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Serum and seminal plasma insulin-like growth factor-1 in male infertility

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Objective: Growth hormone and its mediator, insulin-like growth factor-1 (IGF-1), have been suggested to exert gonadotropic actions in both humans and animals. The present study was conducted to assess the relationship between serum IGF-1 concentration, seminal plasma concentration, and sperm parameter abnormalities.

Methods: A total of 79 men were enrolled in this study from December 2011 to July 2012 and were prospectively analyzed. Patient parameters analyzed included age, body mass index, smoking status, urological history, and fertility history. Patients were divided into four groups based on their semen parameters: normal (A, n = 31), abnormal sperm motility (B, n = 12), abnormal sperm morphology (C, n = 20), and two or more abnormal parameters (D, n = 16). Patient seminal plasma and serum IGF-1 concentrations were determined.

Results: Patient baseline characteristics were not significantly different between any of the groups. The serum IGF-1 levels in groups B, C, and D were significantly lower than the levels in group A; however, the seminal plasma IGF-1 levels were not significantly different between any of the groups.

Conclusion: Men with abnormal sperm parameters had significantly lower levels of serum IGF-1 compared with men with normal sperm parameters. Seminal plasma IGF-1 levels, however, did not differ significantly between the groups investigated here. Further investigations will be required to determine the exact mechanisms by which growth hormone and IGF-1 affect sperm quality.

Keywords: Infertility; Insulin-like growth factor-1; Male; Semen; Serum

Introduction

Infertility is defined as the inability to conceive after 1 year of unprotected intercourse. Approximately 15% of all human couples are recognized as infertile, and about 50% of these cases are associated with male fertility factors. Although overall human fertility does not appear to have declined in recent years, some evidence indicates that sperm quality has declined [1]. To date, multiple factors have been implicated in reduced sperm count and motility. These factors include genito-urinary infections, environmental exposure to hazardous chemicals that are detrimental to spermatogenesis, anatomic and physical obstructions, hormonal imbalances, immunologic deficiencies, and abnormal morphology.

Spermatogenesis is a complex developmental process that requires pituitary gonadotrophins and testosterone, which indirectly regulate spermatogenic cells and testicular functions in autocrine, paracrine, and juxtacrine manners. One of the molecules thought to be involved in the development of germ cells is insulin-like growth factor-1 (IGF-1), a 7.6-kDa polypeptide of 70 amino acids that mediates the cell-proliferating actions of growth hormone (GH). IGF-1 is found in plasma and tissue fluid, and influences steroidogenesis, metabolism, cell proliferation, and differentiation [2,3]. IGF-1 also affects various reproductive processes and plays an essential role in the onset,

progress, and regulation of spermatogenesis [4]. IGFs are produced mainly in the liver, and are released in response to pulsatile secretions of GH from the anterior pituitary [5]. In the male reproductive tract, IGF-1 has been found in the testis [6], where it is secreted by Leydig cells and Sertoli cells [7-9]. Receptors for IGF-1 have been identified on Sertoli cells [10], Leydig cells [8], secondary spermatocytes, spermatids [9,11], and spermatozoa [12,13]. Furthermore, IGF-1 is thought to be involved in spermatogenesis [14-17] and steroidogenesis [18]. IGF-1 also appears in the peripheral circulation, where it exists primarily in a heavy tertiary complex with IGF-binding protein-3 and acid-labile subunit [19,20].

However, only a few studies have investigated the semen and serum levels of IGF-1. One study investigated these levels in normal fertile and vasectomized men, whereas the other identified variable levels of IGF-1 in infertile men [3,21]. To obtain a better understanding of the role of IGF-l in fertility, the present study aimed to determine the relationship between serum IGF-1 concentration, seminal plasma IGF-1 concentration, and sperm parameter abnormalities.

Methods

1. Study design

Data from 79 men who were enrolled in this study from December 2011 to July 2012 were prospectively analyzed. Written informed consent was obtained from each subject before entering the study. This study was performed in accordance with the principles of the Declaration of Helsinki and with the approval of the Cheil General Hospital Institutional Review Board (CGH-IRB-2011-76). All patients received medical care from the same doctor at a single hospital. Patient parameters analyzed included age, height, weight, body mass index (BMI), smoking status, urological history, and fertility history. Exclusion criteria included diabetes mellitus; liver, renal, or thyroid disease; past or current cancer; a history of genito-urinary tract infections; azoospermia; and any surgical history or medications that could have affected semen parameters. According to the fifth edition of the World Health Organization guidelines, the patients were divided into the following four groups based on their semen parameters:

normal (A, n = 31), abnormal sperm motility (B, n = 12), abnormal sperm morphology (C, n = 20), and two or more abnormal parameters (D, n = 16). Seminal plasma and serum concentrations of IGF-1 were determined for each subject.

2. Hormonal assay

Serum and seminal IGF-1 levels were assessed *via* radioimmunoassay. Briefly, samples and standards were incubated in tubes that had previously been coated with the first monoclonal antibody in the presence of a second monoclonal antibody, which was labeled with iodine-125. After incubation, the tube contents were removed and the amounts of bound radioactive iodine-125 were measured. Unknown values were determined by interpolation from a standard curve; standard curve measurements were taken at the same time as those of the samples. The amount of bound radioactivity was directly proportional to the IGF-1 concentration in each sample. All reagents in the kit used were stable until the expiry date, per the kit label, if stored at 2℃ to 8℃. Storage conditions for reagents after reconstitution or dilution are included in the detailed description of the assay procedures.

3. Statistical analysis

The PRISM software package ver. 4.10 (GraphPad, La Jolla, CA, USA) was used for statistical analysis. Significance was set at $p < 0.05$ with a confidence interval of 95%. Spearman's rank correlation analysis was used to assess the relationships between serum IGF-1 levels, semen IGF-1 levels, and other semen variables. Quantitative parametric data are represented as means and standard deviations. Student's *t*-test was used to compare means between groups, whereas one-way analysis of variance was used to compare more than two groups. Intra-group differences were assessed using a post hoc test and by calculating the least significant difference.

Results

The principal characteristics of patients with normal and abnormal semen parameters are listed in Table 1. Body weight is heavier in

Table 1. Baseline characteristics of each group

Characteristics	Group A (n = 31)	Group B (n = 12)	Group C (n = 20)	Group D (n = 16)	*p*-value
Age (yr)	35.2 ± 3.2	34.7 ± 5.3	35.5 ± 3.3	35.4 ± 3.6	0.949
Body weight (kg)	75.0 ± 9.8	70.6 ± 7.0	80.5 ± 8.0	75.8 ± 10.5	0.038
Height (cm)	175.1 ± 5.9	174.2 ± 6.5	175.6 ± 6.0	174.7 ± 5.6	0.922
Body mass index (kg/m²)	24.47 ± 3.2	23.18 ± 1.8	26.05 ± 2.4	24.82 ± 3.0	0.054

Values are presented as mean ± standard deviation.
Group A, normal semen parameters; Group B, abnormal sperm motility; Group C, abnormal sperm morphology; Group D, more than two abnormal semen parameters.

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Table 2. Semen parameters, serum IGF-1 level, and seminal plasma IGF-1 level of each group

Variable	Group A (n = 31)	Group B (n = 12)	Group C (n = 20)	Group D (n = 16)
Semen parameter				
Volume (mL)	2.5 ± 1.0	3.7 ± 1.8	3.2 ± 1.2	3.7 ± 1.6
Concentration (10^6/mL)	153.4 ± 67.5	67.6 ± 42.0	79.8 ± 50.6	34.4 ± 32.9
Motility (%)	68.8 ± 14.5	27.9 ± 5.7	59.7 ± 12.0	27.1 ± 12.8
Morphology (%)	14.0 ± 9.3	8.3 ± 4.2	0.5 ± 0.8	0.5 ± 1.0
Viability (%)	77.1 ± 13.2	47.36 ± 18.2	61.3 ± 13.5	42.6 ± 31.2
IGF-I (ng/mL)				
Serum	175.4 ± 42.7	137.3 ± 7.3[a]	144.4 ± 25.8[b]	130.6 ± 28.2[c]
Seminal plasma	15.4 ± 8.3	15.6 ± 5.8	18.7 ± 4.0	15.9 ± 5.9

Values are presented as mean ± standard deviation.
Group A, normal semen parameters; Group B, abnormal sperm motility; Group C, abnormal sperm morphology; Group D, more than two abnormal semen parameters; IGF-1, insulin-like growth factor-1.
[a]$p < 0.001$; [b]$p < 0.05$; [c]$p < 0.01$; multiple comparison test.

Table 3. Semen parameters, serum IGF-1 levels, and seminal plasma IGF-1 levels of the normal and abnormal semen parameter groups

Parameter	Group A (n = 31)	Groups B+C+D (n = 48)
Semen parameter		
Volume (mL)	2.5 ± 1.0	3.5 ± 1.5
Concentration (10^6/mL)	153.4 ± 67.5	61.1 ± 46.8
Motility (%)	68.8 ± 14.5	40.7 ± 19.4
Morphology (%)	14.0 ± 9.3	2.4 ± 4.0
Viability (%)	77.1 ± 13.2	51.4 ± 23.3
IGF-I (ng/mL)		
Serum	175.4 ± 42.7	137.6 ± 27.1[a]
Seminal plasma	15.4 ± 8.3	17.0 ± 5.2

Values are presented as mean ± standard deviation.
IGF-1, insulin-like growth factor-1; Group A, normal semen parameters; Group B, abnormal sperm motility; Group C, abnormal sperm morphology; Group D, more than two abnormal semen parameters.
[a]$p < 0.001$, two-sided t-test.

Table 4. Correlation analysis between serum IGF-1 and clinical parameters

Parameter	No. of XY pairs	Spearman's r	p-value (two-tailed)	p-value summary
Age	98	−0.035	0.731	NS
Weight	98	0.036	0.720	NS
Height	98	−0.061	0.549	NS
BMI	98	0.105	0.300	NS
TMSC	98	0.140	0.169	NS
Semen parameter				
Volume	98	−0.251	0.012	< 0.05
Concentration	98	0.192	0.058	NS
Motility	98	0.202	0.045	< 0.05
Viability	98	0.254	0.011	< 0.05

IGF-1, insulin-like growth factor-1; BMI, body mass index; NS, not significant; TMSC, total motile sperm count.

group C; however, BMI did not significantly differ between groups. The serum IGF-1 levels of the abnormal sperm motility group (B), the abnormal sperm morphology group (C), and the group with two or more abnormal parameters (D) were significantly lower than those of the normal group (A). However, no significant between-group differences were observed regarding the IGF-1 levels of seminal plasma (Table 2). Next, the IGF-1 levels of the normal (A) and abnormal (B, C, and D) groups were compared. This analysis revealed that the abnormal group showed significantly lower serum levels of IGF-1 compared with the normal group. However, the seminal plasma IGF-1 levels were not significantly different between the normal (A) and abnormal (B, C, and D) groups (Table 3). In a Spearman's correlation test, the serum IGF-1 level is correlated with semen volume, sperm motility, and sperm viability (Table 4).

Discussion

Studies of children and adolescents have indicated an age-related increase in serum IGF-1 levels during the prepubertal and early pubertal stages, followed by a decrease in late puberty [22-25]. Other studies have also shown that serum IGF-1 levels change with age; for instance, Korean men aged 30 to 49 years old were shown to have serum IGF-1 levels that exceeded 180 ng/mL [24]. The mean subject age in our study was 35.2 years, with a range of 30 to 49 years.

Overall, the serum IGF-1 levels measured in our study were relatively low. This finding might be due to the size of our target group, which could have affected the results due to bias in the measuring tools. We found that the serum IGF-1 levels of the abnormal motility group (B, mean = 137.3 ± 7.3 ng/mL), the abnormal morphology group (C, mean = 144.4 ± 25.8 ng/mL), and the group with two or more abnormal parameters (D, mean = 130.6 ± 28.2 ng/mL) were significantly low-

er than the levels in the normal group (A, mean = 175.4 ± 42.7 ng/mL).

Only a few studies have assessed the relationship between serum/seminal IGF-1 and semen quality. In these studies, males with infertility due to oligospermia had the lowest mean and median levels of seminal IGF-1. These levels were significantly different from the levels in the fertile and immuno-infertile groups, as well as from the other male-factor infertile subgroups. These findings suggest that lower seminal IGF-1 levels are associated with oligospermia [2]. However, we did not observe a significant relationship between semen parameters and seminal IGF-1 in this study, although we did identify a significant relationship between semen parameters and the serum level of IGF-1.

Both morphologically normal sperm and the seminal levels of IGF-1 have been reported to be decreased in males who smoke. Specifically, infertile smokers had the lowest seminal IGF-1 values, followed by infertile nonsmokers, fertile smokers, and control subjects [26]. These findings indicate that reduced IGF-1 is associated with both smoking and infertility.

Seminal IGF-1 has also been shown to have a significant positive correlation with the number of motile and rapid linear progressive spermatozoa [24]. IGF-1 production stimulates spermatozoa maturation in a paracrine/autocrine manner, thereby increasing sperm motility [3]. Thus, IGF-1 might function as a differentiation marker in male germ cells. Colombo and Naz [2] also showed that the seminal IGF-1 level exhibited a significant linear correlation with the total sperm count. Although sperm count was not directly investigated in the present study, it is taken indirectly into account for group D. Consistent with other studies, we observed decreased seminal IGF-1 levels in this group; however, this decrease was not significant. A limitation of this study is that subjects in the normal semen parameter group are also infertile male patients; thus, improved fertile male subjects will be needed for comparison of IGF-1 levels.

We found that the groups with abnormal sperm parameters exhibited significantly lower levels of serum IGF-1 compared with the normal group. Seminal plasma IGF-1 levels, however, did not differ significantly between the groups investigated here. Further investigations will be required to determine the exact mechanisms by which GH and IGF-1 affect fertility.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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