Testicular serum chitotriosidase levels in varicocele patients

Varikosel hastalarında testiküler serum kitotirozidaz seviyesi

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Abstract

Objective: We evaluated serum chitotriosidase levels in varicocele patients to investigate the pathophysiology of varicocele.

Materials and methods: In 2008, 38 patients who underwent left varicocelectomy with a diagnosis of left varicocele were divided into two groups based on their sperm counts. Group 1 (n=19) consisted of patients whose sperm counts were ≤20x10^6 sperm/mL (oligospermia), and group 2 (n=19) consisted of patients whose sperm counts were >20x10^6 sperm/mL (normospermia). Serum chitotriosidase levels were determined from samples obtained from the brachial and testicular veins.

Results: The mean patient sperm counts were 13.3±7.2x10^6 sperm/mL in Group 1 and 54.8±23.1x10^6 sperm/mL in Group 2. In Group 1, the mean patient brachial serum chitotriosidase level was 41.1±27.8 nmol/hour/mL, and the mean testicular serum chitotriosidase level was 40.5±27.4 nmol/hour/mL. In Group 2, the mean patient brachial serum chitotriosidase level was 28.6±22.8 nmol/hour/mL, and the mean testicular serum chitotriosidase level was 28.1±22.1 nmol/hour/mL. Although the mean brachial and testicular chitotriosidase levels were higher in Group 1 than in Group 2, this difference was statistically insignificant (p=0.188 and p=0.220 for the brachial and testicular levels, respectively).

Conclusion: The study demonstrates that brachial chitotriosidase levels are representative of testicular chitotriosidase levels. The increase in testicular serum chitotriosidase levels in oligospermic varicocele patients does not achieve statistical significance. However, further studies with larger sample sizes are needed.

Key words: Chitotriosidase; infertility; varicocele.

Özet

Amaç: Varikosel patofizyolojisini araştırmak amacıyla varikosel hastalarında serum kitotirozidaz seviyesini inceledik.

Gereç ve yöntem: 2008 yılında Ankara Askeri Hastanesi'nde sol varikosel tanısı ile sol varikoselektomi ameliyatı olan 38 hasta sperm sayılarına göre iki gruba ayrıldı. Grup 1 (n=19) sperm sayısı ≤20x10^6 sperm/mL olan hastalardan (oligospermik), Grup 2 (n=19) ise sperm sayısı >20x10^6 sperm/mL olan hastalardan (normospermik) oluşmakta idi. Serum kitotirozidaz seviyeleri hem brakial hem de testiküler venlerden elde edildi.

Bulgular: Hastaların ortalama sperm sayısı Grup 1 için 13.3±7.2x10^6 sperm/mL, Grup 2 için 54.8±23.1x10^6 sperm/mL idi. Grup 1'de hastaların ortalama brakial serum kitotirozidaz seviyesi 41.1±27.8 nmol/saat/mL, ortalama testiküler serum kitotirozidaz seviyesi 40.5±27.4 nmol/saat/mL idi. Grup 2'de hastaların ortalama brakial serum kitotirozidaz seviyesi 28.6±22.8 nmol/saat/mL, ortalama testiküler serum kitotirozidaz seviyesi 28.1±22.1 nmol/saat/mL idi. Ortalama testiküler ve brakial serum kitotirozidaz seviyeleri Grup 1'de Grup 2'den daha fazla olmasına rağmen, aradaki fark istatiksel olarak anlamlı seviyeye ulaşmamıştır (brakial için p=0.188, testiküler için p=0.220).

Sonuç: Bu çalışma brakial serum kitotirozidaz seviyelerinin testiküler kitotirozidaz seviyesini yansıttığını göstermektedir. Oligospermik varikosel hastalarının testiküler serum kitotirozidaz seviyelerindeki yükseklik, istatiksel olarak anlamlılığa ulaşmamıştır. Bununla beraber, daha geniş seriler içeren çalışmalara ihtiyaç vardır.

Anahtar sözcükler: İnfertilite; kitotirozidaz; varikosel.

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Varicocele is one of the main causes of male infertility. The incidence of unilateral left varicocele is 15% in the general population and 41% in the infertile population.[1] In several studies, a relationship between varicocele and infertility has been demonstrated, and improvements in semen quality after varicocelectomy have been observed.[2] In a multicenter World Health Organization study on 9,043 infertile couples, varicocele was detected in 25.4% of men with abnormal semen parameters and 11.7% of men with normal semen parameters.[3] Why are some varicocele patients fertile, with normal semen parameters? The answer to this question has been obscured by the pathophysiology of varicocele. Although several etiological factors have been suggested, the pathophysiology of varicocele and related male infertility is still unclear and requires additional investigations.

Rat studies have shown that the testis contains a large population of resident macrophages with a novel cytokine secretion profile and altered response to inflammatory activators.[4,5]

Chitotriosidase, the human analogue of family-18 chitinases, is one of the most abundant proteins synthesized by macrophages and is enzymatically active toward natural chitin.[6] Thus, its activity has been proposed to be a biochemical marker of macrophage accumulation.

Because the testis contains a large population of resident macrophages and chitotriosidase is synthesized by macrophages and secreted into the circulatory system, the serum chitotriosidase level might be significant in some testicular diseases involving macrophages, such as varicocele. We evaluated serum chitotriosidase enzyme levels in varicocele patients. The aim was to gain new insights into the pathophysiological mechanism of varicocele.

Materials and methods

Study population

Between March and December 2008, 38 patients who underwent left inguinal varicocelectomy with a diagnosis of varicocele at Ankara Military Hospital were included in this study. The patients were divided into two groups based on their sperm counts. Group 1 (n=19) consisted of patients whose sperm counts were $\leq 20x10^6$ sperm/mL (oligospermia), and Group 2 (n=19) consisted of patients whose sperm counts were $>20x10^6$ sperm/mL (normospermia). Patients with a history of previous operations or comorbid diseases (hypertension, diabetes mellitus, asthma, atopic allergies, and acute or chronic inflammatory diseases) were excluded from this study. All of the patients were suffering from left inguinal pain, particularly during physical activity. Their histories contained no previous operations or comorbid diseases. Their physical examinations and biochemical features were normal. Their genital examinations revealed grade-III left varicocele.

Operations

All of the procedures were performed in 2008 with permission from the ethics committee and conformed to the provisions of the Declaration of Helsinki. The samples were obtained from the patients' left brachial and testicular veins to estimate the serum chitotriosidase levels, which were compared. The brachial serum samples were obtained prior to the operation (before the induction of anesthesia). The operations were performed under general anesthesia. After a 3 cm inguinal incision, the layers were opened, and the varicose veins around the vas deferens were carefully dissected. An incision into the varicose testicular vein was performed, and testicular serum samples were obtained. The vein was then ligated with 3/0 vicryl sutures, and the layers were closed.

Biochemical procedures

Serum chitotriosidase activity was determined using a fluorogenic substrate, as described previously. Briefly, 5 µl of serum were incubated with 100 µl of 22 µmol/L 4-methylumbelliferyl-β-D-N,N',N''-triacetylchitotriose (Sigma M-5639) in McIlvain's phosphate-citrate buffer (pH=5.2) for 1 hour at 37ºC. The reaction was terminated by adding 120 µl of 0.5 mol/L Na_2CO_3-$NaHCO_3$ buffer (pH=10.7), and the fluorescence of 4 methylumbelliferon was measured using a Microfluor 2® fluorimeter (BIO-TEK Synergy^{HT}; excitation, 355 nm; emission, 460 nm). Chitotriosidase activity was expressed as nanomoles of substrate hydrolyzed per ml per hour (nmol/mL/h).

Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) for Windows (version 15.00). The Kolmogorov-Smirnov test was used to evaluate the distribution of the

data. The Wilcoxon signed-rank test and the Mann-Whitney test were used to compare the data. P values <0.05 were considered to be statistically significant.

Results

The results of the study (based on group, including age, sperm count, and brachial and testicular chitotriosidase levels) are presented in Table 1. The brachial and testicular chitotriosidase levels are also presented in Fig. 1-3. All of the operations were successfully performed with no complications. The patients were discharged approximately 24 hours after the operation. There were no statistically significant differences between the mean brachial and testicular chitotriosidase levels in Group 1 (p=0.721) (Fig. 1) and Group 2 (p=0.066) (Fig. 2). The mean brachial chitotriosidase level in Group 1 (oligospermia) was higher than in Group 2 (normospermia), although this difference was not statistically significant (p=0.188) (Fig. 3). The mean testicular chitotriosidase level in Group 1 (oligospermia) was higher than in Group 2 (normospermia), although this difference was also not statistically significant (p=0.220) (Fig. 3).

Discussion

Chitin, a linear polymer of N-acetylglucosamine, is the second most abundant polysaccharide in nature (after cellulose) and is an indispensable structural component of a variety of organisms, including fungi and arthropods.[7] Family-18 chitinases hydrolyze chitin and have been identified in bacteria, fungi, insects, plants, viruses and protozoan parasites.[8] Because chitin is not present in humans, chitinase activity had not been thought to occur in humans. However, recently, it was discovered that humans also have chitinase activity; chitotriosidase is the human analogue of family-18 chitinases, is synthesized in macrophages and is enzymatically active toward natural chitin.[6] The enzyme was named chitotriosidase because of its capacity to hydrolyze the artificial substrate 4-methylumbelliferly-β-chitotrioside. Although this enzyme has been characterized in detail, information regarding its function and the relationship among its isoforms is still limited. The fact that chitotriosidase is able to degrade colloidal chitin and the chitin in the cell wall prompted the hypothesis that chitotriosidase might play a role in the defense against chitinous human pathogens, such as *Candida albicans*.[9] To our knowledge, the basal chitotriosidase activity in a normal healthy adult is 2-90 nmol/hour/mL.[10] Chitotriosidase is markedly increased in

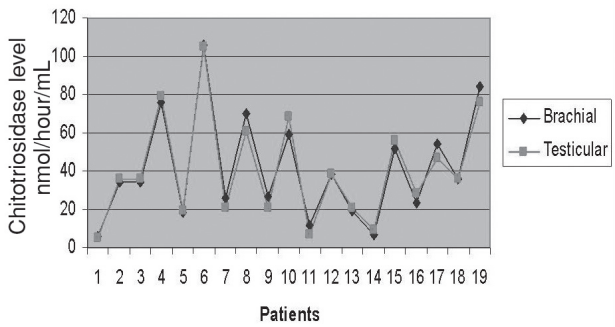

Figure 1 Brachial and testicular serum chitotriosidase levels in oligospermic patients.

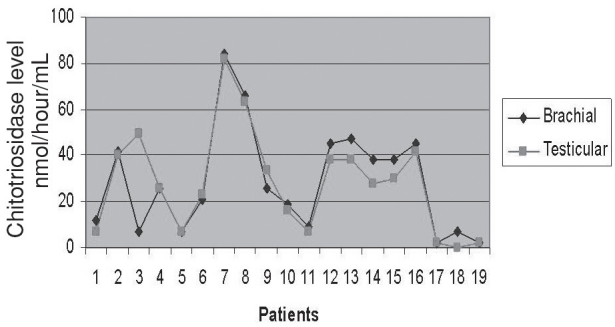

Figure 2 Brachial and testicular serum chitotriosidase levels in normospermic patients.

Table 1. The data of study groups (mean±SD)

	Group 1	Group 2	p value
Age (years)	22.7±3.8	21.8±1.6	-
Sperm count (per mL)	13.3±7.2x10^6	54.8±23.1x10^6	-
Brachial chitotriosidase level (nmol/hour/mL)	41.1±27.8	28.6±22.8	0.188
Testicular chitotriosidase level (nmol/hour/mL)	40.5±27.4	28.1±22.1	0.220

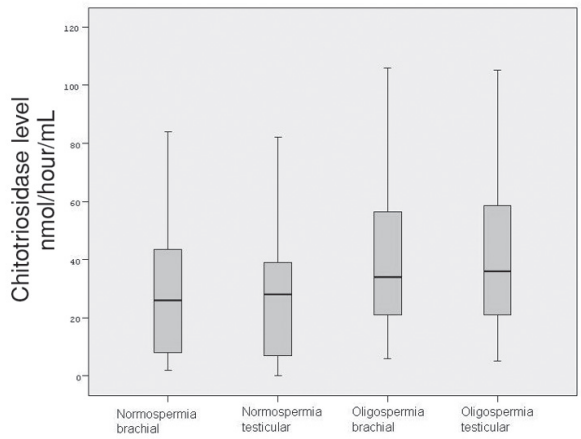

Figure 3 The mean serum testicular and brachial chitotriosidase levels in study groups.

the plasma of patients with Gaucher disease, a rare genetic lysosomal storage disorder that is caused by a mutation in the glucocerebrosidase gene.[11] This is due to the accumulation of abnormal lipid-laden macrophages that massively secrete this enzyme in the various tissues of Gaucher patients. Küçür et al.[12] demonstrated high serum chitotriosidase activity in high Gleason grade prostate carcinoma patients, indicating the possible involvement of macrophages in cancer progression.

In the testis, IL-10 is secreted by macrophages and can significantly downregulate T-cells directly. However, IL-10 can also downregulate T-cells indirectly by suppressing APC (antigen-presenting cell) function.[13] In their study, Veräjänkorva et al.[13] reported the absence of IL-10+ macrophages in mouse testes in which they generated experimental varicoceles. Furthermore, as a conclusion, they stated that the disappearance of IL-10 might decrease local immunosuppression in mouse testes and reflect other changes in testicular macrophage and Leydig cell function under these conditions. In another study, Zhang et al.[14] generated an experimental left varicocele model in rat testes and microscopically identified deformed macrophages in the epididymal lumen.

Over the years, researchers have proposed a number of theories regarding the mechanisms by which varicocele may impair male infertility, including scrotal hyperthermia, hypoxia, retrograde adrenal blood flow, endocrine and testicular paracrine imbalances, and most recently apoptosis.[15] Kılınç et al.[15] found HIF-1α and VEGF expression with angiogenesis in the testes of experimental rat varicocele models, indicating tissue hypoxia in the varicoceles. Furthermore, rat studies have stated that the testis contains a large population of resident macrophages with a novel cytokine secretion profile and an altered response to inflammatory activators.[4,5] With respect to these studies, the higher levels of testicular chitotriosidase in oligospermic varicocele patients compared to normospermic varicocele patients in our study might reflect increased testicular macrophage activity in oligospermic varicocele patients in response to inflammatory activators. However, increased macrophage activity might play a destructive role in the testis and negatively affect spermatogenesis. This is not an exact answer but might provide a clue for posing a new question: Why are some patients with varicocele fertile, with normal semen parameters?

In our study, there were no statistically significant differences between the mean brachial and testicular serum chitotriosidase levels in Group 1 (p=0.721, Fig. 1) and Group 2 (p=0.066, Fig. 2). This result shows that the brachial serum chitotriosidase level should reflect the testicular serum chitotriosidase level.

The mean testicular chitotriosidase level in the oligospermic group was higher than in the normospermic group (Fig. 3). However, this difference was not statistically significant (p=0.220). This statistical insignificance might be due to the small number of patients in each group. This is a weakness of this study.

As a conclusion, our study demonstrates that it is technically feasible to measure chitotriosidase levels in both the brachial and testicular veins. Brachial chitotriosidase levels are representative of testicular chitotriosidase levels. We also demonstrated an increase in testicular serum chitotriosidase levels in oligospermic varicocele patients, although this difference was not statistically significant. A larger sample size may allow us to determine whether there is a significant difference in chitotriosidase levels in oligospermic and normospermic men with varicoceles. We expect that our results might provide new insights into the pathophysiological mechanism of varicocele and related male infertility.

Conflict of interest

No conflict of interest was declared by the authors.

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