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The Effect of Infliximab on Oxidative Stress in Ovarian Tissue of the Rat with Ovarian Hyperstimulation Syndrome

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ABSTRACT

Ovarian hyperstimulation syndrome (OHSS) is a complication that occurs during assisted reproductive techniques. In this study, our aim is to study the effect of Infliximab (IFX) on oxidative stress in ovarian tissue in a rat model of ovarian hyperstimulation syndrome.

A total of 32 immature female rats were divided into four groups randomly: Control, OHSS, OHSS+IFX, and IFX group. OHSS and OHSS+IFX groups were administered 30 IU pregnant mare serum gonadotropin/day on days 22-25 of life and 30 IU hCG on day 26. Plus, intraperitoneal IFX was administered half an hour before hCG administration to the OHSS+IFX group. On the 26th day, solely 7 mg/kg IFX was administered to the IFX group. 48 hours after administration of hCG administration, the rats were sacrificed, and their ovarian tissues were sampled. The levels of MDA, AOPP, TSG as well as catalase activity were measured in these ovarian tissues.

Ovarian tissue MDA and AOPP levels of the OHSS group were determined to be significantly higher compared to the control and OHSS-IFX groups. Ovarian tissue catalase activity and TSG level of the control group was significantly higher compared to the OHSS and OHSS-IFX groups. It was determined that although ovarian tissue catalase activity and TSG level in the OHSS-IFX group were higher compared to the OHSS group, but it was not significant.

The results revealed that IFX could prevent oxidative stress in ovarian tissue induced by OHSS. These effects may be mediated by the anti-inflammatory and antioxidant properties of IFX.

Keywords: Infliximab, Ovarian Hyperstimulation Syndrome, Oxidative Stress

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a rare but life-threatening iatrogenic complication of controlled ovarian hyperstimulation for assisted reproductive techniques by exogenous gonadotropins. It is described by cystic expansion in the ovaries and flow of protein-rich fluid from the vascular area to the peritoneal cavity (1).

Reactive oxygen species (ROS) play a key role in regulating oocyte maturation and fertilization. The development and maturation of oocytes depend on the dynamic balance between oxidant and antioxidant production (2). ROS impact female fertility by modulating a number of reproductive performs. In healthy individuals, antioxidants and ROS are in equilibrium, and when this equilibrium is disturbed in favor of ROS, oxidative stress consists. These product components, including nucleic acids, proteins, lipids are targets of oxidative stress (3).

Infliximab (IFX) is used securely in diverse inflammatory diseases (4). It has been stated that IFX lowers oxidative stress in rats by reducing lipid peroxidation and oxidant status, enhancing antioxidant activities (5). It has been revealed that IFX restores inflammation-induced tissue damage in various tissues by inhibiting the production of free oxygen radicals and TNF-alpha cytokines (6, 7).

In patients with OHSS, increased oxidative stress is not only produced during IVF procedure, but also by inflammatory factors during the pathophysiological processes of OHSS. Oxidative stress is accepted as a valuable parameter in the achievement of controlled ovarian stimulation. Hence, our aim in this study is to research the effect of IFX treatment on oxidant/antioxidant status in OHSS.

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Material and method

Animals: Immature female albino rats (Wistar strain) were used in this study. All rats were weighed 45-50 g and were 22 days old. Animals were kept in steel cages in a temperature-controlled room (22 ± 2 ℃) and a twelve-hour light/dark cycle. The animals were fed ad libitum. The study was conducted following the approval of the Animal Experiments Ethics Committee (Date: 29/04/2021; Decision number: 2021/04-01).

Experimental Design: Thirty-two female rats, 22 days old, were divided into four groups randomly.

Control group was administered 0.1 mL of saline intraperitoneally (IP) from Day 22 to Day 26. OHSS group was administered 30 IU of pregnant mare serum gonadotropin (PMSG) (Folligon, 5x1000IU+diluent, MSD, Animal Health, Intervent International, Netherland) from day 22 to day 26 for 4 days and 10 IU of human chorionic gonadotropin (Chorulon, 5 x 1500 IU+dilunet, MSD, Animal Health, Intervent International, Netherland) on 26th day. Group 3 (OHSS+IFX): In this group, rats were administered 30 IU PMSG from 22nd day to 26th day for 4 days starting from 22nd day, and 10 IU of human chorionic gonadotropin (hCH) (Chorulon, 5th 1500 IU+dilunet, MSD, Animal Health, Intervent International, Netherland) on 26th day and 7mg/kg IFX (Remicade, Janseen, Belgium) was given half an hour before hCG administration. Group 4 (IFX): IFX was administered on 26th day.

All animals were anesthetized by intramuscular administration of 50 mg/kg ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert Ilac Sanayi, Levent, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloric acid (Rompun; Bayer, Sisli, Turkey) 48 hours after hCG administration on Day 28.

Tissue Homogenates: Ovarian tissue was homogenized in 0.05 mm PBS with pH 7.4 and centrifuged at 14000 RPM for 20 minutes. The supernatant was stored at -40 degrees Celsius until the assay day.

Biochemical Parameters: Ovarian tissue MDA level was measured by the method identified by Okhava et al. and MDA level was presented as mmol/gr tissue (8). The AOPP level was worked based on the method of Witko et al. and the AOPP value was expressed as μmol/g tissue (9). CAT activity was spectrophotometrically analyzed at 240 nm according to the Lartillot method[10]. Total sulfhydryl groups were analyzed based on the Ellman method modified by Hu (11).

Statistical Analysis: The statistical analyzes were applied using SPSS v.20. The results are presented as the mean ± standard deviation (M ± SD). All statistical comparisons were performed using one-way ANOVA followed by Duncan's multiple range post hoc analysis. The results were considered significant at $p < 0.05$.

Results

Ovarian tissue Catalase activity and MDA, TSG and AOPP levels are presented in Table1. Ovarian tissue MDA level was found to be significantly higher in the OHSS group when compared to the control and IFX groups ($p<0.05$). Ovarian tissue MDA level was lower in the OHSS-IFX group compared to the control group, but it was not significant ($p>0.05$).

Ovarian tissue TSG level of the OHSS group was found to be significantly lower than the control group ($p<0.05$). Although the ovarian tissue TSG level of the OHSS-IFX group was higher than the OHSS group, but it was not significant ($p>0.05$).

Ovarian tissue catalase activity of the OHSS group and OHSS-IFX group was determined to be significantly lower compared to the control group ($p<0.05$). Catalase activity of the OHSS-IFX group was higher compared to the OHSS group, though the difference was not significant ($p>0.05$).

It was determined that the ovarian tissue AOPP level of the control group was significantly lower compared to the OHSS group ($p<0.05$). Ovarian tissue AOPP levels of the OHSS group were found to be significantly higher compared to the OHSS-IFX and IFX groups ($p<0.05$).

Discussion

Numerous studies have investigated the potential roles of reactive oxygen species in the etiology and pathogenesis of OHSS. This paper reveals the effect of IFX on oxidative stress in the ovarian tissues of rats with simulated-OHSS. Lipid peroxidation and AOPP levels in the ovarian tissue of rats with simulated-OHSS were significantly higher compared to the control group. Catalase activity was significantly lower in the OHSS group compared to the control group. These data demonstrated that oxidative stress is higher in the ovarian tissue of OHSS-stimulated rats. Following IFX treatment, a decrease was

Table 1. The Parameters Studied in Ovarian Tissue In Study Groups

	Control	OHSS	OHSS+IFX	IFX
MDA (nmol/gr tissue)	0.410±0.009b.c*	0.436±0.012a	0.392±0.012c	0.418±0.008a,b
TSG (mmol/gr tissue)	0.068±0.004a	0.038±0.007c	0.043±0.005c	0.052±0.004b
AOPP (mmol/gr tissue)	8.22±0.63b	11.83±1.23a	7.18±0.59c	8.93±0.65b
Catalase (U/L)	546.75±57.18a	377.36±13.16c	397.46±20.65c	447.21±67.74b

* Different letters on the same line represent statistical significance.
MDA: Malondialdehide; TSG: total sulfhydryl groups; AOPP: advanced oxidation protein products; OHSS: over hyperstimulation syndrome; IFX: infliximab

observed in oxidative markers, whereas an elevation was observed in antioxidant parameters. It can be suggested that IFX has antioxidant properties and protects the ovarian tissue from oxidative damage in cases with OHSS.

During IVF, ovarian stimulation induces supra-physiological ROS production while elevated estradiol concentration induces ROS production and mitochondria dysfunction (12). Superovulation increases ROS levels in the ovaries of mice, which indicates that the ovaries are under high levels of oxidative stress following superovulation (13). Excessive formation of free radicals, exceeding the protective effect of the defense system, impacts lipids the most (14). Yet, the activities of oxidant/antioxidants in the body of living things must be in balance (14). Unsaturated bonds of phospholipids in membranes and cholesterol readily react with free radicals to generate peroxidation products (14). The oxidative breakdown of unsaturated fatty acids is termed lipid peroxidation and is remarkably detrimental. The most famous product of lipid peroxidation is MDA. Generally, it is considered as the lipid peroxidation index and is one of the end products of lipid peroxidation (14-16). MDA concentration is directly proportional to cellular damage induced by free radicals (15, 16). It was found out that MDA levels in follicular fluids of women who underwent ovarian stimulation via assisted reproductive techniques were lower in the agonist group compared to the antagonist group (17). Tulic et al. reported that serum MDA and sulfhydryl groups were significantly higher following ovarian stimulation (18). Duraker et al.(19) revealed that patients with severe OHSS had significantly higher serum MDA levels compared to women under controlled ovulation induction without symptoms of OHSS. In a study conducted in rats, it was demonstrated that the ovarian tissue MDA level was lower in the control group compared to the superovulation group; however, this was not significant (20). It has been suggested that ovulation itself is an oxidative stress response and superoxides contribute to the ovulation process (21). Consistent with the above-mentioned remarks, in the present study, the MDA level in the ovarian tissue of the OHSS group rats was found to be significantly higher compared to the control group.

AOPP is produced by the action of chloramines and hypochlorous acid produced by myeloperoxidase in activated neutrophils during oxidative stress. AOPP is considered a reliable marker to predict the oxidative degree of proteins. It is thought that AOPP is closely associated with inflammation (9, 22). High serum AOPP levels have been reported in women with PCOS. (23). It has been demonstrated that the level of AOPP is higher in the serum of patients with uterine leiomyoma (24) and the peritoneal fluids of patients with endometriosis (25). In their study, Shu et al (20) revealed that the AOPP level in the ovarian tissue of rats with OHSS was higher compared to the control group, yet this level was not significant. In our study, the AOPP level of ovarian tissue was found to be significantly higher when the OHSS group was compared with the control group.

Total sulfhydryl groups (TSG) are part of the antioxidant system and are also called thiols. When reactive nitrogen species and reactive oxygen species increase, TSG may be oxidized, thus the antioxidant defense pool is reduced. Hence, the TSG level reflects the whole-body redox status. Its decrease is indicative of elevated oxidative stress and conversely, its increased levels are attributed to detoxification and cell repair of the detrimental effects of ROS/RNS (26). In the present study, ovarian tissue TSG level was significantly lower in the OHSS group than the control group. The low levels of TSG in the

OHSS group may be due to decreased antioxidant defense or increased oxidative stress.

Catalase is an intracellular antioxidant enzyme that catalyzes hydrogen peroxide into water and molecular hydrogen. If restricted glutathione content or decreased glutathione peroxidase activity occurs, this enzyme plays an important role in the development of tolerance to oxidative stress. It has been shown that serum catalase activity is decreased in women with PCOS compared to healthy women. (27). Catalase activity is used as a clinical parameter to determine systemic stress (28). Catalase has a great effect on tissue protection against reactive oxygen species (29). In the present study, ovarian tissue catalase activity was found to be significantly lower in the OHSS group than the control group.

Albeit the pathophysiological mechanism of OHSS is not fully understood, there are two components of OHSS, the first is ovarian enlargement with multiple follicles, luteal cysts, and stromal edema, and the second one is fluid leakage from the intravascular compartment into the third space (30). Cytokines such as TNF-α are mediators that play a role in increased vascular permeability. Immune system mediators such as TNF-α are considered to play a role in the pathogenesis of OHSS.

The interaction between inflammation and oxidative stress is well documented. TNF- α is known to increase the production of ROS in many tissues (31, 32). Thus, inflammation caused by TNF-α may increase oxidative stress in OHSS. Inhibition of TNF-α in OHSS patients could reduce oxidative stress.

IFX is widely used in the treatment of various inflammatory diseases. It has been demonstrated that IFX inhibits TNF-α and reduces ischemia-reperfusion injury in the intestine, liver, and kidney. IFX reduces both cytokines that stimulate tissue damage and tissue damage induced by TNF-α, which increases ROS formation (33). It has been reported that IFX captures free radicals and inhibits inflammation (34). It has been put forward that IFX could suppress oxidative stress by enhancing antioxidant status (35), reducing ROS production (36), and reducing oxidant status and lipid peroxidation (5). It has been demonstrated that IFX treatment reduces MDA concentration in serum and ovarian tissue of rats with ovarian ischemia/reperfusion injury than the control group (37). In our study, AOPP and MDA levels were found to be significantly lower in the OHSS-IFX group than the OHSS group. It was found out that although ovarian tissue catalase activity and TSG level of the OHSS-IFX group were higher compared to the OHSS group, though this elevation was not significant. IFX treatment could protect the ovaries from oxidative stress by reducing the increased oxidative stress and increasing the decreased antioxidant level in OHSS.

The limitations of the study were that we did not measure antioxidant and oxidant parameters and TNF-α levels in follicular fluid and serum. Besides, these parameters can be measured in both follicular fluid and serum in further studies.

OHSS may adversely impact the delicate balance between antioxidants and reactive oxygen species. This may lead to oxidative stress in the ovaries. IFX therapy could suppress oxidative stress and eliminate oxidative stress induced by OHSS. The antioxidant and anti-inflammatory properties of IFX can be considered as a candidate for the treatment of increased oxidative stress in OHSS. Further studies should be carried out to investigate the effects of IFX in OHSS patients.

Conflict of interest: The author declare that they have no conflict of interest.

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