Increased Level of c-kit in Semen of Infertile Patients with Varicocele

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Purpose: Varicocele is the most common risk factor for male infertility, however, not all males with varicocele experience infertility. In fact, most patients with varicocele have normal spermatogenesis. The molecular mechanism of varicocele-associated infertility is yet to be completely understood. The aim of this study is to assess the association of a number of fertility regulatory factors on varicocele associated infertility and to throw light on the mechanism of varicocele-associated infertility.

Materials and Methods: Semen from 30 infertile patients with varicocele and 30 fertile men with varicocele were collected. The concentrations of the following factors in seminal plasma were determined by ELISA: follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), androgen binding protein (ABP), transferrin (Trf), inhibin B (INHB) and stem cell factor (SCF). The expression level of c-kit in seminal precipitate of patients with varicocele was detected by real-time PCR.

Results: The concentrations of sexual hormones, FSH, LH and T, had no differences between infertile patients with varicocele and fertile men with varicocele ($P > 0.05$). Factors secreted by Sertoli cells, ABP, Trf, INHB and SCF, showed no significant differences between the two groups ($P > 0.05$). Interestingly, the expression of c-kit was significant higher in infertile patients with varicocele than that in fertile men with varicocele ($P < 0.01$).

Conclusion: Neither the sexual hormones nor the Sertoli cells was responsible for the infertility induced by varicocele. The aberrant expression of c-kit in infertile patients with varicocele may provide new insight into the mechanism of varicocele-associated infertility.

Keywords: c-kit; infertility; Sertoli cell; varicocele.

INTRODUCTION

Varicocele is prevalent worldwide and is considered by the World Health Organization (WHO) as the first cause for male infertility. However, varicocele is not only a frequent finding in infertile men, but that it is also found in fertile men[1]. In fact, most patients with varicocele have normal spermatogenesis. The exact mechanism of varicocele-associated infertility remains unclear[2]. Many studies have shown that varicocele have negative effect on spermatogenesis[3].

Spermatogenesis is a complex process and is controlled by hormones and many regulatory factors. It is well known that spermatogenesis is under the control of hypothalamic-pituitary-gonadal axis[4]. Hypothalamus secretes GnRH, which stimulates hypophysis to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH has an effect on maturation of testis and promotes sperm maturation. LH stimulates Leydig cells to secrete testosterone (T), which is necessary for spermatogenesis.

In the testis, Sertoli cells play important roles on spermatogenesis. They are responsible for the secretion of numerous proteins into the seminiferous tubular lumen, such as androgen binding protein (ABP), transferrin (Trf) and inhibin B (INHB). ABP binds androgens with high affinity and transports them to the epididymis. Trf is thought to play a critical role in the delivery of iron from the somatic compartment to the germ cells, which is necessary for cell proliferation, differentiation, and metabolism[5]. INHB is a glyco-protein that modulates FSH secretion via a negative feedback loop[6]. It is considered as a biomarker of testicular toxicity[7]. All of these factors play necessary roles in spermatogenesis.

During testicular development, stem cell factor (SCF) and c-kit are critical for reproductive events and important for germ-cell development[8]. FSH secreted by the pituitary stimulates Sertoli cells to secrete SCF, which binds to its tyrosine kinase receptor, c-kit, on the surface of differentiating germ cells, where it induces proliferation and differentiation[9]. The c-kit receptor forms a dimer by binding SCF, and as a result, tyrosine kinase

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Received August 2016 & Accepted February 2017

Table 1. Semen Parameters in Patients with Varicocele

Group	*n*	Sperm volume (ml)	Sperm concentration (*10^6/ml)	Sperms with forward motility (A+B) (%)
Fertile men with varicocele	30	2.72 ± 0.79	45.73 ± 14.51	34.27 ± 16.76
Infertile patients with varicocele	30	2.57 ± 0.85	6.88 ± 4.12	20.28 ± 18.28
P value		0.86	< 0.01	0.02

Data is presented as mean ± SD.

activity is induced[10]. The interaction of SCF and c-kit plays an essential role in primordial germ cell migration and survival, and in spermatogonial adhesion, proliferation[11].

In this study, we detected these relating hormones and regulatory factors, including FSH, LH, T, ABP, Trf, INHB, SCF and c-kit, in infertile patients with varicocele and fertile men with varicocele. The aim of this study is to assess the association of a number of fertility regulatory factors on varicocele associated infertility.

MATERIALS AND METHODS

Patients

We included 60 patients with varicocele aged between 25 and 30 years attending reproductive medicine department of Shanxi Provincial People's Hospital between May 2014 and March 2015. Patients were divided into two groups: infertile patients with varicocele (n=30) and fertile men with varicocele (n=30).

Inclusion criteria: All patients exhibited grade II or grade III clinical varicocele. The following standard grading system was used: grade II, easily palpable, but not visible; and grade III, easily visible. For the group of fertile men with varicocele, they had given birth to children in a year and the sperm count was >39×10^6/ejaculation, sperm concentration was >15×10^6/ml. For the group of infertile patients with varicocele, their wives did not get pregnant after more than 1 year of sexual life without contraceptives and the sperm count was <39×10^6/ejaculation; sperm concentration was <15×10^6/ml. Female infertility was excluded: normal follicular development was monitored by transvaginal ultrasound (TVUS); and patency of the fallopian tube was determined by transvaginal ultrasonography.

Exclusion criteria: Patients with reproductive tract infections, gonad function abnormality, abnormal chromosome karyotype, radioactive and other special professionals, taking drugs which disrupt sperm production and sperm motility, patients with serious cardiovascular, liver, kidney and hematopoietic system disease, or mental illness.

Informed consent was obtained from each patient and approval for the study protocol was granted by the Institutional Review Board of Shanxi Provincial People's Hospital.

Sperm examination

In all cases, after 3-5 days of sexual abstinence, semen samples were collected by masturbation. Then total sperm counts were determined 30 minutes after ejaculation using computer-aided semen analysis (CASA). The sperm examination was performed in accordance with the WHO fifth version of human semen examination standards.

ELISA

Semen samples were collected as previously described and centrifuged to separate seminal plasma and seminal precipitate. The seminal plasma concentration of FSH, LH, T, ABP, Trf, INHB and SCF were examined using commercially available ELISA kits (Jianglaibio, Shanghai, China). The experiment was performed according to the manufacturer's instructions. The optical density of color development was read by a photometer and the concentration was calculated by Gen5 software (BioTek, Winooski, USA).

Real-time PCR

Total RNA from seminal precipitate was isolated using a TaKaRa MiniBEST Universal RNA Extraction Kit (Takara Bio, Inc., Shiga, Japan) and 500ng RNA was reverse transcribed to cDNA using PrimeScript RT Master Mix (Perfect Real Time) (Takara Bio, Inc., Shiga, Japan). Real-time PCR was performed using the SYBR Premix Ex Taq II (Takara Bio, Inc., Shiga, Japan) following the manufacturer's instructions for the CFX96 Real-time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, USA). The used primers were: the forward primer 5'-TCCTCGCCTCCAAGAATTGT-3' and the reverse primer 5'-TCACAGGTAGTCGAGCGTTT-3'. The expression of beta-actin was used as a loading control. Relative transcript abundance was quantified by the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

All statistical analysis was carried out using SPSS version 19.0 (SPSS, Chicago, IL, USA). Values are presented as Mean ± Standard Deviation. Levels of significance for comparisons between groups were determined by Student's *t*-test. *P* value of < 0.05 was considered statistically significant.

RESULTS

Patients

Patients with varicocele were collected as previously described. The mean age of the infertile patients with varicocele was 27.2 ± 2.3 years; and that of fertile men with varicocele was 26.9 ± 2.1 years. There was no difference in the mean age of the two groups ($P > 0.05$).

Semen analysis

The semen from patients with varicocele was analyzed in accordance with the WHO fifth version of human semen examination standards. The result showed that both fertile men with varicocele and infertile patients with varicocele had normal semen volume and showed no difference ($P > 0.05$). Infertile patients with varicocele had significant lower sperm concentration than

Table 2. Concentration of sexual hormones

Group	LH (ng/L)	FSH (IU/L)	T (nmol/L)
Fertile men with varicocele	45.63 ± 7.27	7.27 ± 0.76	7.01 ± 0.96
Infertile patients with varicocele	43.91 ± 6.27	7.07 ± 0.88	7.23 ± 1.38
P value	0.46	0.40	0.61

Data is presented as mean ± SD.

fertile men with varicocele ($P < 0.01$). Sperms with forward motility significantly decreased in infertile patients with varicocele when compared with fertile men with varicocele ($P < 0.05$) (**Table 1**).

Concentrations of sexual hormones

We detected the concentrations of sexual hormones including LH, FSH and T in the seminal plasma of patients with varicocele using ELISA. The result showed that all of the detected sexual hormones showed no differences between fertile men with varicocele and infertile patients with varicocele ($P > 0.05$) (**Table 2**).

Factors secreted by Sertoli cells

Sertoli cells secrete many factors which are crucial for spermatogenesis. We examined the secretory function of Sertoli cells by detecting the factors secreted by Sertoli cells including ABP, Trf, INHB and SCF, in the seminal plasma of patients with varicocele. The result indicated that none of the factors showed significant difference between fertile men with varicocele and infertile patients with varicocele ($P > 0.05$) (**Table 3**).

Expression of c-kit

c-kit is important regulator in spermatogenesis. We detected the expression of c-kit in seminal precipitate of patients with varicocele using real-time PCR. The result showed that the expression of c-kit in infertile patients with varicocele was significantly higher than that in fertile men with varicocele ($P < 0.01$) (**Figure 1**).

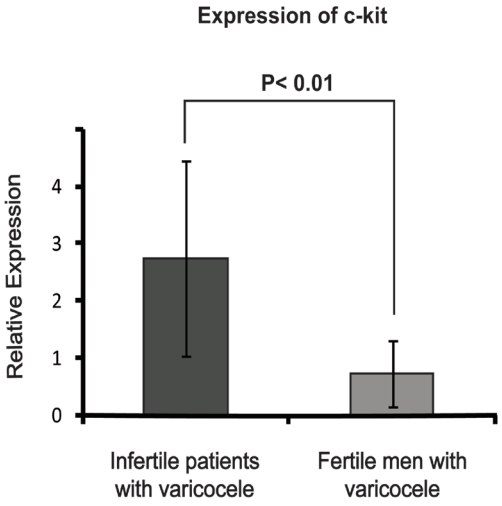

Figure1. The expression of c-kit in patients with varicocele
Data represent the mean of triplicate measurements and are reported as the mean fold change (x-fold) ± SD.

DISCUSSION

Though it is well known that varicocele is the first cause for infertility, it has been found that a portion of patients with severe varicocele do not present with infertility. The cause-effect relationship between varicocele and infertility has not been conclusively established yet. Based on the current knowledge about negative effect of varicocele on spermatogenesis, we postulate that spermatogenesis failure may be responsible for varicocele-associated infertility.

Spermatogenesis is under control of the hypothalamic-pituitary-gonadal axis. It is well known that FSH and LH are the pivotal endocrine regulators of testicular sex steroids production and spermatogenesis. LH stimulated T production of Leydig cells is the key endocrine stimulus of spermatogenesis[12]. Studies have reported that varicocele may be associated with increased FSH and low levels of T[13,14]. In this study, we detected the seminal plasma concentration of FSH, LH and T in patients with varicocele. The reason why we detected the hormones in the seminal plasma is that the operational sites of these hormones are in the testis, not the serum. The seminal plasma concentration of FSH, LH and T may be more valuable for evaluate their roles on spermatogenesis than serum content. Our result showed that there were no differences in the seminal plasma concentrations of FSH, LH and T between infertile patients with varicocele and fertile men with varicocele. It seems that FSH, LH and T may not participate in the process that varicocele induces infertility.

Spermatogenesis is supported and regulated by Sertoli cells. Sertoli cells provide nutritional as well as morphogenetic support for germ cells during spermatogenesis. They are responsible for the secretion of numerous proteins into the seminiferous tubular lumen, such as ABP, Trf and INHB, which regulates or responds to pituitary hormone release and further influences spermatogenesis[15,16]. To evaluate whether the secretory function of Sertoli cells is changed in infertile patients with varicocele, we detected the key secretions of Sertoli cells in seminal plasma of patients with varicocele. The result

Table 3. Concentration of factors secreted by Sertoli cells

Group	ABP (ng/ml)	Trf (nmol/L)	INHB (ng/L)	SCF (pg/ml)
Fertile men with varicocele	6.84 ± 1.34	403.82 ± 78.74	31.58 ± 5.47	87.25 ± 54.69
Infertile patients with varicocele	6.42 ± 1.28	385.2 ± 61.86	31.53 ± 5.30	75.41 ± 24.52
P value	0.30	0.41	0.98	0.31

Data is presented as mean ± SD.

showed that none of these factors, ABP, Trf and INHB, had statistically significant difference between infertile patients with varicocele and fertile men with varicocele. This indicated that Sertoli cells were not damaged in infertile patients with varicocele and Sertoli cells may be not involved in the process of infertility induced by varicocele.

SCF/c-kit system plays an important role in the production of gametes. The interaction of SCF with c-kit is required for germ cell survival and growth, and abnormalities in the activity of the SCF/c-kit system have been associated with human infertility[17]. We detected the concentration of SCF and c-kit, and the result showed that SCF was not altered while c-kit was increased in infertile patients with varicocele compared with fertile men with varicocele. SCF is secreted by Sertoli cells and c-kit is expressed on spermatogonia A1 to A4, spermatocytes and round spermatids. The result of stable expression of SCF is in accordance with the postulation that Sertoli cells may be not responsible for varicocele associated infertility. Ectopic expression of c-kit in infertile patients with varicocele suggests that germ cells may be involved in the process of varicocele associated infertility. Varicocele may cause damage to germ cells and induce infertility. C-kit may be helpful to predict whether varicocele will harm spermatogenesis, and induce infertility clinically. For patients with varicocele who might develop infertility, preventive measures may be taken, such as surgery and sperm freezing.

CONCLUSIONS

Taken together, we screened the sexual hormones, factors secreted by Sertoli cells, and SCF/c-kit system to clarify the mechanism of infertility induced by varicocele. Neither the sexual hormones nor the Sertoli cell was responsible for varicocele-associated infertility. A valuable finding was that the expression of c-kit was significant higher in infertile patients with varicocele than fertile men with varicocele. The aberrant expression of c-kit in infertile patients with varicocele may provide new insight into the mechanism of varicocele-associated infertility.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (No. 81373650), Shanxi Natural Science Foundation (No. 2009011057-3 and No. 2012011045-4) and Science Foundation of Shanxi Provincial Health Department (No. 201201061).

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