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Seminal Plasma Transforming Growth Factor-β (TGF-β) and Epidermal Growth Factor (EGF) Levels in Patients with Varicocele

Üçler KISA[1]
M. Murad BAŞAR[2]
Mehmet FERHAT[2]
Osman ÇAĞLAYAN[1]

[1] Department of Clinical Biochemistry, Faculty of Medicine, Kırıkkale University, Kırıkkale - TURKEY

[2] Department of Urology, Faculty of Medicine, Kırıkkale University, Kırıkkale - TURKEY

Aims: The aim of this study was to analyze seminal plasma *epidermal growth factor* (EGF) and *transforming growth factor-β* (TGF-β) levels in infertile patients with varicocele and normal fertile cases, and to evaluate the relationships between seminal plasma EGF and TGF-β, seminal parameters and serum hormone levels.

Materials and Methods: A total of 100 patients with varicocele were enrolled into the study. Semen analyses were performed after 2 and 5 days of sexual abstinence. These patients were divided into two groups based on the results of semen analysis as oligoasthenoteratozoospermia (Group 1) and normal semen analysis (Group 2). Additionally, 100 fertile cases without any scrotal pathology were accepted as the control group (Group 3), and semen analysis was also performed in these cases. Seminal plasma EGF and TGF-β levels were measured with ELISA. Additionally, serum gonadotropin and serum sex steroid levels were measured in patients and controls. Statistical analysis was performed by using one-way ANOVA test and post hoc Bonferroni test.

Results: There was a statistically significant difference between patients and controls regarding seminal parameters ($P < 0.05$). Seminal plasma EGF and TGF-β levels were higher in patients with varicocele (Group 1 and Group 2) than Group 3; however, there was a statistically significant difference only in TGF-β levels ($P = 0.017$). Seminal plasma EGF and TGF-β levels showed a negative relationship with seminal parameters ($P < 0.005$). However, there was no relationship between these growth factors and serum hormone levels.

Conclusions: This study showed that increased levels of EGF and TGF-β were associated with decreased seminal parameters in patients with varicocele. However, the relationship was more evident with TGF-β.

Key Words: Infertility, epidermal growth factor, transforming growth factor-β, varicocele

Varikoselli Hastalarda Seminal Plazma Transforme Edici Büyüme Faktörü-β (TGF-β) ve Epidermal Büyüme Faktörü (EGF) Düzeyleri

Amaç: Bu çalışmanın amacı varikoselli infertil hastalar ile normal fertil olgular arasında seminal plazma EGF ve TGF-β düzeylerini karşılaştırmak, ve seminal plazma EGF ve TGF-β düzeyleri, semen parametreleri ve serum hormon düzeyleri arasındaki ilişkiyi incelemektir.

Materyal ve Metot: Varikoselli 100 infertil hasta (Grup-1) çalışmaya alındı. İki ile 5 günlük cinsel perhiz sonrası semen analizi uygulandı. Semen analiz sonuçlarına göre bu hastalar oligoastenoeratozoospermisi olanlar (Grup-1) ve normal semen analizi olanlar (Grup-2) olarak iki gruba ayrıldı. İlave olarak, skrotal patolojisi olmayan 100 fertil olgu kontrol grubu (Grup-3) olarak kabul edildi ve semen analizi bunlarda da uygulandı. Seminal plazma EGF ve TGF-β düzeyleri ELİZA yöntemi ile ölçüldü. Ayrıca, tüm olgularda serum gonadotropin ve seks steroid düzeyleri ölçüldü. İstatistiskel analiz tek yönlü ANOVA test ve post hoc Bonferoni test ile yapıldı.

Bulgular: Her iki grup arasında semen analizi parametreleri bakımından anlamlı farklık vardı ($P < 0,05$). Seminal plazma EGF ve TGF-β düzeyleri varikoselli olgularda (Grup-1 ve Grup-2) Grup-3'den daha yüksekti. Ancak, istatistiksel fark sadece TGF-β düzeylerinde saptandı ($P = 0,017$). Seminal plazma EGF ve TGF-β seminal parametreler ile negatik ilişki göstermekteydi ($P < 0,05$). Bununa karşın, bu büyüme faktörleri ile serum hormon düzeyleri arasında ilişki yoktu.

Sonuç: Bu çalışma göstermektedir ki, seminal plasma EGF ve TGF-β düzeylerindeki artış varikoselli hastalarda seminal parametrelerde azalma ile birliktedir. Bununla birilkte, etki TGF-β ile daha belirgindir.

Anahtar Sözcükler: İnfertilte, Epidermal büyüme faktörü, Transfome edici büyüme faktörü-β, Varikosel

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Correspondence
Üçler KISA
GATA Loj. Ural Apt.
No: 8 Etlik,
Ankara - TURKEY

uclerkisa@hotmail.com

Introduction

Varicocele is the venous dilation of the plexus pampiniformis of the spermatic cord. It is observed in approximately 15% of the general population and in 19-41% of infertile males. In a recent study, it was reported that varicocele was found in 35% of

primary infertile and in 75 to 81% of secondary infertile males (1-4).

The most important outcome of varicocele is impaired spermatogenesis. The mechanisms of this damage have been reported as increased scrotal temperature, back-flow of renal and adrenal metabolites in the testicular vein, decreased blood flow in testicular artery, and hypoxia. However, some undefined mechanisms with respect to defective spermatogenesis in varicocele remain (2,5) because some patients with varicocele have normal seminal parameters and furthermore, because seminal parameters do not improve in 40-50% of cases after surgical treatment (5,6).

Recently, several growth factors secreted from the testes or Sertoli cells have been identified in male reproductive tissues and fluids (7,8). *Epidermal growth factor* (EGF) and *transforming growth factor-β* (TGF-*β*) are two of these growth factors, and both have a mitogenic effect on the same receptors in some human tissues (9,10). There are limited studies in the literature about seminal plasma EGF and TGF-β levels in infertile patients, and there are discrepancies among them (10,12,13).

The aims of the present study were to compare seminal plasma EGF and TGF-β levels in infertile patients with varicocele and normal fertile cases, and to evaluate the relationships between seminal plasma EGF and TGF-β levels, seminal parameters and serum hormone levels to determine the effects of these cytokines on spermatogenesis and steroidogenesis in males with varicocele.

Materials and Methods

All patients suffering from infertility were evaluated with detailed history and physical examination in the andrology outpatient clinic. Biochemical (SMA-24) and hormonal analysis including serum follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, total testosterone and estradiol (E_2) levels were measured in blood withdrawn between $08.^{00}$ and $10.^{00}$ am. Semen analyses were done after 2 and 5 days of sexual abstinence and were evaluated according to World Health Organization (WHO) guidelines, and morphological analyses were performed after staining with Sperm Mac (Ferti Net NV, Belgium) stain slide based on Kruger strict criteria (14,15). *Swim up* sperm wash technique was performed if no spermatozoa were observed in direct ejaculate analysis under light microscope.

After the semen analysis, the remaining seminal plasma was centrifuged at 600×g for 10 min, and stored at -80 °C until the analysis of seminal plasma EGF and TGF-β levels was done.

After these analyses, the patients with a history of hormonal treatment in the last 6 months, any scrotal pathology such as cryptorchidism, previous scrotal surgery such as orchiopexy or varicocele ligation, abnormal serum hormone levels, abnormal liver and renal function tests, any findings of obstructive azoospermia, or a systemic disease were excluded from the study. Therefore, a total of 100 infertile patients with varicocele, age range between 22 and 46 years (mean age 31.6 ± 5.1 years) were enrolled into the study. These cases were divided into two groups based on their semen analysis as oligoasthenoteratospermia group (Group 1; age range 23-46 years, mean 31.5 ± 5.2) and as the group with normal seminal parameters (Group 2; age range 22-40 years, mean 31.7 ± 4.5). Oligoasthen-oteratospermia was defined as having one of the following seminal parameters in semen analysis: Spermatozoa number less than 20 million per milliliter, rapid and progressive motility less than 50% and/or normal spermatozoa morphology less than 4% based on Kruger strict criteria.

A total of 100 voluntary fertile cases admitted to the urology outpatient clinic with different urological problems were accepted as the control group (Group 3; age range 22-47 years, mean 31.6 ± 4.9). These cases were chosen from patients having no acute or chronic scrotal pathology in the last 6 months, no varicocele or past varicocele surgery, and no systemic disease or familial infertility history. Moreover, all cases were fertile, and had at least one child or a current pregnancy history.

Seminal plasma cytokine levels were measured by using Human EGF (Biosource, Camarillo, USA) and Multispecies TGF-β1 (BioSource, Camarillo, USA) diagnostic kits based on ELISA principle.

Statistical analysis was performed by using SPSS 8.0 Statistical Package for Social Sciences for Windows (Chicago, IL, USA). Seminal parameters, serum hormone levels and seminal plasma cytokine levels among the three groups were compared by one way ANOVA and using

post hoc Bonferroni tests. Later, the relationships between seminal plasma cytokine levels and age, serum hormone levels and seminal parameters were evaluated with correlation analysis in patients with varicocele. Statistical significance was accepted if p value was less than 0.05.

Results

All cases had normal biochemical values, and there was no statistical difference in age between the three groups (p=0.976).

Serum hormone levels and seminal parameters in all groups are shown in Table 1. There were statistically significant differences in seminal parameters between the three groups (p<0.05), but hormone levels were comparable. The differences in seminal parameters were due to lower spermatozoa number and motility in Group 1 than Group 2 (p_{number}=0.000, $p_{motility}$=0.000) and Group 3 (p_{number}=0.000, $p_{motility}$=0.000). However, there were statistically significant differences in spermatozoa morphology among the three groups (p=0.000).

Only 2 patients (2%) had azoospermia after direct investigation, and no spermatozoa were observed in either after sperm washing procedure.

Seminal plasma TGF-β and EGF levels were 639.2 ± 253.9 pg/ml, and 101.6 ± 49.5 pg/ml in Group 1, 358.7 ± 194.4 pg/ml and 100.3 ± 52.6 pg/ml in Group 2, and 324.2 ± 191.4 pg/ml and 97.7 ± 43.8 pg/ml in Group 3, respectively (Figure 1). While there was no statistically significant difference in EGF levels between the three groups (p=0.890), TGF-β level was statistically higher in Group 1 than Group 2 (p=0.000) and Group 3 (p=0.000).

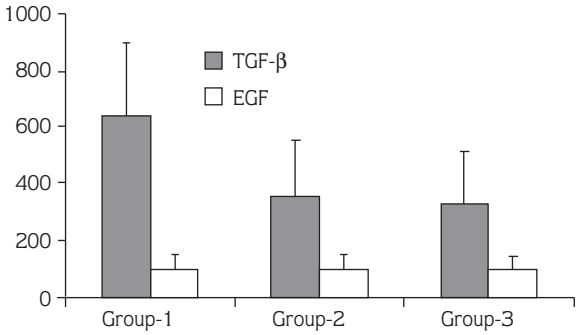

Figure 1. Seminal plasma TGF-β and EGF levels.

In all cases, there was no correlation between age and seminal plasma TGF-β and EGF levels.

The relationship between seminal parameters and seminal plasma TGF-β and EGF levels was evaluated in the varicocele groups and fertile cases separately.

Seminal plasma TGF-β and EGF levels showed a negative correlation with seminal parameters in the varicocele groups. However, only seminal plasma TGF-β level showed significant correlation with spermatozoa number (r=-0.422, p=0.000) and motility (r=-0.231, p=0.021) (Figure 2). On the contrary, there were no correlations between seminal plasma TGF-β level and spermatozoa morphology, or between seminal plasma EGF levels and any of the seminal parameters.

Table 1. Seminal parameters and serum hormone levels in patients with varicocele (Group 1 and Group 2) and control cases (Group 3) (*One way ANOVA *test*, **<0.05).

	Group 1 Mean ± SD	Group 2 Mean ± SD	Group 3 Mean ± SD	p*
Spermatozoa number ($X10^6$/ml)	11.9 ± 4.8	50.1 ± 23.3	51.7 ± 15.7	0.000**
Motility (%)	23.5 ± 14.9	52.1 ± 19.8	61.6 ± 27.0	0.000**
Morphology (%)	2.7 ± 1.2	7.9 ± 3.4	11.7 ± 5.2	0.000**
FSH (mIU/ml)	6.7 ± 4.2	6.4 ± 4.7	6.3 ± 4.7	0.784
LH (mIU/ml)	4.8 ± 1.9	4.7 ± 2.7	5.4 ± 2.8	0.206
Prolactin (ng/ml)	15.7 ± 5.7	14.2 ± 6.2	14.3 ± 5.7	0.316
Testosterone (ng/dl)	5.3 ± 1.4	5.2 ± 1.2	5.7 ± 1.6	0.360
Estradiol (pg/dl)	29.3 ± 8.3	29.6 ± 8.9	30.4 ± 8.1	0.747

SD: Standard deviation.
p* : One-way ANOVA test, <0.05.

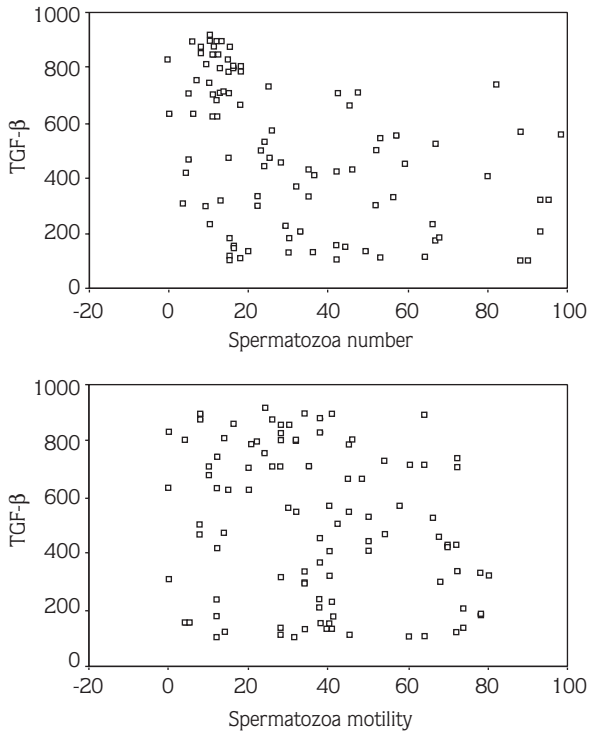

Figure 2. Correlation between seminal plasma TGF-β levels and spermatozoa number (a) and motility (b).

Moreover, seminal plasma GF levels showed no correlation with serum hormone levels in patient groups.

In fertile subjects, there was no correlation between the compared parameters including semen analysis and serum hormone levels.

Discussion

In this study, we observed that increased TGF-β levels were more frequently associated with patients with varicocele with abnormal seminal parameters than healthy individuals. Additionally, seminal plasma EGF and TGF-β levels were higher in the patients with varicocele than fertile subjects.

The exact mechanism of the effects of varicocele on testicular functions has not been identified. Increased testicular temperature, influx of renal and adrenal metabolites in the spermatic vein, decreased blood flow and hypoxia are possible factors in varicocele physiopathology (5,6). Recently, the effects of some growth factors and cytokines released from the testes on spermatogenesis with hormonal pathways have been reported (10,12,13).

Regulation of steroidogenesis in fetal Leydig cells with TGF-β was reported (16,17). Moreover, low TGF-β levels play a stimulating role in steroidogenesis, while increased levels of TGF-β have been shown to inhibit hormone secretions (12,18). In animal studies, it was reported that TGF-β controlled the proliferation of mouse primordial germ cells and the functions of Leydig cells by inhibiting gonadotropin effects and controlled peritubular myoid cells by stimulating extracellular matrix production (12,19). Thus, a dual effect of TGF-β on steroidogenesis has been concluded. Additionally, TGF-β has some effects on spermatogenesis, and regulates DNA synthesis in Leydig cells (17). The effects on germinal cells, such as inhibition of cell proliferation and stimulation of apoptosis, were demonstrated in studies designed in the rat fetus (20,21). Loras et al. (22) measured seminal plasma TGF-β levels in normal cases and in patients with different etiologies; however, they did not find any statistical difference among these groups. Recently, Dobashi et al. (23) concluded that TGF-β1 was related to fibrosis of seminiferous tubules and might lead to spermatogenetic disruption.

Salama et al. (24) evaluated testicular TGF-β levels in diabetic and elderly rats. The authors reported that diabetes had a more harmful effect on testicular TGF-β levels, and was responsible for the impaired seminal parameters in diabetic cases. In our study, although no diabetic patients were enrolled in the study, we also found a positive correlation between seminal plasma TGF-β level and age, like Salama et al. However, this correlation was not statistically significant.

EGF is a 53 amino acid polypeptide originally isolated from mouse submaxillary glands (25). In humans, it was firstly found in urine, and recently has been identified in some biological fluids (26,27). There are some studies in the literature about EGF in human reproductive function. EGF was found to augment the growth of cultured testicular cells in serum-free medium, increase ornithine decarboxylase activity in neonatal mouse testes and stimulate the meiotic phase of spermatogenesis (28). Recently, it has been reported that EGF deficiency might be a cause of male infertility. In these studies, because of lower seminal plasma EGF levels in infertile patients than control cases, no relationship between seminal parameters and spermatozoa function was reported (11,26). On the contrary, there have been some studies showing that seminal plasma EGF levels were higher in

infertile males (28-30). However, only seminal plasma TGF-β levels showed significant difference.

There is only one study concerning these growth factors in patients with varicocele (31). In that study, experimental varicocele was constituted in rats. Later, subcutaneous EGF administration was performed after surgical varicocele ligation, and improvement in spermatozoa number and motility was observed. TGF-β levels in different infertile populations were also evaluated by Loras et al. (22), and they found no statistical difference among the different etiologies. However, there has not been any study about TGF-β levels in patients with varicocele. In our study, we found that both seminal plasma EGF and TGF-β levels were higher in infertile cases with and without varicocele. The effect of TGF-β was more unclear than that of EGF in patients with varicocele.

These findings supported the conclusion that TGF-β likely has a more detrimental effect on spermatozoa than EGF. Hence, we thought that growth factors had an important role on seminiferous tubules in patients with varicocele. However, the main mechanism of this effect has not yet been solved. Since there was not a clear relationship with serum hormone levels, it was thought that the effect was not dependent on Leydig cell function. On the contrary, hypoxia due to varicocele might affect testicular histology and increase the effects of growth factors on seminiferous tubules.

In conclusion, according to this study, varicocele, which is the most important cause of treatable male infertility, deteriorates spermatogenesis via growth factors in addition to the identified effects of varicocele. Our results showed that TGF-β had a more detrimental effect on spermatozoa than EGF. However, the main mechanism of this effect is not yet clear. Therefore, we need further studies, especially histopathologic, to identify the exact mechanism of EGF and TGF-β in the pathophysiology of varicocele.

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