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Original Research

Oxidative stress status in severe OHSS patients who underwent long agonist protocol intracytoplasmic sperm injection cycles

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Purpose of investigation: Current infertility treatment strategies may result in ovarian hyperstimulation syndrome (OHSS), which can present with hemodynamic instability that involves hemoconcentration, hypoxia, and liver and renal dysfunction that may result from thrombosis. This study's purpose was to measure the serum biochemical oxidative stress markers in women with severe OHSS. *Material and methods*: For this prospective controlled study, serum levels of ischemia modified albumin (IMA), total antioxidant capacity (TAC), total oxidative capacity (TOS), oxidative stress capacity (OSI), and serum malondialdehyde (MDA) were measured in women with (n = 25) and without (n = 27) OHSS. *Results*: In our study, we observed significant differences between the two groups in terms of IMA, TAC, TOS, OSI, and MDA levels. High oxidative stress parameter levels in the OHSS group may indicate that OHSS is an oxidative stress condition. A bivariate correlation analysis revealed a significant correlation between serum TOS level, OSI ratio, and embryo or oocyte quality scores. In addition, there was a negative, non-significant tendency among OHSS patients regarding high IMA, OSI, TOS, and MDA levels and low oocyte and embryo scores. Pregnancy results were not affected in a statistically significantly manner. *Conclusion*: These results might indicate that oxidative stress status and oxygen radicals may negatively affect ART cycle outcomes.

Keywords

IMA; ICSI; Infertility; OHSS; Oxidative stress; Ovulation induction

1. Introduction

Current infertility treatment strategies may result in ovarian hyperstimulation syndrome (OHSS), which is considered a thrombotic disease. OHSS affects 5% of patients who undergo IVF and induces microvascular thrombosis. In pathogenesis, patients respond to exaggerated exogenous gonadotropins and experience a change in the hemostatic system and marked hemoconcentration [1]. Following ovulation triggering with human chorionic gonadotrophin, the serum levels of most serum coagulation and fibrinolytic factors increase within 2 to 8 days [2]. Somehow, OHSS can cause microvascular thrombosis and circulation dysfunction that leads to tissue ischemia.

Ischemia-modified albumin (IMA) is a novel marker for assessing tissue ischemia. IMA levels correlate to tissue ischemia [3, 4]. In this study, we expected that microvascular thrombosis caused by OHSS might elevate serum IMA levels that could alert clinicians of severe complications. We also aimed to establish an association between OHSS and changes in total antioxidant capacity (TAC), total oxidative capacity (TOS), oxidative stress capacity (OSI), and serum malondialdehyde (MDA) levels.

2. Materials and methods

This prospective study included women with primary infertility subjected to ICSI-ET cycles with moderate and severe OHSS (study group, group I). The control group (group II) consisted of women with primary infertility subjected to ICSI-ET cycles without any sign of OHSS. Members of the study and control groups were younger than 40 years old and had comparable body mass index (BMI) scores. All patients were screened for inherited or acquired thrombophilia. We excluded women with known inherited or acquired thrombophilia, history or thromboembolism, a history of antithrombotic treatment within the past three months, thrombophilia, systemic diseases, and smoking. Patients in both groups were hyper-responders (PCOS) who underwent ART for oligospermia or azoospermia. The study group contained 25 women, and the control group contained 27. We used the Rotterdam criteria to diagnose PCOS. Two out of three of the following criteria are required for a diagnosis: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries (determined by ultrasound) [5]. Institutional Review Board (Etlik Zubeyde Hanım EA Hospital) approval was obtained on 04 August 2011, and an approval number of 139 was assigned. Informed consent was also obtained for each participant.

The luteal long leuprolide acetate controlled ovarian protocol was used for all women. Pituitary down-regulation with leuprolide acetate (1 mg/day; Lucrin, Abbott Laboratories, North Chicago, IL) began on day 21 of the previous menstrual cycle. Following the second and third day of initial menstruation, subcutaneous administration of recombinant gonadotropin (Gonal-F, 150–225 IU/day, Laboratories Serono S.A., Aubonne, Switzerland) was performed. Serum estradiol measurement and folliculometry via transvaginal ultrasound were used for ovulation induction monitorization. Ovulation was triggered with recombinant human chorionic gonadotropin (0.25 μgr; Ovitrelle subcutaneously, Serono, Istanbul, Turkey) following assessment of at least two or three mature follicles ($>$ 17 mm in diameter). Oocyte pick-up was scheduled 34–36 hours later. Gonadotropin dosage was adjusted according to antral follicular count, age, and serum FSH / E2 levels for each patient. All women underwent day-3 embryo (one or two (for patients age $>$ 35) embryo) transfer. Vaginal progesterone gel (Crinone 8% gel, Serono S. A. Aubonne, Switzerland) was used twice daily for luteal phase support. Four weeks after embryo transfer, visualization of fetal heartbeat with surrounding gestational sac on transvaginal sonography was accepted as clinical pregnancy.

The published classification of OHSS severity was used [6]. Based on this classification, women with complaints of abdominal distension and discomfort, nausea and/or vomiting, and sonographic findings (ovarian size of 8–12 cm, ascites) were diagnosed with moderate OHSS. Women with all moderate OHSS findings (n = 19), at least 2 kg weight gain, and altered laboratory findings (hematocrit $>$ 45%, white blood cell count $>$ 15.000, oliguria, creatinine of 1.0–1.5, creatinine clearance of $>$ 50 mL/min, and high serum ALT and AST results) were diagnosed with severe OHSS (n = 6). Oocyte and embryo quality classifications were based on the current published system [7].

Women with moderate or severe OHSS were hospitalized. Avoidance of physical activity, oral or parenteral hydration, daily laboratory testing (CBC, electrolytes, creatinine, serum albumin, and liver enzymes), and physical and ultrasound examinations were performed. Weight, abdominal circumference, and any worsening signs and symptoms were assessed daily. Disturbed fluid and electrolyte balances were corrected, the secondary complications of ascites and hydrothorax were relieved, and thromboembolic events were prevented with low molecular weight heparin. Ultrasound-guided culdocentesis was performed in women with tense ascites, orthopnea, rapid increase of abdominal fluid, or any other sign of illness progression.

The control group (group II) comprised patients with PCOS who underwent the same controlled ovulation induction protocol but did not demonstrate symptoms of OHSS.

Blood samples were collected on the day on which ovulation was triggered. Antecubital venous blood samples of approximately 5 mL were taken, and the aspirated serum sample was stored at -80 C° until at the end of the experiment. The author who studied the samples did not know whether the samples belonged to the study or control group. Serum levels of IMA, MDA, TOS, TAS, and OSI were measured.

Next, serum IMA [8], MDA [9], TOS [10], and TAC [11] were measured, and the OSI ratio was calculated [12] as described previously.

We hypothesized that OHSS may have detrimental effects on serum oxidative stress markers.

We used Student's *t*-test to compare the parametric variables and Fisher's exact chi-square test to compare the nonparametric variables. *P* values were calculated using SPSS 13.0. The Spearman correlation analysis was used to assess serum IMA, TAC, TOC, OSI, and MDA; $P < 0.05$ was accepted as a statistically significant value.

A post-hoc power analysis was performed for 25 patients in each group, considering the end point as mean serum IMA values (0.67 for the study group and 0.55 for the control group with standard deviation values of 0.1). The calculated power was 0.98 with 0.5% type 1 errors.

This case control study fulfilled the requirements (STROBE) of the Enhancing the Quality and Transparency of Health Research (EQUATOR) network guidelines.

3. Results

The recruited participants included 52 patients requiring IVF because of male factors. There were no significant differences between the study and control groups according to the baseline demographic characteristics (Table 1). A comparison of both groups' serum albumin levels revealed no statistically significantly differences.

Comparison of IMA, TAC, TOC, OSI, and MDA levels between both groups are shown in Table 2.

High numbers of retrieved and mature oocytes and low fertilization rate were found in the OHSS group compared with the control group. However, the clinical pregnancy rate decreased in group I without reaching a statistically significant value. There were no significant differences in the pregnancy rates of women who underwent one or two embryo transfers in OHSS compared with control group.

Bivariate analysis revealed that serum TOC levels were well correlated with the total number of retrieved oocytes (r = 0.515, $P < 0.001$), total number of retrieved MII oocytes (r = 0.439, $P = 0.001$), total number of dominant oocytes on HCG day (r = 0.417, $P = 0.002$), total number of grade I and II embryos (r = -0.437, $P = 0.001$), and serum E2 levels on HCG day (r = 0.483, $P < 0.001$) in the whole group. Similarly, the OSI ratio was well correlated with total number of retrieved oocytes (r = 0.467, $P < 0.001$), total number of retrieved MII oocytes (r = 0.396, $P = 0.004$), total number of dominant oocytes on HCG day (r = 0.346, $P = 0.012$), total number of grade I and II embryos (r = -0.422, $P = 0.002$), and serum E2 levels on ovulation trigger day (r = 0.398, $P < 0.003$) in the whole group. Serum IMA levels were negatively correlated with oocyte quality scores (r = -0.299, $P = 0.031$).

Table 1. Baseline characteristics and clinical data.

	Group I (25 patients)	Group II (27 patients)	*P* value
Age (years)	27.60 ± 4.52	30.03 ± 4.99	NS
Body mass index (BMI) (kg/m^2)	24.98 ± 3.43	25.63 ± 4.78	NS
Basal FSH (mIU/mL)	0.60 ± 0.96	0.30 ± 0.87	NS
Basal LH (mIU/mL)	0.08 ± 0.28	0.00 ± 0.00	NS
Basal oestradiol (pg/mL)	22.68 ± 17.19	15.92 ± 11.63	NS
Total antral follicle count (no.)	20.72 ± 4.30	20.78 ± 3.18	NS
Oestradiol on stimulation day 0 (pg/mL)	14.63 ± 7.17	12.99 ± 7.35	NS
Duration of stimulation (days)	9.28 ± 1.56	10.44 ± 2.54	NS
Oestradiol on HCG day (pg/mL)	4673.00 ± 2231.06	2237.96 ± 0.15	< 0.001
Dominant follicles (14 mm) on HCG day	10.80 ± 3.10	6.74 ± 1.72	< 0.001
Total retrieved oocytes (n)	21.12 ± 8.40	12.55 ± 2.23	< 0.001
No. of MII oocytes retrieved	16.92 ± 6.12	11.70 ± 2.05	< 0.001
Fertilization rate	74.81 ± 22.92	86.69 ± 17.90	0.042
Grade 1 and 2 embryo rate on day 3 (no.)	3.80 ± 1.50	5.26 ± 1.89	0.004
Oocyte quality score (no.)	4.42 ± 0.57	4.82 ± 0.49	0.011
No. of transferred embryo (no.)	1.16 ± 0.47	1.00 ± 0.00	NS
Clinical pregnancy rate (%)	40% (10)	55.6% (15)	NS

NS, not statistically significant; HCG, human chorionic gonadotrophin.

Values are given as mean ± SD or % (number of cases).

Table 2. Comparison of IMA (İschemia modified albumin total antioxidant capacity (TAC), total oxidant capacity (TOC), oxidative stress index (OSI), and malondialdehyde (MDA) level between both groups).

Parameter	Group I (25 patients)	Group II (27 patients)	*P* value
IMA (ABSU)	0.67 ± 0.15	0.55 ± 0.09	$P < 0.05$
TAC (mmol Trolox equiv/L)	0.70 ± 0.19	0.87 ± 0.22	$P < 0.05$
TOC (mmol H2O2/L)	36.12 ± 18.62	7.20 ± 3.85	$P < 0.001$
OSI (%)	5.53 ± 3.50	0.95 ± 0.74	$P < 0.001$
MDA (nmol/mL)	0.81 ± 0.47	0.53 ± 0.12	$P < 0.001$

Student *t* test was used for comparison.

4. Discussion

Controlled ovarian hyperstimulation can significantly affect hepatic and renal functions in patients by causing OHSS [13, 14]. OHSS is characterized by altered capillary permeability, which may result in the transfer of intravascular fluid to extravascular areas, leading to systemic endothelial dysfunction. Fluid escape into a tertiary space can result in hemoconcentration, resulting in thromboembolic events [15]. This phenomenon is similar to sepsis, in that OHSS patients demonstrate vascular permeability. The main cause of this condition is high serum levels of vascular endothelial growth factor (VEGF) [16, 17].

Numerous studies also emphasized the importance of reactive oxygen species in reproduction [18–21]. Unexpected events such as OHSS could negatively affect the delicate balance between antioxidants and ROS. In addition, ROS release may result from oxidative stress. As shown in our study, factors such as OHSS that lead to ischemia may increase serum IMA. Rising IMA levels may be a signal for increased ROS and its likely negative influences over oocyte quality and implantation. Our study also revealed increased TOC, OSI, and MDA levels, which were probably related to increased IMA. Increased TOC, OSI, MDA may result in increased reactive oxygen species levels and oxidative stress.

The interrelationship between the follicular fluid levels of oxidative stress markers and embryos or oocytes is a debatable subject. Some authors suggested that high ROS concentrations in follicular fluid may alter the quality of oocytes in tubal infertile patients [22]. One limitation of our study was that we did not establish ROS levels directly, but attempted to understand pathogenesis indirectly by measuring TAC, TOC, OSI, and MDA levels. Further studies could be designed to examine this point. Another limitation of the study was that we measured only serum levels, not follicular fluid levels of TAC, TOC, OSI, and MDA.

In our study, we found that retrieved oocyte counts were higher in the OHSS group, but the fertilization rate and grade 1 and 2 embryo counts were higher in the control group. This can be explained by variety of factors, including tissue ischemia and increased oxidative stress. The endometrium should be high enough qualified for implantation. This process is very delicate, and follicular development can be dis-

turbed by various factors and may interfere with implantation. Increased IMA levels, which may be a sign of microthrombotic events, might therefore be the cause of changing levels of TAC, TOC, OSI, and MDA. In the literature, lower TAC levels are linked with fertilization failure [23]. In our study, lower TAC levels indicated no significant differences between clinical and biochemical pregnancy rates even if there was a significant difference in grade 1 and 2 embryo counts. The low number of patients might therefore restrict us from making strict suggestion and conclusions.

Microthrombotic effects may also result in chromosomal aberrations in the oocyte or embryo in women with OHSS. This may be related to intrafollicular hypoxia and insufficient angiogenesis in the follicles of OHSS patients [24]. The authors also agreed on the need for balanced oxidative stress in folliculogenesis and oocyte maturation [25].

In the light of recent studies, oxidative stress has been accepted as valuable parameter in the success of controlled ovarian stimulation. Oxidative stress may alter the oocyte quality, sperm and oocyte interaction, fertilization, implantation, and embryonic growth [26]. Some studies show that various factors, including even light exposure, can cause ROS production in cultured media. ROS may decrease the rate of blastocyst development and increase embryo fragmentation and apoptosis, which might explain the detrimental effects of OHSS [23, 27].

Successful IVF may also be related to clinical (e.g., age, AMH, FSH dose), laboratory, and physician associated factors (e.g., low experience, embryo transfer technique) [28, 29]. To date, considerable effort has been focused on identifying a correct algorithm that uses a woman's age and ovarian reserve markers as tools to optimize the follicle-stimulating hormone (FSH) starting dose in IVF procedures. Nevertheless, current available evidence regarding women with PCOS, particularly those with high AMH, appears inadequate [30, 31]. This point has also been an important limitation in preventing OHSS, especially when determining the correct starting FSH dose in IVF patients. In addition, preventing OHSS during controlled ovarian stimulation may increase patient satisfaction and decrease the incidence of severe microvascular complications.

In our study, serum level was assessed on the trigger day, which may not represent the entire OHSS process. This was an additional limitation of the current study. Further studies with serial serum marker results until the OHSS improves would provide better insight into the oxidative effect.

5. Conclusions

In the light of these findings, high oxidative stress might influence oocyte maturation and implantation in women with OHSS. This study also revealed that OHSS could initiate a thrombotic cascade caused by the high oxidative stress condition. IMA elevation might be an indicator of microvascular thrombosis. However, antioxidant supplementation along either with LMWH or aspirin may reduce the detrimental influence of OHSS. Larger clinical trials are necessary to explore this hypothesis further.

Author contributions

RD, ESGG, SD, SA conceived, designed and performed the experiments. SG, ESGG analyzed the data; AM contributed reagents and materials; SG, RD and ESGG wrote the paper.

Ethics approval and consent to participate

Clinical trials registration number: NCT02202278.

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Conflict of interest

The authors declare no conflict of interest.

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