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Original Article

Sperm motility inversely correlates with seminal leptin levels in idiopathic asthenozoospermia

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Abstract: Background: Asthenozoospermia is one kind cause of male infertility. Nevertheless, no specific etiology can be identified by routine tests in some cases. Recently, it has been shown that leptin plays a critical role in male fertility. However, the link between leptin and sperm motility is yet to be determined. The aim of this study was to explore association between seminal and serum leptin levels and sperm motility in idiopathic asthenozoospermia. Methods: Our study included 79 asthenozoospermic men and 77 normozoospermic men. Semen was assessed by volume, sperm concentration, motility and morphology. Serum gonadotropic and sex hormones were determined by a chemiluminescent assay. The leptin levels in serum and seminal plasma were detected with ELISA. Results: The mean seminal leptin level in asthenozoospermic group was significantly higher than that in control group, but there was no significant difference in the serum leptin levels between these two groups. The serum leptin had no significant correlation with sperm motility. The seminal leptin had significantly negative correlation with sperm progressive motility and serum total testosterone. Conclusions: The findings indicate a pathophysiological relevance of seminal leptin in sperm motility.

Keywords: Idiopathic asthenozoospermia, seminal leptin, serum leptin, sperm motility

Introduction

Male infertility is a major social and medical problem globally, which affects people both medically and psychosocially [1]. About 60-80 million couples suffer from infertility worldwide according to the World Health Organization (WHO). The factor from male partner contributes to about 40% of cases of infertility [2]. Asthenozoospermia is one kind cause of male infertility and is involved in 19% of infertile cases [3]. Isolated asthenozoospermia can be found in 24% of all infertile men [4], which may result from prolonged periods of sexual abstinence, sperm dysfunction, partial obstruction of seminal tract, varicocele, infection or genetic factors [5, 6]. Nevertheless, some asthenozoospermia cases could be idiopathic; in other words, no specific etiology can be identified by means of routine medical tests [7]. Present epidemiological studies have found a link between male infertility and lifestyle patterns including dietary habits [8]. A recent animal study showed that high-fat diet rats presented significant increases in serum leptin levels and significant decreases in sperm motility [9].

Leptin is a kind of proteic hormone secreted by white adipose tissue. Its function is to regulate energy balance, providing regulation of body weight balance. Seminal leptin may play a functional role in sperm capacitation [10]. Although there are many documents describing connections between body weight status and the reproductive axis in females, there are few reports investigating the correlation between leptin and problems of fertility in male patients, especially in idiopathic asthenozoospermia cases. The aim of this study was to make an assessment of the association between seminal and serum leptin levels and semen quality in idiopathic asthenozoospermia cases.

Table 1. Clinical and laboratory data of studied groups (mean±SD)

	Asthenozoospermic	Normozoospermic	*P*-value
N	79	77	
Age (y)	30.9±2.7	31.1±2.7	0.722
Body mass index	24.15±2.13	24.32±2.14	0.636
Sperm concentration (10^6/mL)	33.47±11.08	41.84±11.39	0.0001
Sperm progressive motility (%)	20.42±5.78	48.73±7.27	0.0001
Serum FSH (mIU/mL)	8.26±4.99	7.65±4.91	0.441
Serum LH (mIU/mL)	5.30±2.00	4.90±1.97	0.200
Serum T (ng/mL)	7.22±1.54	7.39±1.62	0.505
Serum E_2 (pg/mL)	26.73±6.93	28.05±6.72	0.227
Serum PRL (ng/mL)	10.73±2.62	10.10±1.75	0.079
Serum leptin (ng/mL)	9.48±0.98	9.22±1.15	0.134
Seminal leptin (ng/mL)	4.72±0.99	3.75±0.97	0.0001

Subjects and methods

Patients

Men 25-40 years old admitted to our andrology clinic in Shanghai were included for this study, which was conducted from July 2011 to December 2013. Asthenozoospermia cases and controls were selected after their visit according to the spermatogram results, once the primary infertility examinations were conducted for every couple. Incident cases (n=79) were diagnosed with asthenozoospermia, defined as 'total motility' < 40%, including both progressive motility, sluggish motility and non-progressive motility on the basis of the fifth edition of WHO laboratory manual for the examination and processing of human semen (World Health Organization, 2010) [11]. Asthenozoospermia was defined as progressive motility < 32% in addition, including both rapid, slow progressive and sluggish motility in the same class within 60 min of ejaculation during the past 3 months. The concentration of sperm, and percentage of morphologically normal sperm, was equal to or above the lower reference limits. Thus, incident cases were asthenozoospermic males with normal sperm concentration and morphology, while controls (n=77) were normozoospermic men (≥ 15×10^6 million of sperms/ml, ≥ 40% total motility, ≥ 32% progressive motility and ≥ 4% normal forms, strict criteria). Multiple conditions and aspects of each case's history were used as exclusion criteria, including a history of cryptorchidism, varicocele or endocrine hypogonadism; a history of radiation and/or chemotherapy; and karotype anomalies. Neither group suffered from any nutrition-related diseases such as diabetes, cardiovascular diseases, osteoporosis, renal disease or cancers. Since no possible causes for reduced sperm motility have been detected, conclusive diagnosis of idiopathic asthenozoospermia was reached.

All participating cases and controls were interviewed by particularly trained professional andrology doctors face-to-face. After the initial screening, data were collected from 79 asthenozoospermia cases and 77 controls. The study protocol was approved by the Institutional Review Board of Fudan University Pudong Medical Center. Informed consents were obtained from all participants prior to enrollment.

Determination of serum hormone levels

All the blood samples were collected at 8:00-10:00 AM. Serum was stored at -20°C until analysis after centrifugation at 2000 g. Serum total testosterone (T), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and estradiol (E_2) levels were determined by a chemiluminescent assay (Access Testosterone, hFSH, hLH, Prolactin and Estradiol; Beckman Coulter, USA). All assays were performed according to manufacturer's instructions.

Semen analysis

After a required continence period of 3-5 days, each participant provided semen sample into a plastic sterile wide-mouth and metal-free container via masturbation. After liquefaction the semen specimens were subjected to computer-assisted semen analysis. The seminal plasma was obtained after centrifugation (3000 g/min for 10 min) and then stored at -20°C until use. Semen specimens were obtained and processed by an experienced technician according to the fifth edition of WHO laboratory manual for the examination and processing of human semen (World Health Organization, 2010) [11].

Table 2. Correlations between seminal leptin, serum leptin, and different parameters (r, *P*)

	Serum leptin	Seminal leptin
Age	-0.031 (> 0.05)	0.001 (> 0.05)
Body mass index	0.626 (< 0.001)	0.524 (< 0.001)
Sperm concentration	0.106 (> 0.05)	-0.099 (> 0.05)
Sperm progressive motility	-0.102 (> 0.05)	-0.431 (< 0.001)
Serum FSH	0.038 (> 0.05)	0.092 (> 0.05)
Serum LH	0.083 (> 0.05)	0.056 (> 0.05)
Serum T	-0.251 (0.002)	-0.160 (0.046)
Serum E_2	-0.046 (> 0.05)	-0.068 (> 0.05)
Serum PRL	0.019 (> 0.05)	0.128 (> 0.05)
Seminal leptin	0.388 (< 0.001)	

Serum and seminal plasma leptin assay

The leptin levels in serum and seminal plasma were detected with a solid-phase sandwich ELISA (RECI Diagnos-tics GmbH, Inc., Herrenberg, Germany). The lower detection limit was 0.5 ng/mL and the detection range was 0-100 ng/mL.

Statistical analysis

All data were analyzed using SPSS software (version 17, Chicago, IL, USA). The data were expressed as mean±SD and the differences were compared by unpaired t-test. Relationships between values were analyzed by Spearman correlation test. $P < 0.05$ was considered as statistically significant.

Results

The clinical and laboratory data of the studied two groups were represented in **Table 1**. The mean seminal leptin level in asthenozoospermic group was significantly higher than that in normozoospermic group, but there was no significant difference in the serum leptin levels between these two groups.

The BMI had significantly positive correlation with seminal and serum leptin. The serum leptin had no significant correlation with the patients' age, sperm concentration, sperm progressive motility, serum FSH, LH, E2 and PRL, but had significantly negative correlation with serum T. The seminal leptin had significantly negative correlation with sperm progressive motility and serum T (**Table 2**).

Discussion

The present study about idiopathic asthenozoospermia showed that sperm motility was inversely correlated with seminal leptin levels but not with serum leptin levels. The seminal leptin in asthenozoospermic male was significantly higher than that in normozoospermic male. The BMI had significantly positive correlation with seminal and serum leptin.

Although evaluating the correlation between the BMI and sperm motility was not the aim of this study, we found that both seminal and serum leptin were positively correlated with BMI. Some recent studies have shown that the BMI was negatively associated with sperm motility [5, 12]. Hammoud et al. [13] showed that sperm motility inversely correlated with BMI. Fejes et al. [14] reported the negative association of waist and hip circumference of men with the total sperm motility as well as the rapid progressive sperm motility. Despite these studies, other studies did not include the parameters about sperm motility, and they did not find any relationship between the BMI and sperm motility [15, 16]. Thus, the dispute about whether or not we should advise asthenozoospermic male to reduce weight is still a controversial subject, which needs further research.

Recently, it has been shown that leptin plays a part vital role in female fertility. However, the link between leptin and male spermatogenic function and sperm motility is yet to be determined. In this study, our data reveal that seminal leptin level increased in idiopathic asthenozoospermic males and seminal leptin levels inversely correlated with sperm motility. Some studies reported the presence of leptin receptors in Leydig cells [17] and germ cells [18]. Our previous study also showed that leptin was expressed in the seminiferous tubules and in the intersitium of rat testis, and leptin receptor was expressed mainly in the intersitium [19]. The expression pattern of leptin and its receptor in the testis may imply that leptin may play a part in in male reproduction. Our data reveal that seminal leptin appears to be inversely correlated with sperm motility in idiopathic asthenozoospermic males. This implies that leptin may be involved in the idiopathic asthenozoospermia-related spermatogenic dysfunction.

Similarly, it has been found that leptin is also overexpressed in the testes of the patients with Sertoli cell-only syndrome [20]. Therefore overexpression of leptin might be linked to the spermatogenic dysfunction, including idiopathic asthenozoospermia-related infertility.

However, the mechanism of contribution of leptin to male spermatogenic function is still unknown. In this study, both seminal and serum leptin were inversely correlated with serum testosterone. Some potential effects of leptin on the male gonads have been reported. It is also generally known that spermatogenesis is related to the level of testosterone secretion. It has been shown that leptin can significantly reduce hCG-induced testosterone secretion by rat Leydig cells in vitro [21]. A few upstream factors in the steroidogenic pathway, such as steroidogenic factor 1, may be involved in the leptin-induced inhibition of testosterone [22]. The overexpression of leptin receptors in the testis appears to be related to inhibition of testosterone production. Fombonne *et al*. reported that leptin was shown to suppress the division of prepubertal Leydig cells *in vitro* [23]. According to another study, leptin levels were negatively correlated with the levels of inhibin B, which was secreted by Sertoli cells [24]. It was suggested that leptin may regulate the secretion of inhibin B, or may even be related to the regulation of the function of Sertoli cells. Leptin may affect both Sertoli and Leydig cells. These could be some of the ways through which it affects spermatogenesis.

Our data demonstrated that without a significant difference in BMI, the serum leptin concentration in patients with idiopathic asthenozoospermia was higher than that in controls but the difference is not statistically significant. It was also reported that there was no correlation between serum leptin levels and a history of cryptorchidism or varicocele [25]. It is assumed that the serum leptin changes only in those patients with extremely poor spermatogenic function. Leptin can also be detected in human seminal plasma, though the source of it still remains unclear. We found that the leptin in seminal plasma was positively correlated with that in serum. The leptin level of seminal plasma was significantly higher in patients with idiopathic asthenozoospermia compared with that of the controls and also inversely correlated with sperm motility, consistent with the study of Glander et al [26]. It was proposed that serum leptin was not correlated with the levels of serum FSH and LH, or the seminal parameters, and this has also been confirmed by our study. This may suggest that leptin has certain local effects on the testis, which are independent of the hypothalamus and pituitary. In addition, leptin can also be secreted by human sperm, and leptin receptor has been found on the membrane of spermatozoa [27, 28]. Leptin was also considered to be vital to sperm capacitation [26]. All these results suggest that the sperm may be able to regulate its metabolism independently of systemic serum leptin. However, to know the exact role of leptin on spermatogenesis function, further researches in vitro and in vivo are necessary. On the basis of the current literatures and the results of our study, the link between leptin and sperm motility still lacks a concrete explanation. This is partly on account of the fact that our data are just observational; further experimental studies should be done to explore the specific mechanism.

Another limitation of our study is that we did not evaluate the known genetic mutations and polymorphism involved in the sperm motility; if we had detected the known genetic factors involved in sperm motility, we might have found a stronger association between leptin and sperm motility in asthenozoospermic male with certain gene polymorphisms and/or mutations. What is more, no significant correlations may have been declared significant by chance alone.

Conclusions

This study found that seminal leptin was negatively associated with sperm motility and serum T. The seminal leptin level in asthenozoospermic group was significantly higher than that in control group. The findings imply seminal leptin may play a direct role on sperm motility. We recommend that more studies should be done to address the mechanism of action of leptin on spermatogenesis.

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Disclosure of conflict of interest

None.

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