Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2023.47817

Turk J Obstet Gynecol 2023;20:46-52

An evaluation of the effects on the ovaries of hyperbaric oxygen therapy in a rat model of premature ovarian failure created with cyclophosphamide

Siklofosfamid ile oluşturulmuş erken yumurtalık yetmezliği olan bir sıçan modelinde hiperbarik oksijen tedavisinin yumurtalıklar üzerindeki etkilerinin değerlendirilmesi

Fulya Çağlı[1], Mehmet Akif Baktır[2], Mehmet Dolanbay[1], Esra Balcıoğlu[3], Ahmet Cumaoğlu[4], Mustafa Ermiş[5], Enes Karaman[1], Betül Yalçın[3], Ercan Mustafa Aygen[1]

[1]*Erciyes University Faculty of Medicine, Department of Obstetrics and Gynecology, Kayseri, Turkey*

[2]*Erciyes University Faculty of Medicine, Department of Physiology, Kayseri, Turkey*

[3]*Erciyes University Faculty of Medicine, Department of Histology and Embryology, Kayseri, Turkey*

[4]*Erciyes University Faculity of Pharmacy, Department of Biochemistry, Kayseri, Turkey*

[5]*Erciyes University, Good Clinical Practice and Research Center, Kayseri, Turkey*

Abstract

Objective: To evaluate hyperbaric oxygen therapy (HBO) based on ovarian histology, total antioxidant status (TAS), total oxidant status (TOS), and anti-müllerian hormone (AMH), in the ovarian insufiency (POI) model created with cyclophosphamide (CYP).

Materials and Methods: The rats were separated into 3 groups of the control group (n=6), the CYP group (n=6), and the CYP+HBO group (n=6). The rats in the CYP group and the CYP+HBO group were injected intraperitoneally with 200 mg/kg CYP on day 1, followed by 8 mg/kg/day for 14 days to create POI. From the 15th day onwards, the rats in the CYP+HBO group were placed in a hyperbaric cabin and exposed to 100% oxygen at 2.4 atm pressure for one h, and were then returned to their cages at the end of the hour.

Results: A statistically significant decrease was determined in the primordial and primary follicle counts in the CYP group compared with the control group ($p<0.05$). In the CYP+HBO group, a statistically significant increase was determined in the primordial and primary follicle counts ($p<0.05$). The serum AMH levels were seen to be significantly decreased in the CYP group compared with both the control group and the CYP+HBO groups. The HBO was seen to decrease TOS and increase TAS.

Conclusion: HBO could be an alternative treatment to minimize the effect of ovarian follicle loss caused by CYP, which is used for treating tumors that commonly occur in young females of reproductive age.

Keywords: Anti-müllerian hormone, cyclophosphamide, hyperbaric oxygen, ovarian failure

Öz

Amaç: Bu çalışmanın amacı siklofosfamid (CYP) ile oluşturulan over yetmezliği (POI) modelinde hiperbarik oksijen tedavisini (HBO) over histolojisi, total antioksidan durumu (TAS), total oksidan durumu (TOS) ve anti-müllerian hormon (AMH) bazında değerlendirmektir.

Gereç ve Yöntemler: Ratlar kontrol grubu (n=6), CYP grubu (n=6) ve CYP+HBO grubu (n=6) olmak üzere 3 gruba ayrıldı. CYP grubu ve CYP+HBO grubundaki ratlara 1. gün 200 mg/kg CYP, ardından 14 gün boyunca POI oluşturmak için 8 mg/kg/gün intraperitoneal olarak enjekte edildi. 15. günden

PRECIS: Hyperbaric oxygen therapy in premature ovarian failure.

Address for Correspondence/Yazışma Adresi: Fulya Çağlı MD,
Erciyes University Faculty of Medicine, Department of Obstetrics and Gynecology, Kayseri, Turkey
Phone: +90 505 453 18 78 **E-mail:** f.cagli@yahoo.com **ORCID ID:** orcid.org/0000-0002-6492-3379
Received/Geliş Tarihi: 19.01.2023 **Accepted/Kabul Tarihi:** 15.02.2023

itibaren CYP+HBO grubundaki ratlar hiperbarik kabine alınarak 1 saat 2,4 atm basınçta %100 oksijene maruz bırakıldı ve 1 saatin sonunda ratlar tekrar kafeslerine alındı.

Bulgular: CYP grubundaki primordial ve primer folikül sayılarında kontrol grubuna göre istatistiksel olarak anlamlı azalma saptandı (p<0,05). CYP+HBO grubunda primordial ve primer folikül sayılarında istatistiksel olarak anlamlı artış saptandı (p<0,05). Serum AMH düzeylerinin CYP grubunda hem kontrol grubu hem de CYP+HBO grubuna göre anlamlı olarak düştüğü görüldü. HBO'nun TOS'yi azalttığı ve TAS'yi artırdığı görüldü.

Sonuç: Üreme çağındaki genç kadınlarda sık görülen tümörlerin tedavisinde kullanılan CYP'nin neden olduğu over folikül kaybının etkisini en aza indirmek için HBO alternatif bir tedavi olabilir.

Anahtar Kelimeler: Anti-müllerian hormon, siklofosfamid, hiperbarik oksijen, yumurtalık yetmezliği

Introduction

The total number of immature primordial follicles found in the ovaries is known as the ovarian reserve. Estimation of the ovarian reserve is accepted as a basic marker of fertility With a process known as "activation," the rate at which primordial follicles grow is an important point affecting fertility. From a total of approximately 1-2 million primordial follicles at birth, only approximately 400 will mature into primary oocytes with the capability for ovulation and fertilization in the reproductive years of a woman. The vast majority are victims of atresia.

When approximately 1.000 primordial follicles remain, this causes fertility to stop and menopause to start[1]. Information about the renewal of primordial follicles related to the depletion of the ovarian reserve has started to be discussed with the emergence of evidence related to the identification of oogonial stem cells, which can produce new oocytes in mouse ovaries[2], and their presence in human ovaries[3]. However, there is limited knowledge about the underlying molecules and biochemical processes of follicular activation in humans. Moreover, exposure to exogenous factors such as stress, radiation, anti-neoplastic agents, and cigarette smoke cause the formation of reactive oxgen species, which can develop in the body under physiological conditions. Oxidative stress occurs because of an increase in the oxidant level and/or a decrease in the antioxidant level in the body. Oxidative stress causes damage to cellular mitochondria, nucleus, and membranes. Free radicals cause irreversible damage in the organism with the effect of damage to deoxyribonucleic acid (DNA). It is thought that reactive oxygen species (ROS) plays an important regulatory role in folliculogenesis, oocyte maturation, and luteolysis.

Chemotherapeutic agents have a negative effect on the reproductive potential of young women, but the mechanisms through which this occurs are still unclear. Cyclophosphamide (CYP) is used to treat several cancer types, primarily leukemia and lymphoma, which are the most frequently seen cancers in adolescent females[4]. Alkylating agents, especially CYP, are extremely toxic for the gonads because the toxicities are independent of cell proliferation[5]. CYP directly damages DNA, induces follicular apoptosis, and produces ROS, which damage ovarian cells[6]. CYP also leads to over-activation of primordial follicles by actvating the P13K/AKT/mTOR pathway[7]. This is accepted as a factor that is effective in the early reduction of follicles.

Premature ovarian insufficiency (POI), which is a cause of female infertility, is defined as failure in ovarian functions for a period of longer than 4 months before the age of 40 years, and is characterized by amenorrhea, elevated gonadotropin levels, and hypooestrogenism[8]. This condition affects 0.3-1% of women[9]. POI can occur with the depletion of the ovarian reserve associated with acceleration in the rate of primordial follicle activation[10], primordial follicle loss[11], or iatrogenic causes such as chemotherapeutic agents[12]. These patients experience physiological symptoms associated with hypooestrogenism, infertility, and a series of psychological problems[13]. Simultaneously, there is an increased risk of cardiovascular disease, osteoporosis, urogenital atrophy, and neurodegenerative disease in these patients[14]. Therefore, the prevention of POI is important to protect women against infertility, and other systemic problems brought about by early menopause.

Hyperbaric oxygen therapy (HBO) is a treatment method in which 100% oxygen is breathed by exposure to increasing atmospheric pressure. Recently, it has been used as an effective adjuvant treatment method for treating several different pathologies. The oxygen present can re-oxygenate areas where hypoxia or hypoperfusion has occurred.

This study aimed to determine the possible effects that could formed after HBO therapy in rats applied with CYP to create a premature ovarian failure model, by examining ovarian histology and the serum parameters of Anti-müllerian hormone (AMH), total antioxidant status (TAS), and total oxidant status (TOS).

Materials and Methods

This study was approved by the Erciyes University Animal Experiments Ethic Committee (no: 21/168; date: 07.07.2021). The work was funded by the Erciyes University Scientific Research Projects Coordination Unit (TSA- 2022- 11550).

The study material comprised 18 adult, female Wistar rats, aged 10-12 weeks, each weighing 200±20 gr. The rats were kept under observation for 3 days, for adaptation, for signs of any health problems. Throughout the study, the animals were kept under stable conditions of 22 °C environmental temperature, 30-70% humidity, a 12-hour light/dark cycle, with food of dry pellets and tap water.

The rats were separated into 3 groups of 6 animals in each. The control group was named group 1, the CYP group, group 2,

and the CYP+HBO group, group 3. The control group rats were administered 1 mL/kg physiological saline intraperitoneally (IP) for 15 days. The rats in group 2 and group 3 were injected IP with 200 mg/kg CYP on day 1, based on the protocol in the study by Melekoglu et al.[15] followed by 8 mg/kg/day for 14 days to create POI. From the 15th day onwards, the rats in the CYP+HBO group were placed in a hyperbaric cabin and exposed to 100% oxygen at 2.4 atm pressure for one h, and were then returned to their cages at the end of the hour.

For the HBO therapy, the cabin was filled with 100% oxygen until the pressure reached 2.4 atmosphere at the rate of 0.1 bar/min in 15 min. At the end of 1 h of treatment, the air in the cabin was safely released until it fell to a normal pressure of 1 atmosphere at a rate of 0.1 bar/min. This procedure was continued at the same duration and pressure for 14 days.

On day 30, all the rats in all groups were administered general anesthesia of xylazine (10 mg/kg live weight, IP) and ketamine (60 mg ketamine hydrochloride/kg live weight, IP). First, an intracardiac blood sample of approximately 5 cc was taken. The serum was separated by centrifugation at 3.000 rpm for up to 10 min. While the animals were under deep anesthesia, a 2 cm skin incision was made over the linea alba in the abdominal region. The subcutaneous connective tissue and abdominal muscles were opened, and oophorectomy was performed by dissecting the left and right ovaries. The rats were then sacrificed with the cervical dislocation method.

Ovarian tissues were fixed in 10% formaldehyde solution for 48 h. The tissues were washed under running tap water, and then passed through increasing graded alcohol series. After being made transparent with xylene, the tissues were embedded in paraffin blocks. Slices 5 µm in thickness were cut from the paraffin blocks, stained with Hematoxylin and Eosin and Masson Trichrome, and then blinded examined under an Olympus BX51 microscope. Evaluations were made according to the determined criteria. The germinal epithelium and tunica albuginea were measured using ImageJ software.

Determination of TAS and TOS Levels

Serum TAS and TOS levels were determined using commercial Rel Assay kits (Rel Assay Kit Diagnostics, Turkey). Trolox was used as a calibrator for TAS tests, and the results were expressed in mmol Trolox equivalent/L. Hydrogen peroxide was used as a calibrator for TOS tests and the results were expressed in mmol H_2O_2 equivalent/L.

ELISA Analysis of AMH Levels

Serum samples were collected by centrifugation (5000 rpm for 10 min) and stored at -80 °C. The procedure was performed with an AMH ELISA Kit (AFG Bioscience, USA) following the manufacturer's instructions. Optical density (OD) values were measured at 450 nm using a spectrophotometer (BioTek, Synergy H, TX, USA). The concentrations of AMH in the serum samples were determined by comparing the OD values of the samples to the standard curve.

Statistical Analysis

The SPSS 22 software was used for statistical analysis. Statistical differences between groups were tested using One-Way ANOVA, and comparisons between the control and treated groups were performed using an unpaired Student's t-test. The data werre presented as mean ± standard error of the mean values. A value of $p<0.05$ was accepted as statistically significant.

Results

Serial slices (5 µm) were taken from the ovarian tissues obtained. One in five of the slices were stained, and follicles were counted. A statistically significant decrease was determined in the primordial and primary follicle counts in group 2 compared to group 1 ($p<0.05$). A statistically significant increase was determined in the primordial and primary follicle counts in group 3 compared to group 2 ($p<0.05$). When the groups were evaluated according to pre-antral and secondary follicle counts, the only statistically significant difference was between group 1 and group 2 ($p<0.05$). A statistically significant difference was determined between group 2 and both groups 1 and 3 with respect to secondary follicle count ($p<0.05$) (Table 1).

When the groups were evaluated with respect to the germinal epithelial thickness, the difference between group 1 and group 3 was statistically significant ($p>0.05$). An increase occurred in the mean germinal epithelial thickness of group 2, and this increase was determined to be statistically significant compared to groups 1 and 3 ($p<0.05$) (Table 1). In the evaluation of tunica albuginea thickness, a statistically significant difference was determined between all groups ($p<0.05$) (Table 1).

In group 1, tunica albuginea formed of fibrous connective tissue fibers and cells was observed below the germinal epithelium. Below the tunica albuginea was the cortex section, which was in the more peripheral part and contained various numbers and types of follicles, and the medulla, containing a rich vascular bed and with a loose connective tissue structure in the inner section. The germinal epithelium in group 3 was similar to that in group 1, but the tunica albuginea layer was seen to be thicker than in the control group. In group 2, in the whole tunica albuginea layer, including the cortex, there was more blood vessels and wider diameter of the vessels. In the primordial, primary, pre-antral, secondary, and tertiary follicles, the oocytes and the granulosa cells surrounding the oocytes were irregular. There was also observed to be an evident increase in the number of atretic follicles in group 2. The connective tissue sheath (theca) surrounding the follicle, and which differed from the stromal cells that began to be observed from the primary follicles, was enlarged and irregular. The granulosa lutein cells in the corpus luteum, which develops after ovulation, were observed to be irregular in shape with large gaps between them. There was a significant increase in the number of blood vessels in the medulla layer, and widening of the blood vessel diameters could be significantly differentiated. The histopathological findings in group 3 were close to those in group 1.

The number of blood vessels was decreased and the diameters of the vessels were narrower. Fewer atretic follicles were observed. The theca layer of the connective tissue surrounding the follicles was more regular in shape (Figure 1).

When the serum AMH levels of the groups were examined, the serum AMH levels in group 2 were seen to be significantly decreased compared with both group 1 and group 3 ($p<0.05$) (Table 2). The highest mean serum AMH level was determined in group 3 (8.83±0.87 ng/mL) but this was not at a statistically significant level compared to the control group (8.79±1.37 ng/mL). These results showed that CYP lowered the AMH level significantly, but the application of HBO therapy to these patients with CYP could prevent a fall in serum AMH levels.

When the TOS values were examined, there was determined to be statistically significantly higher oxidant status in group 2 than in group 1 (7.02±2.29 vs. 3.85±0.62, p=0.0083) (Table 2). In group 3, the HBO therapy was seen to have statistically significantly reduced the oxidant status (3.70±1.19, p=0.010). This status can be considered protective with respect to the ovarian follicles.

Table 1. Comparisons of the follicle counts between the groups

	Group 1	Group 2	Group 3	p
Primordial	102.80±8.57[a]	71.10±10.33[b]	81.80±8.91[c]	0.000
Primary	67.40±9.35[a]	49.90±6.33[b]	60.60±8.27[a]	0.000
Preantral	35.40±7.13[a]	26.90±7.06[b]	30.70±5.79[ab]	0.029
Secondary	18.00±2.44[a]	12.50±2.71[b]	16.40±3.59[a]	0.001
Tertiary	8.60±1.71[a]	6.40±2.11[b]	7.30±1.88[ab]	0.026
Germinal epithelial	5.81±1.58[a]	6.35±2.12[b]	5.70±1.72[a]	0.001
Tunica albuginea	15.60±5.07[a]	28.92±11.10[b]	17.52±5.13[c]	0.000

The same letters in the same rows indicate similarity between the groups, and different letters indicate a difference

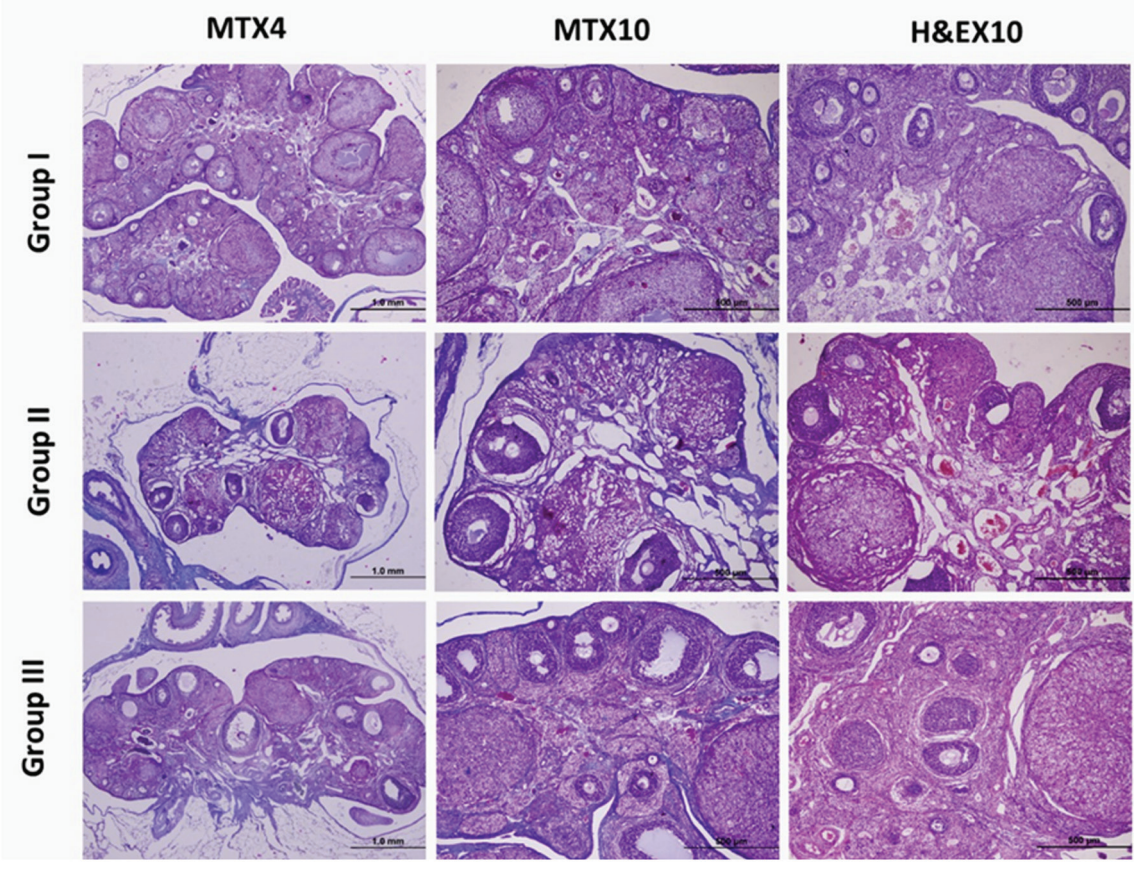

Figure 1. Light microscopic images of MT and H&E staining of all three groups

MT: Masson Trichrome, H&E: Hematoxylin and Eosin

The TAS levels were determined to be statistically significantly higher in group 1 than in group 2 (1.10±0.11 vs. 0.79±0.12, p<0.001) (Table 2). In group 3, HBO was determined to have statistically increased the TAS values (1.27±0.22, p=0.0008).

Discussion

In this study, the effect of HBO therapy was examined on the ovarian reserve in a rat model of ovarian failure created with CYP. The results of the study showed that there was a significant decrease in ovarian reserve with CYP, then with HBO therapy after CYP, and a statistically significant increase was determined histopathologically in the primordial and primary follicle counts in these rats. From an examination of the literature, it can be seen that the current study is the first to have evaluated the effects of HBO in preventing CYP-related ovarian damage, and to have shown that HBO has a therapeutic effect against ovarian failure of CYP origin.

Chemotherapeutic agents have a negative effect on the reproductive potential of young women, but the mechanisms through which this occurs are still unclear. Alkylating agents, especially CYP, are toxic for the gonads because they are independent of cell proliferation[5]. CYP is used to treat several cancer types, primarily leukemia and lymphoma, which are the most frequently seen cancers in adolescent females[4]. CYP directly damages DNA, induces follicular apoptosis, and produces ROS, which damage ovarian cells[6]. CYP also leads to over-activation of primordial follicles by activating the P13K/AKT/mTOR pathway[7].

Morgan et al.[16] concluded that acute loss of the growing follicle population during chemotherapy results in increased intake of primordial follicles to the growing follicle pool. This damage shows two clinical phenotype stages: 1) amenorrhea, which is generally reversible and usually occurs a short time after chemotherapy, and 2) early menopause, which is generally irreversible and related to over-activation of primordial follicles by chemotherapy. In this study, a statistically significant decrease was determined in the number of primordial and primary follicles in the group administered CYP compared with the control group.

There is a great deal of evidence linking exposure to CYP with follicle atresia and granulosa cell apoptosis[17,18]. It has also been reported that inflammation and vessel expansion with CYP create secondary vascular damage[19]. In this study, a thickened tunica albuginea, and increased vessel structure and vessel expansion in the germinal matrix were determined. This finding of increased vascularization and expansion is consistent with the literature. Considering that venous congestion is thought to cause ovarian insufficiency, free oxygen radicals and inflammatory cytokines can be considered to accumulate more intensively in the ovarian tissue.

Table 2. Comparisons between the groups of the AMH, TAS, and TOS levels

	Group 1	Group 2	Group 3	p
AMH (ng/mL)	8.79±1.37[a]	7.03±0.47[b]	8.83±0.87[a]	<0.05
TOS (µmol/L)	3.85±0.62[a]	7.02±2.29[b]	3.70±1.19[a]	<0.05
TAS (mmol/L)	1.10±0.11[a]	0.79±0.12[b]	1.27±0.22[a]	<0.05

AMH: Anti-müllerian hormone, TAS: Total antioxidant status, TOS: Total oxidant status. The same letters in the same rows indicate similarity between the groups, and different letters indicate a difference

When the literature is examined, it can be seen that there has been an investigation of potential protective agents (eg., Curcumin and capsaicin, Tamoxifen, Sphingosine1-phosphate) against the direct loss or accelerated activation of primordial follicles, increasing atresia of growing follicles, and damage to the vascular system in the ovaries, caused by CYP[15,20,21]. In the study by Melekoğlu et al.[15], it was revealed that CYP causes premature ovarian failure, and it was concluded in this study that Curcumin (CRC) and Capsaicin (CPS) can be used to prevent premature ovarian failure in rats. It was observed that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels decreased in rats receiving CRC and CPS, and simultaneously, amh levels increased with estrogen.

The results of the current study clearly showed that CYP reduced the number of primordial and primary follicles in rat ovaries. The numbers of pre-antral and secondary follicles in the rats administered CYP were also observed to be lower compared with the control group and the rats that received HBO.

The ovaries express the vascularity of primordial and pre-antral follicles from the stromal blood vessels. Additionally, the growth of primary follicles leads to the development of the vascular network in the theca layer. Embryos developing from oocytes obtained from well -vascularised follicles have a higher implantation rate than embryos obtained from oocytes developing from follicles with poor vascularity[22].

The positive effects of HBO on wound healing have been documented in animal experiments. There are many studies showing that HBO increases collagen production, supports angiogenesis and leads to the development of the vascular network causing the formation of new capillaries by inducing the release of stem cells from the bone marrow into circulation, thereby promoting mechanisms that trigger regeneration and healing[23,24].

HBO therapy triggers the angiogenesis mechanism by providing the oxygen needed and with increased release of several cytokines such as vascular endothelial growth factor[25]. Angiogenesis is of critical importance for follicular development, oocyte quality, and early embro development. This showed that adjuvant HBO therapy after chemotherapy could regulate ovarian function and hormone levels. Mitrović et al.[26] showed that HBO therapy could be a potential treatment option for infertility by increasing endometrial receptivity through a change in blood vessel resistance. In this study, HBO therapy was seen to correct the abnormal expansions in the blood vessels created by CYP, reduced congestion, and provided a normal vascular structure

close to that of the control group. This correction formed in the vascular structure suggests that follicle loss is prevented by reducing oxidative stress and inflammatory cytokines in the ovary.

In addition to reducing follicle loss, that HBO showed an effect on serum AMH levels was among the main aims of this study. Yu et al.[27] examined the effect of HBO therapy on ovarian function following cystectomy. A total of 60 patients with ovarian cysts were treated with laparoscopic ovarian cystectomy. HBO was added to patients in the observation group in addition to the treatment in the control group. The AMH, FSH, LH, estradiol (E2), and antral follicle count (AFC) serum levels were detected in both groups before the operation and at the first and third menstrual cycles postoperatively to evaluate ovarian function. After the operation, AMH, E2, and AFC serum levels in the observation group were significantly higher than in the control group. FSH and LH serum levels were significantly lower than in the control group, and the differences were statistically significant[27]. In a study of 4 patients by Pineda et al.[28], the effect of HBO was examined on serum AMH levels. In 2 of the 4 patients, the serum AMH level showed an increase of 40% and 116%. In one patient, no change was observed in the serum AMH level, and the other patient became pregnant during the HBO therapy. When the serun AMH levels were examined in the current study, the AMH levels were found to be significantly higher in group 3 than in group 2, which was consistent with the literature[28].

Although some studies have shown that HBO-induced oxidative damage[29,30], several others have stated that HBO has a protective effect against oxidative damage[31,32]. The debate is ongoing on whether HBO acts as an oxidant promoter or as an antioxidant agent. It has been shown that ROS and mitochondrial DNA (mtDNA) affect cellular aging in the human body, including the female reproductive system. More importantly, studies have suggested that ovarian aging could be negatively affected by excessive ROS[33]. Van Blerkom et al.[34] showed the importance of oxygen in oocyte meiosis. It was reported in that study that a decrease in oxygen content in the ovarian follicular fluid in humans is associated with an increase in abnormalities in the organization of chromosomes on the metaphase spindle, and it was emphasized that a sufficient oxygen source is necessary to allow the acceleration of chromosomes and normal oocyte maturation[34,35]. In this study, TOS was seen to be increased in group 2 compared with group 1, and TAS was statistically significantly higher in group 3 than in group 2. These results suggest that follicle loss in the ovaries was reduced and the negative effects of CYP were prevented with HBO therapy.

Study Limitations

The strength of our study is that it evaluated both tissue oxidative stress markers, ovarian reserve markers, and histopathological parameters together. The limitation of our study is that this study is an animal experiment, and further clinical studies should be done to show its effect on humans.

Conclusion

CYP negatively affects the ovarian reserve and causes severe follicle loss. HBO therapy could be an alternative treatment to minimize the effect on ovarian follicle loss of these alkylating agents, which must be used for treating tumors that commonly occur in young females of reproductive age. Although different pressures and durations of HBO were not applied in this study, the dose applied suggests that HBO could be protective for the ovaries. There is a need for further clinical trials to provide more clarification on this subject.

Ethics

Ethics Committee Approval: This study was approved by the Erciyes University Animal Experiments Ethic Committee (no: 21/168; date: 07.07.2021).

Informed Consent: Not necessary.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.Ç., M.A.B., A.C., M.E., Concept: F.Ç., M.A.B., E.M.A., Design: F.Ç., M.A.B., M.D., Data Collection or Processing: E.B., A.C., B.Y., Analysis or Interpretation: E.B., A.C., B.Y., Literature Search: F.Ç., M.A.B., M.D., E.M.A., Writing: F.Ç., M.A.B., M.D., E.B., A.C., E.M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The work was funded by the Erciyes University Scientific Research Projects Coordination Unit (TSA-2022- 11550).

References

1. Findlay JK, Hutt KJ, Hickey M, Anderson RA. How Is the Number of Primordial Follicles in the Ovarian Reserve Established? Biol Reprod 2015;93:111.
2. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004;428:145-50.
3. Clarkson YL, McLaughlin M, Waterfall M, Dunlop CE, Skehel PA, Anderson RA, et al. Initial characterisation of adult human ovarian cell populations isolated by DDX4 expression and aldehyde dehydrogenase activity. Sci Rep 2018;8:6953.
4. Ahmad SS, Reinius MA, Hatcher HM, Ajithkumar TV. Anticancer chemotherapy in teenagers and young adults: managing long term side effects. BMJ 2016;354:i4567.
5. De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. Lancet 2010;376:911-21.
6. Deng J, Zhong YF, Wu YP, Luo Z, Sun YM, Wang GE, et al. Carnosine attenuates cyclophosphamide-induced bone marrow suppression by reducing oxidative DNA damage. Redox Biol 2018;14:1-6.
7. Roness H, Kashi O, Meirow D. Prevention of chemotherapy-induced ovarian damage. Fertil Steril 2016;105:20-9.
8. Shelling AN. Premature ovarian failure. Reproduction 2010;140:633-41.

9. Grossmann B, Saur S, Rall K, Pecher AC, Hübner S, Henes J, et al. Prevalence of autoimmune disease in women with premature ovarian failure. Eur J Contracept Reprod Health Care 2020;25:72-75.
10. Nelson LM. Clinical practice. Primary ovarian insufficiency. N Engl J Med 2009;360:606-14.
11. Depalo R, Nappi L, Loverro G, Bettocchi S, Caruso ML, Valentini AM, et al. Evidence of apoptosis in human primordial and primary follicles. Hum Reprod 2003;18:2678-82.
12. Ben-Aharon I, Shalgi R. What lies behind chemotherapy-induced ovarian toxicity? Reproduction 2012;144:153-63.
13. van der Stege JG, Groen H, van Zadelhoff SJ, Lambalk CB, Braat DD, van Kasteren YM, et al. Decreased androgen concentrations and diminished general and sexual well-being in women with premature ovarian failure. Menopause 2008;15:23-31.
14. Podfigurna-Stopa A, Czyzyk A, Grymowicz M, Smolarczyk R, Katulski K, Czajkowski K, et al. Premature ovarian insufficiency: the context of long-term effects. J Endocrinol Invest 2016;39:983-90.
15. Melekoglu R, Ciftci O, Eraslan S, Cetin A, Basak N. Beneficial effects of curcumin and capsaicin on cyclophosphamide-induced premature ovarian failure in a rat model. J Ovarian Res 2018;11:33.
16. Morgan S, Anderson RA, Gourley C, Wallace WH, Spears N. How do chemotherapeutic agents damage the ovary? Hum Reprod Update 2012;18:525-35.
17. Chen XY, Xia HX, Guan HY, Li B, Zhang W. Follicle Loss and Apoptosis in Cyclophosphamide-Treated Mice: What's the Matter? Int J Mol Sci 2016;17:836.
18. Yuksel A, Bildik G, Senbabaoglu F, Akin N, Arvas M, Unal F, et al. The magnitude of gonadotoxicity of chemotherapy drugs on ovarian follicles and granulosa cells varies depending upon the category of the drugs and the type of granulosa cells. Hum Reprod 2015;30:2926-35.
19. Pascuali N, Scotti L, Di Pietro M, Oubiña G, Bas D, May M, et al. Ceramide-1-phosphate has protective properties against cyclophosphamide-induced ovarian damage in a mice model of premature ovarian failure. Hum Reprod 2018;33:844-59.
20. Piasecka-Srader J, Blanco FF, Delman DH, Dixon DA, Geiser JL, Ciereszko RE, et al. Tamoxifen prevents apoptosis and follicle loss from cyclophosphamide in cultured rat ovaries. Biol Reprod 2015;92:132.
21. Li S, Chen J, Fang X, Xia X. Sphingosine-1-phosphate activates the AKT pathway to inhibit chemotherapy induced human granulosa cell apoptosis. Gynecol Endocrinol 2017;33:476-9.
22. Gordon JD, Shifren JL, Foulk RA, Taylor RN, Jaffe RB. Angiogenesis in the human female reproductive tract. Obstet Gynecol Surv 1995;50:688-97.
23. Oscarsson N, Ny L, Mölne J, Lind F, Ricksten SE, Seeman-Lodding H, et al. Hyperbaric oxygen treatment reverses radiation induced pro-fibrotic and oxidative stress responses in a rat model. Free Radic Biol Med 2017;103:248-55.
24. Thom SR, Bhopale VM, Velazquez OC, Goldstein LJ, Thom LH, Buerk DG. Stem cell mobilization by hyperbaric oxygen. Am J Physiol Heart Circ Physiol 2006;290:H1378-86.
25. Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z, Hunt TK. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. Arch Surg 2000;135:1293-7.
26. Mitrović A, Nikolić B, Dragojević S, Brkić P, Ljubić A, Jovanović T. Hyperbaric oxygenation as a possible therapy of choice for infertility treatment. Bosn J Basic Med Sci 2006;6:21-4.
27. Yu J, Qi YL, Lu DW, Fang QJ, Li L, Sang L. Multiple Protocols Combined with Hyperbaric Oxygen Therapy on the Maintenance of Ovarian Function in Patients After Ovarian Cystectomy. Front Surg 2022;20;9:877114.
28. Pineda JF, Ortiz CG, Moguel Gde J, Lopez CR, Alcocer HM, Velasco ST. Improvement in Serum Anti-Müllerian Hormone Levels in Infertile Patients after Hyperbaric Oxygen (preliminary results). JBRA Assist Reprod 2015;1;19:87-70.
29. Benedetti S, Lamorgese A, Piersantelli M, Pagliarani S, Benvenuti F, Canestrari F. Oxidative stress and antioxidant status in patients undergoing prolonged exposure to hyperbaric oxygen. Clin Biochem 2004;37:312-7.
30. Oter S, Korkmaz A, Topal T, Ozcan O, Sadir S, Ozler M, et al. Correlation between hyperbaric oxygen exposure pressures and oxidative parameters in rat lung, brain, and erythrocytes. Clin Biochem 2005;38:706-11.
31. Yasar M, Yildiz S, Mas R, Dundar K, Yildirim A, Korkmaz A, et al. The effect of hyperbaric oxygen treatment on oxidative stress in experimental acute necrotizing pancreatitis. Physiol Res 2003;52:111-6.
32. Ozden TA, Uzun H, Bohloli M, Toklu AS, Paksoy M, Simsek G, et al. The effects of hyperbaric oxygen treatment on oxidant and antioxidants levels during liver regeneration in rats. Tohoku J Exp Med 2004;203:253-65.
33. Rizzo A, Roscino MT, Binetti F, Sciorsci RL. Roles of reactive oxygen species in female reproduction. Reprod Domest Anim 2012;47:344-52.
34. Van Blerkom J, Antczak M, Schrader R. The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. Hum Reprod 1997;12:1047-55.
35. Van Blerkom J. Intrafollicular influences on human oocyte developmental competence: perifollicular vascularity, oocyte metabolism and mitochondrial function. Hum Reprod 2000;15(Suppl 2):173-88.