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Research Article

Oxidative Stress and Inflammation Markers in Undescended Testes Patients

Semih Lutfi Mirapoglu[1], Aytekin Kaymakci[2], Sumeyye Koc[3,4], Fatih Gultekin[4,5], Eray Metin Guler[4,6]

[1]Department of Pediatric Surgery, Umraniye Training and Research Hospital, University of Health Sciences, İstanbul, Türkiye
[2]Haydarpasa Numune Training and Research Hospital, University of Health Sciences, İstanbul, Türkiye
[3]Department of Medical Biochemistry, Institue of Health Sciences, University of Health Sciences, İstanbul, Türkiye
[4]Department of Medical Biochemistry, Hamidiye Faculty of Medicine, University of Health Sciences, İstanbul, Türkiye
[5]Department of Medical Biochemistry, Faculty of Medicine, Lokman Hekim University, Ankara, Türkiye
[6]Department of Medical Biochemistry, Haydarpasa Numune Health Application and Research Center, University of Health Sciences, İstanbul, Türkiye

Abstract

Aim: Undescended testis is a congenital genitourinary system pathology characterized by the absence of testis in the scrotum. In this disease, the heat stress caused by the testes not being at the optimal temperature can trigger oxidative stress and inflammation. Our study investigated the status of oxidative stress and inflammation markers between patients with undescended testes and healthy infants.

Materials and Methods: Fifty pediatric patients with undescended testes and a control group who applied to Pediatric Surgery Clinic were included in the study. From the blood samples, oxidative stress and inflammation status were examined. Interleukin 1 beta, interleukin 6, and tumor necrosis factor alpha levels of inflammation parameters were measured by the ELISA method using commercial kits. Total oxidant status, total antioxidant status, total thiol, and native thiol levels were measured photometrically with commercial kits. Oxidative stress index and disulfide levels were calculated with a mathematical formula. Oxidative stress and inflammation marker levels of the patient and healthy groups were compared statistically.

Results: Total antioxidant status, total thiol, and native thiol levels were statistically significantly lower in the patient group than the healthy group ($p<0.05$). Total antioxidant status, oxidative stress index, disulfide levels, and interleukin 1β, interleukin 6 levels were also statistically significantly higher in the patient group ($p<0.05$). There was no difference in tumor necrosis factor-α levels between the groups.

Conclusion: In our study, it was observed that oxidative stress and inflammation were higher in patients with undescended testes. Since this situation may lead to systemic diseases in the future, more extensive studies are needed.

Keywords: Undescended testes, Oxidative Stress, Inflammation, Cryptorchidism

INTRODUCTION

Undescended testes (UT) are one of the most common (1-2%) congenital pathologies of the genitourinary system, characterized by the lack of at least one testicle in the scrotum (1). In congenital or acquired UT cases, the risk of developing infertility (2) and testicular cancer (3) is relatively high. It has been reported that the risk of developing infertility is six times higher in those with bilateral UT (4). A diagnosis of UT is found in 5-10% of males with testicular cancer (5).

Studies in experimental animals have shown that UT causes spermatogenic damage by increasing oxidative stress (OS) and inflammation, which in turn causes infertility (6). In cases of UT, the testis is prevented from reaching the optimal temperature. In this situation, an increase in temperature is observed. Elevated temperature can trigger cell death. It has been suggested that excessive death of the gonocytes and spermatogonia is the cause of infertility seen in UT (7). Elevated scrotal temperature can lead to male infertility by impairing spermatogenesis and steroidogenesis. According to research, OS plays a significant role in the pathophysiology of male infertility (8). The increase in scrotal temperature in UT leads to oxidative damage (9). It has also been reported that OS may cause testicular cancer by changing the environment

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Corresponding Author: Sumeyye Koc, Department of Medical Biochemistry, Institue of Health Sciences, University of Health Sciences, İstanbul, Türkiye and Department of Medical Biochemistry, Hamidiye Faculty of Medicine, University of Health Sciences, İstanbul, Türkiye **E-mail**: sumeyyeakin16@gmail.com

of the testis and sperm parameters (10,11).

Total oxidant status (TOS) is commonly used to measure the body's total oxidation state (12). Similarly, total antioxidant status (TAS) is used to assess the body's overall antioxidant level (13). It is challenging to measure directly because of the low half-life and serum concentrations of ROS. Oxidative protein damage causes an increase in protein carbonyl levels and a reduction in protein thiol levels. This decrease is associated with reduced antioxidant levels. Therefore, thiol-disulfide hemostasis is used as a novel method for evaluating OS. Cytokines (e.g., interleukin 1 beta, Tumor Necrosis Factor-alpha) are biomolecules that mediate inflammatory responses, provide intercellular communication, and play an essential role in reproductive physiology. It is released in inflammatory cells in response to infection and has local and systemic effects (14).

UT can affect male infertility and the risk of developing cancer by increasing OS and inflammation. TAS, TOS, disulfide (DS) homeostasis, interleukin 1 beta (IL1ß), and Tumor Necrosis Factor-alpha (TNF-α) levels, which are OS and inflammatory indicators, have not been studied in patients with UT. This study evaluated the OS and inflammation parameters biochemically in children with UT.

MATERIAL AND METHOD

Study Population

The study was initiated after approval of the Ethics Committee (No: 21/564). Patients with a diagnosis of UT who underwent elective undescended testis operation in the pediatric surgery operating room were included after obtaining written informed consent from their parents. Cryptorchid patients with high scrotal and inguinal canal localization were included. It was calculated that there should be at least 50 volunteers in each group, to achieve 80% power at the α=0.05 significance level by power analysis of the groups. The case group consisted of patients aged 12-24 months, whereas the control group included on healthy infants with the same demographic characteristics. The study did not include patients with neurological, neuromuscular, convulsive, and bleeding coagulation disorders, opioid and local anesthetic drug allergy, and additional pathology.

Approximately 3mL of blood was taken into BD Vacutainer® blood collection tubes in the study. After the routinely requested blood was studied, the remaining inert blood was studied. The blood samples were centrifuged at 3000x g for 10 minutes.

Biochemical Analyses

Oxidative Stress Analyses

Serum samples were analyzed spectrophotometrically in the multi-plate reader for OS parameters. TAS and TOS in serum samples were measured using commercially available kits. According to the standard curve, the serum TAS levels were presented as mmol ascorbic acid Eq/mL, and the tissue TOS levels were computed as μmol H_2O_2 Eq/mL protein. Oxidative stress index (OSI) was calculated as TOS/TAS (15).

Thiol-Disulfide Homeostasis

As new OS biomarkers, serum and disulfide are tested. NaBH4 converted dynamic disulfide links (−S−S−) in the blood sample to NT groups (−SH). Commercial kits were used to assess serum total thiol (TT) and NT levels (Rel Assay, Gaziantep, Turkey). A spectrophotometer was used to measure biomarkers. The molar extinction coefficient of 14.100 mol/L-1 cm-1 of 5-thio-2-nitrobenzoic acid (TNB) calculated total and free thiol levels. The disulfide level (DS) was calculated as μmol/L using the formula (total thiol-free thiol)/2.

Inflammation Biomarkers

IL1β, IL6, and TNFα cytokines, which are inflammation biomarkers, were investigated in serum samples. Cytokines levels were measured spectrophotometrically with ELISA kits in a multi-plate reader.

Statistical Analysis

The IBM SPSS 25.0 version application was used to perform statistical analyses. The data distribution was determined using the Kolmogorov-Smirnov test. Mean ± standard deviation values were used to express continuous variables. When the variable was normally distributed, the independent sample t-test was performed to compare variables between the two groups. The confidence intervals (95%) were used to show the differences between groups. Statistical significance was defined as $p<0.05$.

RESULTS

The mean age of all participants was 17.48 ± 3.69 months. In the classification of undescended testicles, 40 patients had bilateral and 10 unilateral undescended testes. OS and thiol-disulfide hemostasis parameters were compared between infants with UT and the control group (Table 1). TOS and OSI values in the undescended testes group were higher, but TAS was lower. It was found that TT and NT values were lower in patients with UT, but DS levels were higher.

Table 1. Oxidative stress biomarkers between UT and controls

Oxidative Stress Biomarkers	UT (mean±SD)	Control (mean±SD)	p[a]
TAS (mmol Ascorbic Acid Eq/L)	1.02±0.07	1.23±0.06	0.001*
TOS (μmol H2O2 Eq/L)	11.1±2.2	8.4±0.8	0.006*
OSI (AU)	10.9±2.7	6.8±1.1	0.001*
TT (μmol/L)	475±126	545±95	0.008*
NT (μmol/L)	300±100	455±75	0.001*
DS (μmol/L)	87.5±2.2	45.0±0.8	0.001*

TAS: Total Antioxidant Status, TOS: Total Oxidant Status, OSI: Oxidative Stress Index, TT: Total Thiol, NT: Native Thiol, DS: Disulphide, a: Independent sample t-test, *: p<0.05, SD: Standard deviation

The difference between the levels of inflammation parameters IL1ß, IL6, and TNFα between the two groups was compared (Figure 1). IL1ß (p=0.001) (Figure 1A) and IL-6 (p=0.031) (Figure 1B) levels were found to be significantly higher in patients with UT compared to controls. Although the TNFα level (Figure 1C) was higher in the UT group, this difference was not statistically significant (p>0.05).

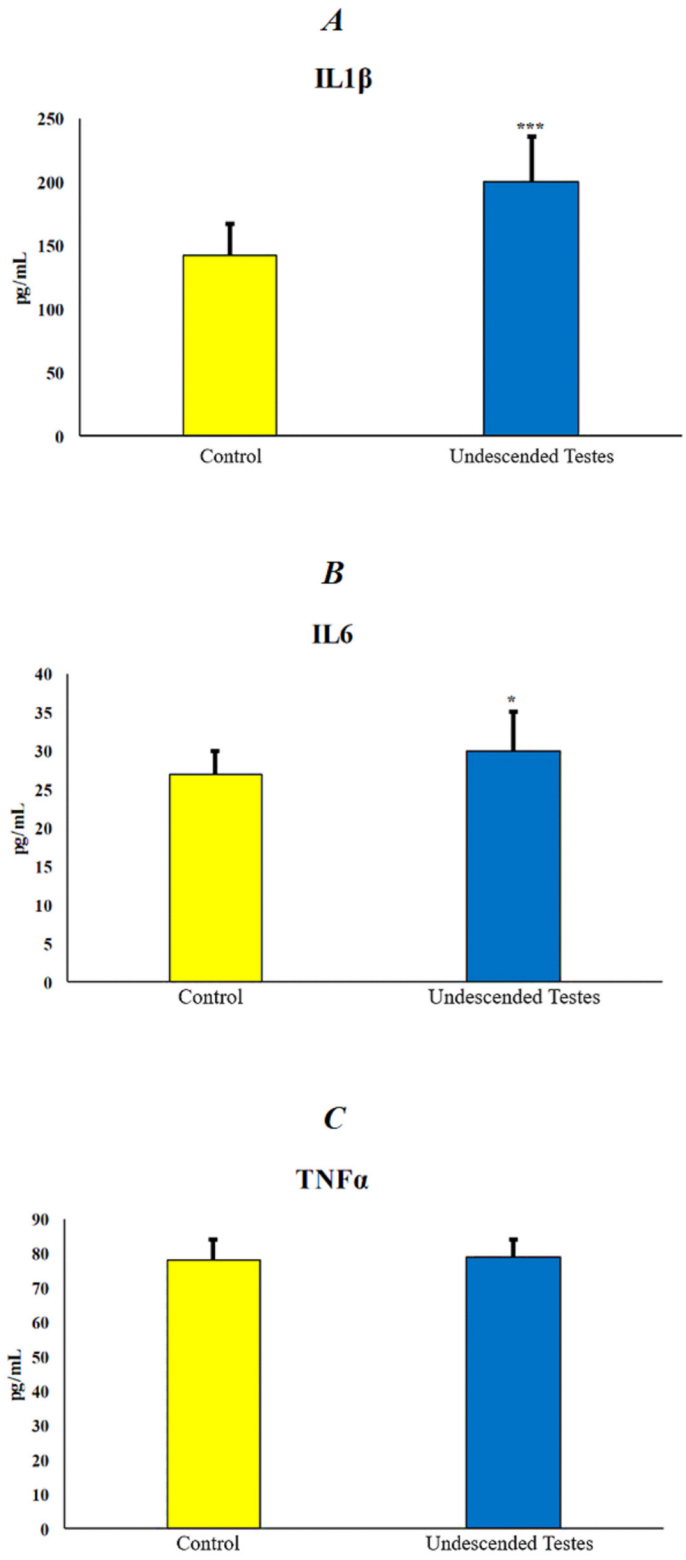

Figure 1. IL1ß, IL6, and TNFα levels between undescended testes and the control group. *: p<0.5, **: p<0.01, ***: p<0.001; Independent Sample t-test

DISCUSSION

Undescended testes are one of the congenital genitourinary system diseases seen in 1-2% of boys worldwide (1). Increased OS and inflammation may occur in UT because the testis is not at optimal temperature (9). In our study, it was observed that OS and inflammation were higher in patients with UT.

The testis should be at a lower temperature than body temperature. In cases of UT, the testis does not reach the scrotum. In UT, heat stress occurs because the testis cannot reach the optimal temperature. Heat stress can contribute to elevated intracellular reactive oxygen species (ROS) levels, creating a signal that triggers apoptosis (16). The imbalance between ROS generation and elimination by current antioxidant mechanisms causes OS to develop. Temperature increase in the testis in UT cases is associated with OS (17). Li et al., in their study (18), examined ROS production and gene expression in adult mice with a UT model. Increased germ cell death was identified in the research, as well as alterations in the expression of genes linked to redox reactions, stress response, energy, and lipid metabolism.

TAS, TT, and NT levels were low in infants with UT in our research, whereas TOS, OSI, and DS levels were high. In parallel with our findings, Imamoğlu et al., In their case-control study, found that IL-6 and malondialdehyde (MDA) levels were higher in blood samples from patients with UT compared to the control (8). These findings show that OS and inflammatory responses are increased in patients with UT. Similarly, MDA levels were greater in individuals with UT in a study of 59 cases and 30 controls (19). Avci et al. planned on 30 patients with UT and 40 healthy controls, they found high levels of lipid peroxidation, oxidative DNA damage, ischemia-modified albumin, and nicotinamide adenine dinucleotide phosphate oxidase 4, which are parameters associated with OS (20).

OS, which occurs when ROS concentrations exceed physiological needs, may cause infertility. OS induced by increased scrotal temperature in UT cases may lead to deterioration of spermatogenesis and steroidogenesis at later ages (7). In addition, OS can negatively affect the structural and functional integrity of sperm. It damages the proteins and lipids in the plasma membrane of the sperm cell, and as a result, the DNA integrity is impaired. Thus, OS can affect the cell membrane fluidity and permeability, leading to disruptive effects on sperm function (21). These damages to spermatogenesis, steroidogenesis, and sperm cells can cause male infertility. Indeed, it is known that OS is associated with many conditions related to male infertility. OS can affect male infertility by causing changes in testicular blood flow, endocrine pathways, and germ cell apoptosis (17). It involves the plasma membrane integrity and causes premature capacitation. These conditions may inhibit the fertilization of spermatozoa (22).

ROS also initiates inflammation by inducing the activation of transcription factors and pro-inflammatory genes (23).

In our study, IL-1β and IL-6 cytokine levels, which are inflammation parameters, were found to be high. IL-6 is a cytokine that regulates temperature during inflammation (22). Koçak et al. In their study, it was observed that IL-6 levels in the seminal plasma of infertile men were higher than those of fertile ones (24). It is reported that IL-1β induces apoptosis in semen and decreases sperm motility (25). Inflammation can reduce semen quality by causing impaired gland functions, inhibiting sperm transport, and affecting spermatogenesis (25). Therefore, inflammation in UT may also affect infertility at later ages.

CONCLUSION

OS and inflammation are more prevalent in people diagnosed with UT, according to the findings of this study. This situation poses a risk in terms of infertility in older ages. It may also pave the way to forming other systemic disorders in the long term. For this reason, children diagnosed and treated with UT should be followed at later ages in terms of possible risks.

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