Int J Clin Exp Med 2015;8(11):21669-21673
www.ijcem.com /ISSN:1940-5901/IJCEM0011448

Original Article

Relation between the testicular sperm assay and sex hormone level in patients with azoospermia induced by mumps

Shuiwen Zhang, Yulin An, Junguo Li, Junhong Guo, Guoping Zhou, Jianhua Li, Ye Xu

Reproductive Medical Center, The General Hospital of Beijing Military Region, Beijing 100700, China

Received June 15, 2015; Accepted November 8, 2015; Epub November 15, 2015; Published November 30, 2015

Abstract: This study aimed to investigate the relation between the testicular sperm assay (TESA) and sex hormone level or testicular volume in patients with azoospermia induced by mumps. Samples from 52 patients with mumps-induced azoospermia were subjected to TESA, and then the sperm activity was observed microscopically. The sex hormone level was detected with an electrochemical assay, and ultrasound was used to calculate the testicular volume. Of the 52 azoospermia patients, 38 were found to have active sperms through testicular sperm extraction from the opened testis; furthermore, the serum follicle-stimulating hormone (FSH) and luteinizing hormone levels were obviously higher in the non-sperm group than in the sperm group ($P < 0.05$). Moreover, the testicular volume was smaller in the non-sperm group than in the sperm group; however, there was no significant difference between the two groups ($P > 0.05$). With the FSH value as a standard, the quantity of sperms was found to be within two times of, or more than two-fold of the normal range. With the testicular volume as a standard, sperms were found in testes with a volume of > 6 mL or < 6 mL. The FSH value and the testicular volume were indicators of the ability of the TESA to obtain sperms. To allow the performance of intracytoplasmic sperm injection, all patients need to undergo TESA.

Keywords: Mumps, azoospermia, sex hormone, testis volume

Introduction

Mumps is an acute respiratory disease caused by the mumps virus that has a great affinity to the testis [1]. Generally, in patients with mumps who are older than 10 years, about 25% also have orchitis [2]. The mumps virus mainly invades the seminiferous tubules and interstitium of the testis, damaging the seminiferous tubules and resulting in the lack of spermatogenic cells [3]. Furthermore, orchiatrophy and small testis, and even the reduction in the amount of sperms, occur in mumps patients. About 10%-30% of patients develop azoospermia due to infection of both testes [4]. Thirty years ago, there was little understanding on the testicular injury caused by the mumps virus in China, and treatment was not given in a timely manner, especially in remote rural areas. According to our statistical data, there were about 21% patients with non-obstructive azoospermia caused by mumps at our hospital, which was a much lower number than that reported in Western countries [5].

With the development of assisted reproductive technology, including intracytoplasmic sperm injection (ICSI), it is now possible for azoospermic men to have offspring [6]. Through testicular biopsies, some studies found that sperms are still produced by spermatogenic cells in some azoospermic men; however, these sperms were not discharged because of peritubular fibrosis in most seminiferous tubules [7, 8]. Thus, it is essential to find sperms in the testes for the success of ICSI. Because testicular biopsy can be a traumatic experience for patients, it is important to find an indicator that can predict whether the patients' testes produce sperms.

The level of follicle-stimulating hormone (FSH) was believed to be a variable that significantly predicts positive sperm retrieval in non-obstructive azoospermia patients [9]. Positive sperm recovery was achieved in 20.83% of patients with an FSH level of > 3 × the maximum value of the normal range (N), in 42.5% patients with 2 N ≤ FSH < 3 N, and in 62.5% patients with N < FSH < 2 N. Some researchers commonly con-

sidered that it was not necessary to perform biopsy when the testes were < 6 mL in size or when the FSH value was twice as high as the normal range, because these conditions make it impossible to obtain mature sperms [10].

Azoospermia caused by mumps is different from other types of non-obstructive azoospermia [11] because the chromosome is normal in patients with mumps-induced azoospermia. In our clinical practice, we also found mature sperms through the testicular biopsy of patients with mumps-induced azoospermia whose testes were < 6 mL in size or whose FSH value was twice as high as the normal range, and these patients were able to reproduce successfully through the test-tube-baby method [12]. Here, we investigated the relation between the testicular sperm extraction assay (TESA) of azoospermic patients and their sex hormone level and testicular volume, which are important factors in predicting whether testicular sperm extraction was successful. There is no related research to report at present.

Materials and methods

Patients

Male patients who had a history of mumps and visited the reproductive medicine center of our hospital from March 2006 to February 2012 were enrolled in this study. These patients had normal chromosomes and had more than three failures of sperm detection with routine semen analysis. Their mean age was 34.7 ± 6.1 years (range, 22-47 years). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of General Hospital of Beijing Military. Written informed consent was obtained from all participants.

TESA

The bigger testicle was chosen and injected with anesthesia (2% lidocaine). Then, approximately 1.5 cm incision was made on the skin of the scrotum with a sharp knife, followed by blunt dissection up to the albuginea testis. Next, an about 0.5 cm incision was done with a sharp knife, and the testicle was lightly squeezed until testicular parenchyma was obtained. Relative extended tissue from the seminiferous tubules was cut with ophthalmic scissors under microscopic guidance, and then the specimen was directly added to culture medium. Finally, the seminiferous tubule was torn up under a dissecting microscope, and we found sperm by observing under an inverted microscope.

Measurement of testicular volume

The testicular length, width, and thickness were measured through ultrasound, and the testicular volume was calculated according to the Macomber formula: $v = \Pi d^2/4 \times L \times k$, where k, L, and d represent 0.9, length, and the average of width and thickness, respectively.

Measurement of sex hormone level

Blood samples were collected, and chemoluminescence was used to detect the sex hormone level (IMMULTIE 2000; SEMES Company, Germany). The normal ranges of luteinizing hormone (LH), FSH, estradiol (E2), prolactin (PRL), and testosterone (T) were 1.7-8.6 Miu/mL, 1.5-12.4 Miu/mL, 7.63-42.6 pg/mL, 86.0-324.0 uIU/mL, and 2.8-8.0 ng/mL, respectively.

Statistical analysis

Statistical analysis was performed by using the SPSS13.0 software. The measured data were presented as means ± standard deviation (SD). Analysis of variance was used to analyze the correlation among testicular volume, sex hormone level, and TESA result.

Results

Active sperms were found in 38 testes, and the success rate of TESA was 73.1% (38 of 52).

Relation between sex hormone and TESA result

Table 1 shows the results of LH, FSH, E2, PRL, and T in the sperm group and the non-sperm group. The mean serum FSH and LH levels were obviously higher in the non-sperm group than in the sperm group, with a statistical significance ($P < 0.05$); however, there were no significant differences in the T, E2, and PRL levels. When the FSH level was used as the standard, 23 cases (44.2%) were within the normal range (N), 11 cases (21.2%) were N < FSH < 2 N, and 4 cases (7.7%) were > 2 N.

Table 1. Relationship between TESA result and parameters of sex hormone and testis volume

Group	Testis vol (ml)	PRL (uIU/ml)	E2 (pg/ml)	T (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)
Non-sperm group	10.3 5± 1.62	127.5 ± 45.8	18.58 ± 9.34	4.37 ± 1.48	12.71 ± 3.58	18.74 ± 9.59
Sperm group	11.73 ± 1.84	136.7 ± 7.67	17.17 ± 7.67	5.04 ± 1.27	9.85 ± 4.37*	14.58 ± 8.43*

Vs. non-sperm group, *$P < 0.05$.

Relation between testicular volume and TESA result

The testicular volume was smaller in the non-sperm group than in the sperm group, with no statistical significance ($P > 0.05$). **Table 1** shows that the testes of 35 patients (67.3%) were > 6 mL in size, and 3 testes (5.8%) were < 6 mL in size.

Discussion

Azoospermia caused by mumps is the most common non-obstructive azoospermia in China. Thirty years ago, people had no knowledge that the mumps virus causes testicular disorder and treatment was not given in a timely manner, especially in poor rural areas. At our hospital, there were about 21% men with non-obstructive azoospermia caused by mumps. After ICSI became popularized in 1992, it became possible to cure azoospermia caused by mumps because some studies indicated, through testicular biopsy, that sperms are still produced by spermatogenic cells in some azoospermic patients. Although sperms were not discharged because of peritubular fibrosis in most seminiferous tubules [7, 8], very few sperms are needed for ICSI [13].

In this study, 38 of 52 patients had active sperms, and the testicular sperm extraction rate was 73.1%. What was the relation between the testicular sperm extraction rate and sex hormone level or testicular volume? Previously, researchers in China commonly considered that it was not necessary to perform biopsy when the testes were < 6 mL in size or when the FSH value was twice as high as the normal range because it was impossible to obtain mature sperms in these conditions. Foreign researchers also suggested that testicular volume and FSH concentration were closely related to the results of TESA [14]. Indeed, testicular volume and FSH concentration were used to judge testicular spermatogenic condition in clinical practice; however, some researchers thought that the testicular sperm extraction rate was not associated with testicular volume and serum FSH concentration [4, 15].

Both FSH and LH are glycoprotein hormones secreted by the gonadotrophs of the anterior pituitary gland [16]. Furthermore, FSH and LH act synergistically in reproduction [17]. A high serum FSH level indicates seminiferous epithelium injury and dyszoosperima [18]. Additionally, the degree of elevated serum FSH level has a positive correlation to the extent of the injury of the blood-testis barrier, and first presents in clinical practice when testicular function is impaired; then, the FSH level increases, followed by an increase of LH level. Thus, the FSH level is usually an indicator of seminiferous tubule function [19]. Here, the average of the serum FSH and LH concentrations was significantly higher in the non-sperm group than in the sperm group, which was statistically significant ($P < 0.05$). The mumps virus mainly invades the seminiferous tubules and interstitium of the testes, thus reducing the testosterone level; however, there was no significant difference in testosterone level between the sperm group and the non-sperm group ($P > 0.05$), and there were no statistical differences in serum E2 and PRL levels between the two groups.

Colpi and his team found that FSH concentration was a variable significantly predicting positive sperm retrieval in non-obstructive azoospermia patients [20]. A positive sperm recovery was achieved in 20.83% patients with FSH level ≥ 3 × the maximum value of the normal range (N), in 42.5% patients with 2 N ≤ FSH < 3 N, and in 62.5% patients with N < FSH < 2 N. This study showed that active sperms were found in 38 cases, of which 23 (44.2%) were within the normal range (N), 11 (21.2%) were N < FSH < 2 N, and 4 (7.7%) were > 2 N. Our results demonstrated that the previous theory (i.e., that testicular biopsy need not be performed when the FSH level was twice the normal level because this condition means there are no mature sperms) is doubtful, although researchers in China agree on that theory,

especially in patients with azoospermia induced by mumps. This study proved that active sperms were found in four azoospermic men with FSH levels twice as high as the normal level, which implied that azoospermic men with very high FSH concentrations should not to give up the opportunity to undergo ICSI.

In addition, the average of the testicular volume was smaller in the sperm and non-sperm groups than in the normal group. Although the testicular volume was smaller in the non-sperm group than in the sperm group, there was no statistical significance ($P > 0.05$). Moreover, the testes of 35 of 38 patients (67.3%) were > 6 mL in size, and the testes of 3 patients (5.8%) were < 6 mL in size. Therefore, testicular volume could not be used as an effective indicator of whether testicular sperm extraction will be successful or not in patients with mumps-induced azoospermia. Nevertheless, we suggest performing testicular biopsy even when the testes were < 6 mL in size, as this could potentially increase the reproductive success rate.

In sum, the testicular volume and FSH level are potential indicators of the success of testicular sperm extraction in patients with azoospermia induced by mumps; however, these are not the gold standard. Presently, only testicular biopsy is the gold standard. To create more opportunities for ICSI, patients with mumps-induced azoospermia should be subjected to testes biopsy.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shuiwen Zhang, Reproductive Medical Center, The General Hospital of Beijing Military Region, Beijing 100700, China. Tel: +86 10 66721552; Fax: +86 10 66721552; E-mail: shuiwenzhangcn@163.com

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