



## Assessment of sperm quality, oxidative stress injury as well as ACP, AC and PDE expression in patients with oligoasthenozoospermia before and after qilin pill treatment

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### ABSTRACT

**Objective:** To analyze the sperm quality, oxidative stress injury as well as ACP, AC and PDE expression in patients with oligoasthenozoospermia before and after qilin pill treatment.

**Methods:** A total of 60 patients with oligoasthenozoospermia were randomly divided into observation group and control group, control group received routine western medicine treatment, observation group received qilin pill + conventional western medicine treatment, and then differences in sperm quality, oxidative stress injury, ACP, AC and PDE expression, etc. were compared between two groups after treatment. **Results:** Semen volume and sperm density in semen samples of observation group after qilin pill treatment were higher than those of control group; serum FSH and LH levels were lower than those of control group, and the T level was higher than that of control group; ROS and MDA levels in seminal plasma were lower than those of control group, and SOD level was higher than that of control group; ACP, AC, -Glu and Fru levels in seminal plasma were higher than those of control group, and PDE level was lower than that of control group. **Conclusion:** Qilin pill can improve sperm quality and optimize testicular internal environment in patients with oligoasthenozoospermia, and it has positive clinical significance.

## 1. Introduction

Oligoasthenozoospermia is a major cause of male infertility, and is directly related to low quality and small number of sperms. Targeted solutions for western medicine treatment of oligoasthenozoospermia are short at present, and some scholars put forward that combine traditional Chinese and western medicine treatment is expected to break the current predicament of oligoasthenozoospermia treatment[1,2]. Qilin pill inherits the traditional Chinese medicine theory of "kidney controlling reproduction", is composed of epimedium, semen cuscatae, cynomorium songaricum and other drugs that reinforce the kidney to replenish essence, and main exerts the efficacy of invigorating the kidney and strengthening Yang,

nourishing yin and replenishing essence as well as soothing the liver and regulating qi[3,4]. In the study, qilin pill was applied in the treatment of patients with oligoasthenozoospermia, and the changes in sperm quality, oxidative stress injury, the internal environment of seminal plasma, etc. were mainly stated.

## 2. Information and methods

### 2.1. General information

A total of 60 patients with oligoasthenozoospermia treated from May 2014 to May 2015 were included in the study, conformed to the clinical diagnostic criteria and signed informed consent. According to random number table, the included patients were divided into observation group and control group ( $n=30$ ). Control group were 23-35 years old, the average age was  $(28.34 \pm 6.11)$  years, the course of disease was 1-8 years and the average course

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was (3.28±0.43) years; observation group were 24-37 years old, the average age was (29.18±6.75) years, the course of disease was 1-7 years and the average course was (3.41±0.47) years. The two groups were not statistically different in the distribution of age and course of disease ( $P>0.05$ ) and could be subsequently compared.

## 2.2. Dosage regimen

Control group received levocarnitine treatment, levocarnitine oral liquid 10 mL/time, 3 times/d. On the basis of levocarnitine treatment, observation group received qilin pill treatment, specifically as follows: qilin pill (90 g/bottle, Guangdong Taiantang Pharmaceutical Co., LTD., approved by Z10930034), oral administration, 6 g/time, 3 times/d, 12 weeks as one course of treatment.

## 2.3. Testicular ultrasonography

Color Doppler ultrasonic developing-out unit was used, spectrum was set to 11 MHz and convex array probe was used. Patients took horizontal position, the probe was coated with coupling agent and then directly touched the skin, and the varicocele, testicular dysplasia and so on were observed.

## 2.3. Observation indexes

### 2.3.1. Sperm quality and sex hormone levels

Patients were abstinent for 7 d, semen samples were collected through masturbation, stored in 37 °C incubator for about 20 min and moved to graduated centrifuge tube to detect the volume, and the WHO operating methods were followed to analyze sperm quantity, sperm density, etc. Fasting peripheral venous blood was extracted from patients after treatment, and chemiluminescence analyzer was used to determine serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T) and other sex hormones.

### 2.3.2. Oxidative stress indexes

Semen specimen were collected from patients after treatment to separate seminal plasma, flow cytometer was used to determine reactive oxygen species (ROS) level, and pyrogallol autoxidation colorimetry was used to determine the levels of superoxide dismutase (SOD) and malondialdehyde (MDA).

### 2.3.3. Biochemical indexes

Levels of biochemical indexes in seminal plasma in semen sample of two groups were analyzed after treatment, and disodium phenyl

phosphate was used to determine acid phosphatase (ACP), adenylate cyclase (AC), phosphodiesterase (PDE), -glucosidase (-Glu) and fructose (Fru) levels.

## 2.4. Statistical methods

Obtained data was input in SPSS21.0 for analysis and processing, measurement data was in terms of average ± standard deviation (Mean ± SD), comparison between two groups was by t test, and  $P<0.05$  was the standard of statistical significance in differences.

## 3. Results

### 3.1. Color Doppler ultrasound

Patients with oligoasthenozoospermia are mostly accompanied by different degree of abnormal functional anatomy, which is an important factor of disease. In the study, color Doppler ultrasound was used to inspect testicular tissue, and it was found that among patients with oligoasthenozoospermia before treatment, 47 cases were accompanied by varying degrees of varicocele, and included 22 cases in observation group and 25 cases in control group; 30 cases were accompanied by testicular dysplasia, and included 17 cases in observation group and 13 cases in control group.

### 3.2. Sperm quality and sex hormone levels

Semen sample detection can intuitively reflect the sperm quality, sex hormones are directly related to the changes of sperm treatment (quality), semen and peripheral blood specimens were detected after treatment in the study, and it was found that semen volume and sperm density in semen samples of observation group after qilin pill treatment were higher than those of control group; serum FSH and LH levels were lower than those of control group, and the T level was higher than that of control group ( $P<0.05$ ), shown in Table 1.

### 3.3. Oxidative stress indexes

Oxidative stress injury is one of the important factors causing the decline in sperm treatment (quality), flow cytometer and pyrogallol autoxidation colorimetry were used in the study to detect the levels of ROS, SOD, MDA and other oxidative stress indexes in seminal plasma of two groups after treatment, and the following results were obtained: after observation group received qilin pill treatment, ROS and MDA levels in seminal plasma were lower than those of

**Table 1.** Comparison of sperm quality and sex hormone levels after treatment.

Groups	n	Sperm quality		Sex hormone levels		
		Semen volume (mL)	Sperm density ( 106/mL)	FSH (mIU/mL)	LH (mIU/mL)	T (ng/mL)
Observation	30	2.82±0.41	22.84±2.59	6.02±0.75	6.16±0.75	5.79±0.62
Control	30	2.37±0.32	19.73±2.14	6.21±0.78	7.32±0.84	5.03±0.58
t		5.382	7.293	5.092	6.281	7.293
P		<0.05	<0.05	<0.05	<0.05	<0.05

control group, and SOD level was higher than that of control group ( $P<0.05$ ), shown in Table 2.

**Table 2.**

Comparison of oxidative stress index levels in seminal plasma after treatment.

Groups	<i>n</i>	ROS ( $\mu\text{mol/L}$ )	SOD (U/mL)	MDA (nmol/mL)
Observation	30	5.47 $\pm$ 0.59	173.28 $\pm$ 20.59	10.03 $\pm$ 1.17
Control	30	7.31 $\pm$ 0.85	141.59 $\pm$ 17.63	13.47 $\pm$ 1.93
<i>t</i>		7.293	12.372	6.393
<i>P</i>		<0.05	<0.05	<0.05

### 3.4. Biochemical indexes in seminal plasma

Change in the levels of biochemical indexes in blood circulation and semen is one of the important causes of oligoasthenozoospermia, the levels of biochemical indexes in seminal plasma of two groups were detected after treatment, and the specific results were as follows: ACP, AC, -Glu and Fru levels in seminal plasma of observation group after treatment were higher than those of control group, and PDE level was lower than that of control group ( $P<0.05$ ), shown in Table 3.

## 4. Discussion

Oligoasthenozoospermia is one of the main causes of clinical infertility, and the causes are complex and the treatment is difficult. The proportion and count of normal morphology sperm as well as the proportion and count of forward movement sperm in semen are the necessary premise to ensure that the sperm arrives at female fallopian tube and combines with egg[5]. Less content of active sperm in male semen is a major cause of reduced fertility, and how to optimize the sperm quality and increase the number of active sperm is the key link in treatment of oligoasthenozoospermia, and is also the current focus of clinical research. Western medicine treatment of oligoasthenozoospermia can obtain certain curative effect, but the proportion of sensitive population is low and the overall treatment effect is limited, and some scholars have proposed to introduce traditional Chinese medicine therapy to the treatment of the disease, which optimizes the overall illness in patients from different mechanisms of action[6,7].

Chinese medicine includes oligoasthenozoospermia in the category of "kidney vacuity and essence depletion" and holds that kidney is the most important organ to sustain life and nourish the viscera, the theory that the kidney stores the essence has lasted for thousands of years, and the core idea is that human reproduction and fertility,

growth and senescence are closely related to the kidney function[8]. The pathogenesis of oligoasthenozoospermia is in kidney, and therefore, traditional Chinese medicines for invigorating the kidney should be applied to improve gonad nerve conduction and activate testicular androgen secretion and spermatogenic cell function. Qilin pill is made from prepared fleece flower root, epimedium, cynomorium songaricum, Patasbeej, semen cuscutae, salvia miltiorrhiza, medlar, Chinese yam and other drugs that reinforce the kidney to replenish essence, the active components of epimedium are icariin and VitE, and it has the estrogen-like effect; Purified material of semen cuscutae can exert the synergetic effect of gonadotropin; the core component of medlar is lycium barbarum polysaccharide and can delay the spermatogenic cell apoptosis and inhibit lipid peroxidation; astragalus contains trace elements that can improve sperm concentrations and vitality[9,10]. Domestic scholars have found in the rat models that qilin pills can increase the relative quality of ovary and uterus of rats, and play a positive role in stimulating semen secretion in seminal vesicle, strengthening the function of hypothalamus gonad axis and other aspects[11]. In the study, qilin pill was added in the treatment of patients with oligoasthenozoospermia, and its role in improving sperm quality, reducing oxidative stress, optimizing the levels of various biochemical indexes and other aspects was mainly analyzed.

The occurrence of oligoasthenozoospermia is related to varicocele, testicular dysplasia and so on, and the testicular ultrasonography of the two groups in the study also confirmed this. Detection of semen samples of two groups showed that semen volume of observation group increased after treatment and sperm count per unit semen also increased dramatically, indicating that qilin pill treatment macroscopically increased the patients' sperm count, which was a clear sign of improved sperm quality in patients [12]. Reproductive hormones play an important role in spermatogenesis and function generation, follicle-stimulating hormone (FSH) acts on testicular seminiferous tubule epithelial sertoli cells, luteinizing hormone (LH) is combined with interstitial cell membrane receptor and then maintains interstitial cell generation, and high level of testosterone (T) is the prerequisite for sperm production [13]. In the study, FSH and LH levels of observation group after treatment were lower and the T level was higher, which might because that qilin pill repaired the testicular seminiferous tubule epithelial and interstitial cells injury and optimized testicular spermatogenesis function.

The theory of oxidative stress injury of sperm is the one of the important mechanisms of oligoasthenozoospermia proposed in recent years, and has received more and more attention from scholars. Study

**Table 3.**

Comparison of biochemical index levels in seminal plasma after treatment.

Groups	<i>n</i>	ACP (U/mL)	AC (mmol/L)	PDE ( $\mu\text{g/L}$ )	-Glu (U/mL)	Fru (mg/L)
Observation	30	95.38 $\pm$ 10.41	73.29 $\pm$ 8.12	1.27 $\pm$ 0.18	68.34 $\pm$ 7.12	2 884.36 $\pm$ 301.77
Control	30	87.62 $\pm$ 8.49	59.43 $\pm$ 6.27	2.69 $\pm$ 0.31	55.82 $\pm$ 6.14	2 417.59 $\pm$ 264.12
<i>t</i>		7.293	8.273	6.173	8.394	12.932
<i>P</i>		<0.05	<0.05	<0.05	<0.05	<0.05

believes that oxidative stress injury can affect sperm membrane structure an motility ability, and directly lead to the sperm vitality decrease and quality damage. The study results showed that ROS and MDA levels in seminal plasma of observation group decreased and SOD level increased. Reactive oxygen species (ROS) are the general term of active substances produced by the body's metabolism and with strong oxidizing capacity, spermatids are rich in mitochondria, the generation of ROS is quite active in the mitochondria, and they form balance with large number of antioxidants in seminal plasma [14]. Superoxide dismutase (SOD) is the main antioxidant in the seminal plasma and malondialdehyde (MDA) has high oxidation activity. Research has confirmed that the imbalance of oxidation and anti-oxidation system such as ROS, MDA and SOD, especially the ATP depletion caused by a large number of ROS is the important reason causing oligoasthenozoospermia, and the above study results indicated that qilin pill could restore the oxidative stress balance of sperm and seminal plasma, reduce the damage effect of oxidation products on sperm and effectively restore the sperm vitality.

Acid phosphatase (ACP) is the specific secretion of the prostate that plays an important role in maintaining the spermatid membrane stability, and study have found that ACP is lowly expressed in men with low fertility function. Adenylate cyclase (AC) is the effector molecule in signal transduction pathway, and its product cAMP plays an important role in the in sperm capacitation and acrosome reaction [15]. Phosphodiesterase (PDE) can transform cAMP into inactive 5-AMP, and overexpressed PDE in seminal plasma can reduce cAMP and cGMP concentration in cells, thus affecting sperm function. -glucosidase (-Glu) and fructose (Fru) constitute the internal environment of accessory sex gland and provide enough ability for the sperm to ensure its vitality, and study has shown that levels of -Glu and Fru is one of the important symbols of low sperm vitality. In the study, detection of the levels of above biochemical indexes in seminal plasma showed that ACP, AC, -Glu and Fru levels of observation group increased after treatment and PDE level decreased, indicating that qilin pill could optimize the internal environment for sperm survival and play a positive role in improving sperm vitality.

To sum up, it is concluded as follows: qilin pill can improve sperm quality and optimize testicular internal environment in patients with oligoasthenozoospermia, and it's worth popularization and application in clinical practice in the future.

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