive function group, MCI group and dementia group.

2.2.2. Diagnostic Criteria

The MMSE score of patients in the normal cognitive function group was ≥27, the other patients lower than that was diagnosed with cognitive dysfunction. Different levels of education affect the degree of cognitive function. The MMSE score of patients in dementia group was ≤17 for illiterate, ≤20 for primary school, ≤22 for junior high school, high school and technical secondary school, ≤23 for college and above. And the MMSE score of patients in MCI group was higher than that but lower than normal cognitive function group.

2.2.3. Quality Control

The MMSE scale of patients was evaluated by the members of the research group. All pre-evaluation inspectors have received uniform professional training. During the evaluation, it was necessary to explain the purpose of the evaluation to the research object. And we also created a quiet, comfortable, light-appropriate, well-ventilated environment for the patients. More importantly, we should conduct the evaluation on the research object with a neutral and objective attitude. All data entry are double-checked and double entered to ensure the data is accurate.

2.2.4. Statistical Method

Data input and management were performed using Excel software, and data analysis was performed using SPSS 21.0 statistical software. The measurement date was represented by X ± S. Multiple sets of measurement date were analyzed by one-way ANOVA or Welch's ANOVA. And the enumeration data were ex-

pressed as count and percentage. The enumeration data were analyzed by chi-square test. We used the Spearman rank correlation for two continuous variables. Otherwise, the Pearson rank correlation was used. The risk factors with statistical significance in ANOVA were analyzed by ordinal and multinomial logistic regression. We adopted a significance level of $P < 0.05$ in all analyses.

3. Results

3.1. The Basic Situation of Research Object

A total of 150 patients with atrial fibrillation were enrolled in this study, and the average age of these patients was 65.05 ± 8.74 years old. We included 78 males (52%) and 72 females (48%), and the mean of MMSE score was 23.42 ± 4.65. According to MMSE score, 86 cases (57.3%) of cognitive dysfunction occurred in 150 patients with atrial fibrillation, which included 41 cases (27.3%) of mild cognitive impairment and 45 cases (30%) of dementia.

3.2. The Comparison of Demographic Data of Patients with Atrial Fibrillation in Three Groups

The three groups were compared in age, marital status, gender, BMI, daily smoking, daily drinking and the level of education, among which there were significant differences in age, daily smoking and the level of education ($P<0.05$), while significant differences were not found in BMI, marital status, gender and daily drinking ($P > 0.05$). As shown in Table 1.

Table 1. The comparison of demographic data in three groups (BMI: body mass index; MCI: mild cognitive impairment).

	Normal Cognitive function n = 64	MCI n = 41	Dementia n = 45	p
Age	59.07 ± 5.29	67.39 ± 5.30	71.42 ± 9.74	0.000
Marital status				0.061
Married	60 (93.8)	32 (78.0)	38 (84.4)	
Unmarried	4 (6.3)	9 (22.0)	7 (15.6)	
Gender				0.968
Men	33 (51.6)	22 (53.7)	23 (51.1)	
Women	31 (48.4)	19 (46.3)	22 (48.9)	
BMI	22.64 ± 1.27	22.42 ± 1.48	24.06 ± 1.98	0.070
Daily smoking	27 (42.2)	36 (87.8)	37 (82.2)	0.000
Daily drinking	11 (17.2)	15 (36.6)	15 (33.3)	0.052
Level of education				0.000
Illiteracy	3 (4.7)	6 (14.6)	12 (26.7)	
Primary school	10 (15.6)	12 (29.3)	12. (26.7)	
Junior high school	5 (7.8)	8 (19.5)	8 (17.8)	
High school and technical secondary school	8 (12.5)	6 (14.6)	6 (13.3)	
College and above	38 (59.4)	9 (22.0)	7 (15.6)	

3.3. The Comparison of Cardiac Color Doppler Ultrasound and Laboratory Test Results in Three Groups

In this study, significant differences were found in left ventricular ejection fraction, left atrial diameter, d-dimer, fibrinogen, homocysteine and platelet among the three groups ($P < 0.05$), as shown in Table 2.

3.4. The Comparison of Previous Use of Taking Drugs in Three Groups

In this study, there were significant differences in the previous use of taking warfarin, dabigatran etexilate, CCB and statins among the three groups ($P < 0.05$), as shown in Table 3.

3.5. The Linear Correlation Analysis between Risk Factors of Cognitive Dysfunction and MMSE Score in Three Groups

In this study, the results of linear correlation analysis showed that there was a positive correlation between left ventricular ejection fraction and MMSE score.

Table 2. The comparison of cardiac color Doppler ultrasound and laboratory test in three groups.

	Normal cognitive function n = 64	MCI N = 41	Dementia N = 45	p
LVEF	53.70 ± 9.49	46.26 ± 7.73	39.05 ± 4.94	0.000
LAD	36.18 ± 3.11	36.17 ± 4.06	40.01 ± 4.8	0.000
NT-proBNP	565.15 ± 484.18	756.89 ± 616.80	759.63 ± 462.87	0.081
D-dimer	0.36 ± 0.15	0.41 ± 0.36	0.57 ± 0.61	0.027
FIB	2.40 ± 0.43	2.19 ± 0.48	2.79 ± 0.96eu	0.001
PT	10.87 ± 0.82	10.84 ± 0.96	10.82 ± 1.15	0.954
CHOL	4.05 ± 1.33	4.19 ± 1.28	4.51 ± 0.98	0.159
TG	1.54 ± 0.77	1.30 ± 0.72	1.39 ± 0.75	0.252
HDL	2.15 ± 0.50	2.03 ± 0.44	2.14 ± 0.45	0.403
LDL	3.33 ± 1.34	3.93 ± 1.48	3.84 ± 1.42	0.059
ALT	29.53 ± 7.93	28.70 ± 10.51	29.53 ± 11.04	0.906
AST	32.17 ± 7.53	30.78 ± 9.40	31.00 ± 9.13	0.650
TBIL	18.19 ± 4.29	18.36 ± 4.81	17.47 ± 3.29	0.494
Alb	44.80 ± 4.17	44.17 ± 4.43	42.90 ± 5.64	0.168
Hcy	8.40 ± 4.73	16.88 ± 6.95	19.40 ± 6.82	0.000
SCr	79.96 ± 14.76	77.67 ± 13.86	83.17 ± 14.84	0.213
BUN	7.00 ± 0.25	7.01 ± 0.29	6.95 ± 0.25	0.606
Plt	156.26 ± 36.06	211.97 ± 22.40	243.40 ± 41.31	0.000

(LVEF: left ventricular ejection Fractions; LAD: left atrial diameter; NT-proBNP: N-terminal pro-brain natriuretic peptide; FIB: fibrinogen; PT: prothrombin time; CHOL: cholesterol; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; Alb: albumin; Hcy: hemocyanin; SCr: serum creatinine; BUN: blood urea nitrogen; Plt: platelet; MCI: mild cognitive impairment).

Table 3. The comparison of previous use of taking drugs in three groups.

	Normal cognitive function n = 64	MCI N = 41	Dementia N = 45	p
Previous use of medication				
Aspirin	17 (26.6)	14 (34.1)	22 (48.9)	0.055
Warfarin	16 (25)	16 (39)	7 (15.6)	0.045
Dabigatran etexilate	22 (34.4)	5 (12.2)	3 (6.7)	0.001
Digoxin	8 (12.5)	7 (17.1)	10 (22.2)	0.406
Beta-blockers	10 (15.6)	12 (29.3)	9 (20.0)	0.240
Amiodarone	1 (1.6)	3 (7.3)	3 (6.7)	0.251
ACEI/ARB	14 (21.9)	13 (31.7)	12 (26.7)	0.530
CCB	6 (9.4)	17 (41.5)	15 (33.3)	0.000
Statins	11 (17.2)	23 (56.1)	13 (28.9)	0.000

(ACEI/ARB: angiotensin converting enzyme inhibitors/angiotensin receptor blocker; CCB: calcium-channel blocker; MCI: mild cognitive impairment).

but age, left atrial diameter, homocysteine, low density lipoprotein, platelet, BMI, NT-proBNP, and D-dimer were negatively correlated with MMSE score, as shown in Table 4.

3.6. The Results of Risk Factors with Statistical Significance in ANOVA Analyzed by Ordinal and Multinomial Logistic Regression

The results showed that age (OR = 1.174, 95% CI: 0.091 - 0.231), level of education (illiteracy OR = 4.162, 95% CI: −0.032 - 2.955, primary school OR = 2.751, 95% CI: −0.172 - 2.197, junior high school OR = 3.539, 95% CI: −0.048 - 2.577, senior high school and special secondary school OR = 1.332, 95% CI: −1.080 - 1.655), no CCB (OR = 1.174, 95% CI: 0.091 - 0.231), no warfarin (OR = 13.749, 95% CI: 1.480 - 3.762), no dabigatran (OR = 16.395, 95% CI: 1.462 - 4.131), D-dimer (OR = 2.745, 95% CI: −0.611 - 2.631), fibrinogen (OR = 3.228, 95% CI: 0.399 - 1.946) were related to the high occurrence of cognitive dysfunction, as shown in Table 5.

4. Discussion

Cognitive dysfunction is a kind of disease that influenced by many factors, and the pathogenesis of cognitive dysfunction is very complex. People found that atrial fibrillation may be associated with cognitive dysfunction. Therefore, this study intended to analyze the clinical data of patients with atrial fibrillation, explore the risk factors and protective factors of cognitive dysfunction, and improve the quality of life in patients with atrial fibrillation.

The results of this study found that age was an independent risk factor for cognitive impairment in patients with atrial fibrillation, and with the age increasing, MMSE score showed a decreasing trend. Not only that, in a study by

Table 4. The linear correlation analysis between risk factors of cognitive dysfunction and MMSE score.

Risk factors	r	p
Age	−0.525	0.000
LAD	−0.371	0.000
Hcy	−0.530	0.000
LDL	−0.237	0.004
Plt	−0.669	0.000
BMI	−0.215	0.008
LVEF	0.628	0.000
NT-proBNP	−0.350	0.000
D-dimer	−0.228	0.005

(LAD: left atrial diameter; Hcy: hemocyanin; LDL: low density lipoprotein; Plt: platelet; BMI: body mass index; LVEF: left ventricular ejection fractions; NT-proBNP: N-terminal pro brain natriuretic peptide).

Table 5. The results of risk factors analyzed by ordinal and multinomial logistic regression.

Risk factors	Regression coefficient	Standard error	Wald χ2	P	OR	95% CI
Age	0.161	0.036	20.105	0.000	1.174	0.091 - 0.231
No Smoking	−0.608	0.572	1.131	0.287	0.544	−1.729 - 0.512
Smoking (reference group) level of education	-	-	-	-	1	-
Illiteracy	1.426	0.762	3.680	0.055	4.162	−0.032 - 2.955
Primary school	1.012	0.604	2.806	0.094	2.751	−0.172 - 2.197
Junior high school	1.264	0.670	3.563	0.059	3.539	−0.048 - 2.577
High school and technical secondary school	0.287	0.698	0.169	0.681	1.332	−1.080 - 1.655
College and above (reference group)	-	-	-	-	1	-
No Warfarin	2.621	0.582	20.263	0.000	13.749	1.480 - 3.762
Warfarin (reference group)	-	-	-	-	1	-
No dabigatran etexilate	2.797	0.681	16.864	0.000	16.395	1.462 - 4.131
Dabigatran etexilate (reference Group)	-	-	-	-	1	-
No CCB	-0.751	0.535	1.971	0.160	0.471	−1.800 - 0.297
CCB (reference group)	-	-	-	-	1	-
No Statins	-0.074	0.474	0.024	0.876	0.982	−1.003 - 0.855
Statins (reference group)	-	-	-	-	1	-
LVEF	−0.019	0.041	0.217	0.641	0.981	−0.099 - 0.061
D-dimer	1.010	0.827	1.492	0.222	2.745	−0.611 - 2.631
LAD	0.034	0.074	0.206	0.650	1.034	−0.112 - 0.179
Hcy	0.138	0.040	11.734	0.001	1.148	0.059 - 0.216
Plt	0.042	0.008	26.126	0.000	1.042	0.026 - 0.058
FIB	1.172	0.395	8.819	0.003	3.228	0.399 - 1.946

(CCB: calcium-channel blocker; LVEF: left ventricular ejection fractions; LAD: left atrial diameter; Hcy: hemocyanin; Plt: platelet; FIB: fibrinogen; OR: odds ratio).

Mathuranath, it was pointed out that age was an independent risk factor for cognitive impairment, this may be related to the fact that elderly patients are prone to hypertension, diabetes, atrial fibrillation and other diseases [2]. At present, most studies support that the harmonious and stable marriage has a protective effect on cognitive function, and Widowhood can reduce the patient's interest of the world, which may increase the risk of cognitive impairment [3] [4]. However, this study did not find an exact relationship between marital status and cognitive dysfunction in patients with atrial fibrillation.

This study also found that daily drinking could not increase the risk of cognitive impairment in patients with atrial fibrillation, which may be related to the fact that these patients were daily drinkers but rarely had an excessive state of drinking. In addition, we found significant difference in daily smoking among the three groups, but it did not become an independent risk factor. Previously, some scholars believed that smoking would not aggravate the impairment of cognitive function and had a protective effect on cognitive function [5]. The protective effect of smoking on cognitive function may be related to the fact that nicotine enhances the antioxidant activity of neurons [6]. In recent years, many studies have also found that smoking may aggravate cognitive impairment. A prospective study showed that smokers had a 1.59-fold higher risk of Alzheimer's disease than non-smokers (95% CI: 1.15 - 2.20) [7], and the pathogenesis of it may be related to the fact that smoking fume can cause vascular endothelium damage, which aggravates the impairment of cognitive function [8]. The level of education is associated with vocabulary expression, visuospatial structure, the ability of memory and other areas of cognitive function, so it may have significant influence on some neuropsychological tests [9]. The result of this study indicated that high level of education was a protective factor for cognitive functions in patients with atrial fibrillation. The process of education may increase the synaptic connections of neurons, which enable nervous system to tolerate the changes of structure and function [9]. In addition, people with higher level of education are more likely to engage in activities that stimulated cognitive function, and thus people with high level of education have lower rates of cognitive dysfunction compared with low level of education [10]. So moderate mental exercise may help reduce the risk of cognitive impairment in patients with atrial fibrillation. In this study, left atrial diameter was not found to be a risk factor for cognitive dysfunction in patients with atrial fibrillation. As is known to all, left atrial diameter is closely related to many cardiovascular diseases, but it is not clear that whether the increase of left atrial diameter will aggravate the cognitive impairment in patients with atrial fibrillation. Now, in a study by Carlson, it was pointed out that the increase in diameter of left atrium can lead to the formation of thrombosis, and increase the risk of thromboembolic events that affected cognitive function [10]. We also found that there was significant difference in left ventricular ejection fraction among the three groups. The linear correlation analysis also showed a positive correlation between left ventricular ejection fraction and MMSE score, but it was not to the extent of an independent risk factor.

In a study by Woo, it was pointed out that the structural integrity of synapses in heart failure patients was lower than that in healthy people, and the midbrain circuits were also have changed in the patients [11]. In addition, brain atrophy and demyelination are also common in patients with heart failure, these structural changes are important factors that affected cognitive function and it may contribute to the diagnosis and treatment in patients with cognitive impairment [12] [13].

High blood coagulation state is an important pathogenesis of cerebral vascular embolism caused by atrial fibrillation [14]. Both fibrinogen and D-dimer are important factors involved in the blood coagulation process, and the results of this study indicated that both were risk factors for cognitive dysfunction in patients with atrial fibrillation. Activated platelets can provide phospholipid surfaces for the activation of coagulation factors, and can also release coagulation factors such as fibrinogen. We found that there was a significant difference in platelet among the three groups, the linear Correlation Analysis showed a negative correlation between platelets and MMSE score, and the ordinal and multinomial logistic regression showed that platelet was not a risk factor for cognitive dysfunction in patients with atrial fibrillation. The cognitive impairment caused by high blood coagulation state should be worthy of being attended, which may be an important part of the prevention of cognitive dysfunction in patients with atrial fibrillation. Previously, some scholars found that homocysteine may increase the risk of cardiovascular and cerebrovascular diseases [14]. However, this study not found an exact relationship between homocysteine and cognitive impairment in patients with atrial fibrillation. Because of the high incidence of homocysteine in China, the correlation between homocysteine and cognitive function has become a hot topic in recent years.

Patients with atrial fibrillation are often accompanied by excessive inflammatory response and endothelial cell injury [15], but statins can reduce the level of inflammatory mediators. Therefore, some scholars speculated that statins could have a protective effect on the cognitive function of patients with atrial fibrillation. In a study by Indira Tendolkar, it was pointed out that previous use of taking lipid-regulating drugs in patients with atrial fibrillation could slow the cognitive impairment [16]. In a study by Chao, it was pointed out that, the incidence of non-vascular dementia was lower in patients with atrial fibrillation who received statins, compared with patients who were not received statins [17]. But in this study, we do not find any protective effect of statins on cognitive function in patients with atrial fibrillation, which may be related to many factors such as insufficient time of using statins and different types of statins could have different effects on the cognitive function in patients with atrial fibrillation.

Anticoagulant therapy has long been considered as the cornerstone of atrial fibrillation treatment. In a study by Mavaddat, it was not found that warfarin had protective effect on cognitive function [18]. However, another study involved 2605 patients showed that the time of INR in the treatment range (TTR)

was significantly correlated with cognitive impairment in patients with atrial fibrillation. It means that the patients with TTR < 25% could have a higher risk of dementia than with TTR > 70% [19]. The effect of warfarin on cognitive function may be influenced by many factors and closely related to the time of INR in the treatment range (TTR). However, the new oral anticoagulant has certain advantages in safety and efficacy, and the compliance of that is higher than warfarin. A meta-analysis published by Wenke Cheng showed that previous use of taking dabigatran etexilate had a better protective effect on cognitive function in patients with atrial fibrillation than warfarin. And for patients with atrial fibrillation whose INR was controlled at 2 - 3, the increase of TTR was inversely proportional to the incidence of cognitive dysfunction [20]. In this study, the ordinal and multinomial logistic regression showed that dabigatran etexilate and warfarin were protective factors for cognitive dysfunction in patients with atrial fibrillation. Previously, some studies have shown that antiplatelet drugs could be used to prevent stroke in patients with atrial fibrillation, but the results of this study not found that aspirin had a protective effect on cognitive function in patients with atrial fibrillation. So the effect of aspirin on cognitive function in patients with atrial fibrillation needs to be further confirmed by more large-scale multicentre studies.

5. Conclusion

1) Patients with atrial fibrillation had a higher risk of cognitive dysfunction (the incidence of 57.4%). 2) There was a positive correlation between left ventricular ejection fraction and MMSE score. But age, left atrial diameter, homocysteine, low density lipoprotein, platelet, BMI, NT-proBNP, and D-dimer were negatively correlated with MMSE score. 3) The results showed that high level of education, previous use of taking warfarin and dabigatran etexilate were protective factors for cognitive function in patients with atrial fibrillation; age, previous use of taking CCB, D-dimer and fibrinogen were risk factors for cognitive function in patients with atrial fibrillation.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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