Hipogonad Hastalarda Lipid Profili ve Oksidatif Stres Markerlarının Tedavi ile Değişimi

Change of Lipid Profile and Oxidative Stress Markers with Treatment in Hypogonad Patients

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ÖZET

Amaç: İdiyopatik Hipogonadotropik Hipogonadizm (İHH) hastalarında kardiyovasküler riski artıran oksidatif stres ve dislipidemi varlığını ve gonadotropin replasman tedavisinin bu parametreler üzerindeki etkisini araştırmak.

Materyal ve Metod: Çalışmamız faz IV, randomize olmayan, vaka kontrollü bir çalışmaydı. Çalışmaya, İHH tanılı erkek hastalar (n=30) ile benzer yaş ve vücut kitle indeksine (VKİ) sahip sağlıklı kontrol grubu (n=20) dahil edildi. Oksidatif stres belirteçleri, lipidler ve apolipoprotein seviyeleri, gonadotropin tedavisinden önce ve sonra ölçüldü ve sağlıklı kontrollerle karşılaştırıldı.

Bulgular: Tedavi sonrası yüksek dansiteli lipoprotein (HDL), düşük dansiteli lipoprotein (LDL), ileri oksidasyon protein ürünleri (AOPP), malondialdehit (MDA) ve nitrik oksit (NO) seviyelerinde dramatik bir düşüş görülürken tiyol seviyesinde önemli bir artış tespit edildi ($p<0.05$).

Sonuç: Elde edilen veriler hipogonadizm olgularında dislipidemi varlığına ve oksidatif stres artışına işaret etmiştir ve gonadotropin tedavisinin bu hastaların oksidatif stresini azalttığını ve lipid profilini olumlu yönde etkilediğini göstermiştir.

Anahtar Kelimeler: Hipogonadizm, oksidatif stres, gonadotropin

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ABSTRACT

Aim: To investigate the presence of oxidative stress and dyslipidemia, which increase cardiovascular risk, and the effect of gonadotropin replacement therapy on these parameters in patients with Idiopathic Hypogonadotropic Hypogonadism (IHH).

Material and Methods: This was a phase IV, non-randomized, case-controlled study. Male patients diagnosed with IHH (n=30), and a control group of healthy participants with similar age and body mass index (BMI) (n=20) were included. Oxidative stress markers, lipids and apolipoprotein levels were measured before and after gonadotropin treatment and compared with healthy controls.

Results: After treatment, a dramatic decrease was observed in high-density lipoprotein (HDL), low-density lipoprotein (LDL), advanced oxidation protein products (AOPP), malondialdehyde (MDA) and nitric oxide (NO) levels, while a significant increase in thiol level was detected ($p<0.05$).

Conclusion: The data obtained indicated the presence of dyslipidemia and an increase in oxidative stress in hypogonadism cases, and showed that gonadotropin treatment reduced the oxidative stress of patients and positively affected their lipid profile.

Keywords: Hypogonadism, oxidative stress, gonadotropin

INTRODUCTION

Within living organisms, the production and extinguishment speed of free oxygen radicals (FOR) are proportioned and this regulated state is called oxidative balance. Excess production of radicals or a decrease in their extinguishment speed can cause this balance to be disturbed, resulting in a state called oxidative stress. It is well understood that oxidative stress leads to many pathological conditions, such as atherosclerosis, chronic renal failure, diabetes, respiratory distress syndrome, rheumatoid arthritis, sepsis and Alzheimer's disease by causing tissue damage in the body (1, 2).

Hypogonadism is a disease characterized by a decreasing number of spermatozoa and/or a defect in one or more levels of the hypothalamo-pituitary-testicular axis, causing a condition in which the testis cannot synthesize testosterone physiologically. If the disease arises due to a defect at the hypothalamic or pituitary level, it is called Hypogonadotropic Hypogonadism (HH). Previous literature supports the notion that oxidative stress plays a key role in male infertility and the pathogenesis of hypogonadism (3-8). These studies concentrated on the investigation oxidative stress markers in semen samples. In addition to enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase, the existence of non-enzymatic antioxidants including ascorbate, urate, α-tocoferole, pyruvate, glutathione, and taurine were demonstrated in the semen sample (9). Although idiopathic infertile males have significantly higher seminal FOR levels and a lower antioxidant capacity than those of the healthy control group, the underlying pathophysiological mechanism is not being understood clearly. Therefore, it is claimed that the existence of oxidative stress may be one of the source of the conditions observed in infertile males that could not be explained before (3). A limited number of studies show a decrease in oxidative stress with gonadotropin therapy (5).

In numerous studies, an important relationship has been suggested between hypogonadism and central obesity, insulin resistance, dyslipidemia and the risk of cardiovascular disease. Due to the favorable effects of testosterone therapy on insulin resistance and lipid profiles, it has been specified in many studies that such therapy has a preventive potential on the development of cardiovascular disease (10-13). On the other hand, the number of studies indicating the effects of gonadotropin replacement therapy on insulin resistance

and cardiovascular risk factors of the patients diagnosed with HH is limited (5).

Accordingly, in this study we aimed to evaluate the effect of gonadotropin replacement on oxidative stress and lipid parameters in patients diagnosed with IHH by measuring such parameters in serum/plasma samples.

METHODS

Study design and patient selection

Thirty male participants (mean age: 29.3 ± 8.0 years) were recruited from the Endocrinology and Metabolic Diseases outpatient clinic of Medical Faculty of Erciyes University between May 2010 and December 2012. Diagnoses of IHH were confirmed through the physical examinations and laboratory tests. Twenty healthy controls (mean age: 29.4 ± 3.8 years) were also included in the observational, case-control study.

Inclusion criteria for patients included undeveloped secondary sex characteristics, low levels of total testosterone (t-Tes) and free testosterone (f-Tes), low/normal levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and a normal pituitary MRI. The patients were informed on study procedures that would be conducted. Those who agreed to participate in the study were included after their signed consent documents were obtained in accordance with the Helsinki Declaration and the local research ethics committee approved the protocol (Erciyes University Clinical Research Ethics Committee Date: 16/09/2010 Resolution Number: 2010 / 107).

Any patients with additional concurrent hormonal or systemic diseases who received or who received related therapy within the last 6 months were excluded from the study.

Anthropometric measurements and body composition analysis

The same physician made all measurements. BMI was measured via the formulation of weight/height^2 (kg/m^2). Waist (W) circumference was measured at the mid-level between the 10th rib and the iliac crest. Hip (H) circumference was measured at the level of the great trochanters, with the legs closed. W/H ratio (WHR) was found by proportioning waist circumference (cm) to hip circumference (cm).

Treatment and follow-up

At our facility, gonadotropin therapy is the standard treatment for the patients who have hpogonadotrophic hypogonadism and desire fertility. For this reason, the patients were subjected to gonadotropin replacement therapy. Firstly, 1500 IU of human chorionic gonadotropin (hCG) (Pregnyl ® Organon hCG 1500 IU) therapy was administered three days per week for 12 months. Afterward, 75 IU of human menopausal gonadotropin (Merional ® ARIS 75 IU) was administered 3 days per week along with hCG for 6 months. Except for routine therapies, no additional therapy was applied to the patient group. The patients returned every 12 weeks to assess the effectiveness of the therapy, to evaluate side effects, and to determine if they should continue therapy.

Biochemical analyses

Blood samples of the patients were collected at two time-points; once before starting the therapy, and once after completing therapy. Blood samples of the control group were taken only once. 6 patients who were initially included in the study did not complete the therapy, so the post-treatment blood samples of these patients were not collected. Venous blood samples were drawn between 08:00 and 09:00 a.m. after 12-h fasting periods. Blood samples were collected in accordance with the biochemical measurements by using anticoagulant and plain tubes. The tubes were centrifuged for 10 minutes at 4° C for 4000 cycle/minute. Separated plasma and serum samples were put in aliquots and stored in Eppendorf tubes after being frozen at -70° C.

TG, TC, HDL and LDL measurements were analyzed using Sentinel-branded commercial kits in an Architect C8000-branded auto-analyzer. Siemens-branded commercial kits were used for FSH, LH, and Estradiol (E_2) measurements in a Siemens ADVIA Centaur XP-branded auto-analyzer. Sex hormone binding globulin (SHBG) and Insulin like growth factor (IGF-1) levels were measured through immunoradiometric assay (IRMA) method using Immunutech-branded commercial kit; t-Tes levels and f-Tes levels were measured through radioimmunoassay (RIA) method using Biosourche-branded and DSL-branded commercial kits, respectively.

Plasma AOPP levels were determined by means of spectrophotometric method developed by Witko-Sarsat et al (14); serum thiol levels were detected through the method modified by Hu et al (15); serum NO levels, serum MDA levels, serum 8-hydroxydeoxyguanosine (8-OHdG) levels, and serum Apolipoprotein A1 (ApoA1) and Apolipoprotein B100 (ApoB100) levels were measured using Cayman-branded commercial kit (Catalog No. 780001), Immundiagnostik-branded commercial kit (Catalog No. KC1900) in an HPLC device, JaIca-branded commercial kit (Catalog No. KOG-HS10) through competitive ELISA method and USCN-branded commercial kit (Catalog No. E90519Hu) through sandwich-type ELISA method, respectively.

Statistical analysis

All data were recorded on a computer database and statistical analyses were performed with SPSS software (version 15.0, SPSS Inc., Chicago, IL, USA). Kolmogorov–Smirnov test was used to determine the distribution characteristics of variables. Results are expressed as mean ± S.D or median (25%-75%) Within-group changes at two time points were analyzed by paired samples t-test or Wilcoxon signed-rank test as appropriate. Inter-group differences were analyzed by Student's t-test and Mann–Whitney U as appropriate. Pearson and Spearman correlation coefficients were used to test inter-variable relationships as appropriate. Differences were considered significant at $p < 0.05$.

RESULTS

Baseline characteristics

The demographic and biochemical features of patients (n=30) and healthy controls (n=20) are displayed in Table 1. The two groups were similar with regard to age, height, WHR and BMI. t-Tes, f-Tes, FSH, LH, E_2, HDL, ApoA1, AOPP and thiol levels were lower, whereas serum total cholesterol (TC), LDL, ApoB100, AOPP, MDA and NO levels were significantly higher in patients with IHH ($p < 0.05$, for all). 8-OHdG, TG, IGF-1 and SHBG levels were similar between the two groups ($p > 0.05$).

Baseline versus post-treatment demographics and biochemical parameters of patients

WHR, SHBG, HDL, LDL, AOPP, MDA and NO levels decreased, whereas t-Tes, f-Tes, E_2, IGF-1, and thiol levels increased significantly after gonadotrophin treatment ($p < 0.05$, for all) (Table 1). No significant changes in BMI, TC, TG, ApoA1, ApoB100, and 8-OHdG levels were observed in the patient group after gonadotropin treatment.

Simple correlation analysis

There were significant positive correlations of t-Tes and f-Tes with thiol, HDL and ApoA1 ($p < 0.05$, for all). Serum t-Tes and f-Tes levels were negatively correlated with AOPP, MDA, NO, TC, LDL and ApoB100 ($p < 0.05$, for all) (Table 2). There were no correlations of t-Tes and f-Tes with TG and 8-oHDG levels ($p > 0.05$, for all).

Table 1. Anthropometric and biochemical parameters of patients (before/after treatment) and control group

Parameters	Controls (n=20)	Patients		p1	p2
		Pretreatment (n=30)	Posttreatment (n=24)		
Age (year)	29.4±3.8	29.3±8.0		0.979	
Height (cm)	1.77 ± 0.03	1.79±0.1	1.80± 0.1	0.320	0.130
BMI (kg/m2)	24.74 ± 1.34	26.2±5.4	26.60 ± 3.88	0.243	0.061
WHR	0.89 ± 0.04	0.89±0.09	0.86 ± 0.06	0.271	**0.002**
Total Testosteron (ng/dL)	530.50 (463-556)	41.0 (4-225)	498.50 (400-679)	**0.0001**	**0.001**
Free Testosteron (pg/mL)	21.02 (19.57-23.85)	1.75(0.1-9.0)	17.87 ± 11.59	**0.0001**	**0.001**
E2 (pg/mL)	30.75 ± 4.25	17.3±10.4	46.07 ± 31.35	**0.0001**	**0.001**
SHBG (nmol/L)	43.80 ± 8.92	40.9±19.1	22.50 (18.50-35.50)	0.303	**0.001**
IGF-1 (ng/mL)	249.50 (212-280)	198.0 (46-761)	378.45 ± 207.59	0.172	**0.001**
TC (mg/dL)	137.70 ± 9.90	184.5±39.1	156.29 ± 35.56	**0.0001**	0.977
TG (mg/dL)	75.95 ± 21.27	116.3±102.7	84.50 (70.50-125.50)	0.091	0.099
HDL (mg/dL)	54.65 ± 7.38	45.47±9.8	40.95 ± 8.51	**0.003**	**0.004**
LDL (mg/dL)	67.86 ± 12.28	112.5±30.6	91.87 ± 27.62	**0.0001**	**0.001**
ApoA1 (mg/dL)	159.08±13.87	97.2±16.9	108.07 ± 29.67	**0.0001**	0.164
ApoB100 (mg/dL)	92.25±26.55	168.0±56.7	138.85 ± 64.69	**0.0001**	0.355
AOPP (μmol/L)	133.70 ± 42.73	272.67 ± 111.79	132.92 ± 40.39	**0.001**	**0.001**
Thiol (μmol/L)	291.38 ± 39.83	210.03 ± 47.43	290.27 ± 71.63	**0.001**	**0.001**
MDA (μmol/L)	1.71(1.55-1.71)	2.79(2.17-4.76)	1.86(1.75-2.09)	**0.001**	**0.001**
NO (μmol/L)	17.05(13.75-19.99)	20.28(13.89-25.13)	15.32(12.51-19.45)	**0.034**	**0.001**
8-OHdG (ng/mL)	0.17 (0.15-0.19)	0.18 (0.16-0.21)	0.17 ± 0.03	0.661	0.398

E_2: Estradiol, SHBG: Sex hormone binding globulin, IGF-1: Insulin like growth factor, TC: Total cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein, Apo: Apolipoprotein, AOPP: Advanced oxidation protein products MDA: Malondialdehyde, NO: Nitric oxide, 8-OHdG: 8-hydroxydeoxyguanosine
p1: Controls versus pretreatment, p2: Pretreatment versus posttreatment

Table 2. Biochemical Correlates of total testosterone and free testosterone of all study group

Variable	Total Testosterone		Free Testosterone	
	r	*p value*	r	*p value*
AOPP (μmol/L)	-0.583	**0.0001**	-0.566	**0.0001**
Thiol (μmol/L)	0.673	**0.0001**	0.680	**0.0001**
MDA (μmol/L)	-0.572	**0.0001**	-0.556	**0.0001**
NO (μmol/L)	-0.288	**0.047**	-0.331	**0.022**
TC (mg/dL)	-0.554	**0.0001**	-0.546	**0.0001**
HDL-C (mg/dL)	0.420	**0.002**	0.443	**0.001**
LDL-C (mg/dL)	-0.610	**0.0001**	-0.613	**0.0001**
ApoA1 (mg/dL)	0.883	**0.0001**	0.900	**0.0001**
ApoB100 (mg/dL)	-0.620	**0.0001**	-0.617	**0.0001**

AOPP: Advanced oxidation protein products MDA: Malondialdehyde, NO: Nitric oxide, TC: Total cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein Apo: Apolipoprotein

DISCUSSION

After the treatment, there was no statistical difference in BMI, while the waist-hip ratio decreased. In the literature, there are studies showing that testosterone therapy affects the lipid metabolism, body composition and many other cardiovascular and metabolic parameters positively (16-18). Bhasin et al. reported that body fat mass of young male patients with hypogonadism who were administered testosterone replacement therapy decreased and their body muscle mass increased (19). Furthermore, Naharci et al. and Kapoor et al. showed in their studies that after testosterone replacement therapy, weight, BMI and body muscle index of male patients with hypogonadism increased and their body fat mass and waist-hip ratio decreased statistically (17, 20). We think that the results are different from other studies since different therapy techniques were applied. Unlike other studies, gonadotropin replacement therapy was administered instead of testosterone therapy in our study.

Gonadotropin replacement therapy was applied in our study and after the therapy TC, HDL and LDL levels decreased significantly and TG levels decreased insignificantly. In the literature, it is asserted that low testosterone levels of the patients with hypogonadism are associated with their atherogenic lipid profiles. Such patients are reported to have increasing levels of TC, LDL, TG and decreasing HDL levels (21). Similar results were obtained in our study as well. It is known that the disrupted lipid profiles of patients with hypogonadism are related to body fat distribution and insulin resistance. Tan et al. found that of TG, LDL, and HDL levels were decreased significantly after testosterone therapy, but TC levels did not change (22). Kapoor et al. and Malkin et al. observed a statistically significant decrease in TC levels of male IHH patients who received testosterone replacement therapy, but they did not find any statistical difference in LDL and TG levels (17, 23). Testosterone replacement raises insulin sensitivity by reducing abdominal fat tissue and it affects the lipid profile positively (22-24). In our study the changes of lipid profile that was observed after the therapy was due to effects of testosterone increasing with gonadotropin replacement.

We found in our study that the levels of ApoA1 and ApoB100 of IHH patients were significantly low and significantly high respectively compared to control group. Such data indicate the increased cardiovascular risk of patients with hypogonadism. There are numerous studies suggesting that ApoA1 and ApoB100 are strong descriptors of atherosclerosis and coronary artery disease (25, 26). Serum ApoB100 level shows a strong correlation with the atherogenic lipoprotein profile. On the other hand, ApoA1, which exists in primary HDL structure, reflects the potential of cardio-protective lipoprotein (25). Tan et al. measured ApoA1 and total ApoB levels of patients with hypogonadism after testosterone enanthate therapy. There was a significant decrease in ApoA1 levels while there was no significant difference in total ApoB levels (22). Although it is not statistically significant, we discovered an increase in ApoA1 levels in our study in which we administrated gonadotropin replacement therapy, unlike previous studies. We also measured ApoB100 levels instead of total ApoB, and found that there was a decrease in ApoB100 levels, although it was not significance. If the sample size were larger, such changes would likely be significant. These results give us clues that, increased cardiovascular risk in patients with hypognadism may decrease depending on the improving lipid levels after gonadotropin therapy.

In our study, we measured AOPP levels and detected a dramatic increase in AOPP levels in IHH patients. After gonadotropin replacement therapy there was a significant decrease in AOPP levels. Although there are many studies indicating the existence of the increased oxidative stress in seminal plasma of IHH patients (3, 6), the number of studies

evaluating the oxidative stress in the serum/plasma of IHH patients is limited. Unluhizarci et al found that after gonadotropin replacement therapy there was a significant decrease in AOPP levels (5). In this study, plasma AOPP levels of the patients with hypogonadism were almost two times more than the normal values and this makes us consider the existence of serious protein oxidation. The decrease in AOPP levels after the therapy is an important finding showing that gonadotropin replacement therapy prevents the emergence of protein oxidation.

In our study we observed a dramatic decrease in thiol levels of IHH patients and a significant increase after the therapy. Free thiol, which is found locally as cysteine residues in the albumin structure, is an important antioxidant in plasma (27). Himmelfarb et al. named the loss of thiol that develops secondary to oxidative stress as "thiol stress" (28). Oxidation of thiol group may be the indicator of protein oxidation. Determining thiol stress may reflect both the loss of antioxidant strength and the degree of protein oxidation (29). Unluhizarci et al. found that thiol levels in control group were significantly higher than IHH patients. They found that thiol levels increased after gonadal replacement therapy (5).

In light of these findings, we can say there is a weak antioxidant defense system in patients with hypogonadism, but thiol levels become normal and oxidative stress decreases after gonadotropin replacement therapy.

In our study, serum MDA levels of IHH patients were statistically significant than control group, but these levels showed a significant decrease after the therapy. MDA levels have a good correlation with the degree of lipid peroxidation (30). There are some studies investigating MDA levels in patients with hypogonadism. Haymana et al found that MDA levels were statistically significant in male patients with hypogonadism (31). High levels of MDA show the existence of increased oxidative stress in patients with hypogonadism and decreased MDA levels after the therapy reveals that gonadotropin replacement therapy inhibits lipid peroxidation.

We detected in our study that NO levels, which were high before the therapy, decreased significantly after gonadotropin replacement therapy. NO is a free radical, regulating numerous functions of male reproduction system. If NO level is above the physiological levels, it has negative effects on the functions of sperm and testis by affecting steroid production (32). In hypogonadism, it is thought that reactive nitrogen species (RNS) are as responsible as reactive oxygen species (ROS) (32). The increasing studies on NO revealed that this molecule has a wide range of important functions in many systems of the organism. Some of these functions include prevention of platelet aggregation, vasodilatation and the inhibition of vessel-smooth muscle proliferation. Moreover, NO should be at physiological levels for normal endothelial function (32-35). Although NO is a cardio-protective agent, its effect emerges only in the physiological concentrations. In contrast, it is characterized with being a free radical because of its unpaired electron in the last orbit. As a result of the excess superoxide ion, NO reacts with the superoxide ion and produces peroxynitrite. Thus, the physiological effect of NO is inhibited and a much more potent oxidant material emerges. Therefore, both the lowness and highness of NO create a pathological situation. Our study suggested that IHH patients are exposed to oxidative stress due to high NO levels, and they benefit from gonadotropin therapy in this sense.

In our study the 8-OHdG levels of the patient group were not significantly different in comparison with the healthy controls' and did not significantly change after the therapy. The measurement of 8-OHdG is accepted as the direct indicator of oxidative destruction in DNA (36). 8-OHdG is the base destruction product of hydroxyl radical in DNA and it causes mutations. Yasuda et al. studied the

serum 8-OHdG levels of 128 patients with hypogonadism (37). In this study serum 8-OHdG levels of patients with hypogonadism were significantly higher than normal patients. It was asserted that serum 8-OHdG levels can be used to show the oxidative stress in patients with hypogonadism. In the literature, among 8-OHdG measurement methods, HPLC method was reported to have the highest specificity and sensitivity. In our study, because of the technical difficulties, ELISA method was used instead of HPLC method. Since this method is not sufficiently sensitive, it may be the reason for our insignificant results.

This study had several limitations. Our first limitation was our small sample size, due to strict eligibility criteria. Retention rate was another limitation as 6 patients did not take their treatment at regular intervals and could not be included in the analysis. Another limitation is that we couldn't show the direct relationship between oxidative stress/lipid parameters and coronary artery disease. If we could follow up the patients for a longer time it might be possible to show this relationship. In addition, antioxidant status should have been evaluated while planning the study.

CONCLUSION

Consequently, the data that we obtained show the existence of dyslipidemia and increased oxidative stress in IHH cases and gonadotropin replacement therapy contributes to these parameters positively. The data also provides important clues that gonadotropin replacement therapy may be effective in reducing cardiovascular risks in the long term. Since the number of studies on this subject is insufficient, we think that it shall contribute to the literature considerably. In our opinion, further studies are required in which the benefits of the existing changes are shown in the same patient group. Additionally, more patients should participate and longer therapies should be administered.

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REFERENCES

1. Alkadi H. A Review on Free Radicals and Antioxidants. Infect Disord Drug Targets. 2020;20(1):16-26
2. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006;160 (1):1-40.
3. Mannucci A, Argento FR, Fini E, Coccia ME, Taddei N, Becatti M, et al. The Impact of Oxidative Stress in Male Infertility. Front Mol Biosci. 2022; 8:799294.
4. Leisegang K, Roychoudhury S, Slama P, Finelli R. The Mechanisms and Management of Age-Related Oxidative Stress in Male Hypogonadism Associated with Non-communicable Chronic Disease. Antioxidants. 2021;18(11):1834.
5. Unluhizarci K, Sık SK, Keti DB, Kose K, Hacıoglu A, Karaca Z. Treatment of male hypogonadism partially reverses oxidative stress in patients with hypogonadism. Endocr J. 2020;67(9):935-940
6. Mancini A, Leone E, Festa R, Grande G, Silvestrini A, de Marinis L, et al. Effects of testosterone on antioxidant systems in male secondary hypogonadism. J Androl. 2008;29(6):622-9.
7. Cicek OSY, Kaya G, Alyuruk B, Doger E, Girisen T, Filiz S. The association of seminal oxidation reduction potential with sperm parameters in patients with unexplained and male factor ınfertility. Int Braz J Urol. 2021;47(1):112-119.
8. Matsumoto AM, Bremner WJ. Testicular disorders. In: Melmed S, Polansky KS, Larsen PR, Kronenberg HM, eds. Williams Textbook of Endocrinology. 13th ed. New York, NY: Elsevier; 2016:688–777.
9. Aitken RJ, Baker MA. Oxidative stress, sperm survival and fertility control. Mol Cell Endocrinol. 2006;250 (1-2):66-9.
10. Rastrelli G, Filippi S, Sforza A, Maggi M, Corona G. Metabolic Syndrome in Male Hypogonadism. Front Horm Res. 2018; 49:131-155.
11. Kloner RA. Testosterone and Cardiovascular Health: Safety of Treatment of Hypogonadism. Sex Med Rev. 2015;3(1):56-62.

12. Doulamis IP, Tzani A, Konstantopoulos P, Daskalopoulou A, Spinos T, Bletsa E, et al. Experimental hypogonadism: insulin resistance, biochemical changes and effect of testosterone substitution. J Basic Clin Physiol Pharmacol. 2019;4;30(3).

13. Anawalt BD, Yeap BB. Conclusions about testosterone therapy and cardiovascular risk. Asian J Androl. 2018;20(2):152-153.

14. Witko-Sarsat V, Friedlander M, Nguyen Khoa T, Capeillere-Blandin C, Nguyen AT, Canteloup S, et al. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. J Immunol . 1998;161(5):2524-32.

15. Hu ML, Louie S, Cross CE, Motchnik P, Halliwell B. Antioxidant protection against hypochlorous acid in human plasma. J Lab Clin Med. 1993;121 (2):257-62.

16. Kim SH, Park JJ, Kim KH, Yang HJ, Kim DS, Lee CH, et al. Efficacy of testosterone replacement therapy for treating metabolic disturbances in late-onset hypogonadism: a systematic review and meta-analysis. Int Urol Nephrol. 2021;53(9):1733-1746.

17. Kapoor D, Goodwin E, Channer KS, Jones TH. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolemia in hypogonadal men with type 2 diabetes. Eur J Endocrinol. 2006;154 (6):899-906.

18. Cai X, Tian Y, Wu T, Cao CX, Li H, Wang KJ. Metabolic effects of testosterone replacement therapy on hypogonadal men with type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. Asian J Androl. 2014;16 (1):146-52.

19. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. J Endocrinol. 2001;170 (1):27-38.

20. Naharci MI, Pinar M, Bolu E, Olgun A. Effect of testosterone on insulin sensitivity in men with idiopathic hypogonadotropic hypogonadism. Endocr Pract. 2007;13 (6):629-35.

21. Haymana C, Sonmez A, Aydogdu A, Tapan S, Basaran Y, Meric C, et al. Visceral adiposity index and triglyceride/high-density lipoprotein cholesterol ratio in hypogonadism. Arch Endocrinol Metab. 2017;61(3):282-287.

22. Tan KC, Shiu SW, Pang RW, Kung AW. Effects of testosterone replacement on HDL subfractions and apolipoprotein A-I containing lipoproteins. Clin Endocrinol (Oxf). 1998;48 (2):187-94.

23. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. J Clin Endocrinol Metab. 2004;89 (7):3313-8.

24. Bolu E, Sonmez A, Tapan S, Taslipinar A, Aydogdu A, Meric C, et al. HDL cholesterol subfractions and the effect of testosterone replacement in hypogonadism. Horm Metab Res. 2013;45 (6):443-8.

25. Erqou S, Thompson A, Di Angelantonio E, Saleheen D, Kaptoge S, Marcovina S, et al. Apolipoprotein (a) isoforms and the risk of vascular disease: systematic review of 40 studies involving 58,000 participants. J Am Coll Cardiol. 2010;55(19):2160-7.

26. Kargin R, Emiroglu Y, Pala S, Akcakoyun M, Aung SM, Candan Ö, et al. Association Between Apolipoprotein-B100 and Apolipoprotein-A1 in Patients with Coronary Slow Flow. Kosuyolu Kalp Derg. 2010;13 (2):11-15.

27. Comini MA. Measurement and meaning of cellular thiol:disufhide redox status. Free Radic Res. 2016;50(2):246-71.

28. Himmelfarb J, McMonagle E, McMenamin E. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. Kidney Int. 2000;58 (6):2571-8.

29. Ulrich K, Jakob U. The role of thiols in antioxidant systems. Free Radic Biol Med. 2019;20;140:14-27.

30. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2017;1;524:13-30.

31. C. Haymana, A. Aydoğdu, B. Soykut, O. Erdem, T. Ibrahimov, M. Dinc, et al. Oxidative stress status in congenital hypogonadism. Toxicology Mechanisms and Methods, 2017; 27(6), 451-7.

32. Lee NP, Cheng CY. Nitric oxide/nitric oxide synthase, spermatogenesis, and tight junction dynamics. Biology of reproduction. 2004;70 (2):267-76.

33. Tenopoulou M, Doulias PT. Endothelial nitric oxide synthase-derived nitric oxide in the regulation of metabolism. F1000Res. 2020;9: F1000 Faculty Rev-1190.

34. Totzeck M, Hendgen-Cotta UB, Rassaf T. Nitrite-Nitric Oxide Signaling and Cardioprotection. Adv Exp Med Biol. 2017; 982:335-46.

35. Preedy MEJ, Baliga RS, Hobbs AJ. Multiplicity of Nitric Oxide and Natriuretic Peptide Signaling in Heart Failure. J Cardiovasc Pharmacol. 2020;75(5):370-384.

36. Gholinezhad M, Aliarab A, Abbaszadeh-Goudarzi G, Yousefnia-Pasha Y, Samadaian N, Rasolpour-Roshan K, et al. Nitric oxide, 8-hydroxydeoxyguanosine, and total antioxidant capacity in human seminal plasma of infertile men and their relationship with sperm parameters. Clin Exp Reprod Med. 2020;47(1):54-60.

37. Yasuda M, Ide H, Furuya K, Yoshii T, Nishio K, Saito K, et al. Salivary 8-OHdG: a useful biomarker for predicting severe ED and hypogonadism. J Sex Med. 2008;5 (6):1482-91.