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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

LETTER FROM THE PRESIDENT

Dear Turkish Gynecology and Obstetrics Family,

I am proud and honored to share the latest issue of our international and well-respected scientific journal in 2024 with my esteemed colleagues. In this issue, I congratulate our department editors, especially our editors, and scientific reviewers taking part in the article evaluation process.

Dear colleagues, TJOD is not only an association of obstetricians, but also an organization giving reaction to professional events in our country in a timely manner. TJOD has contributed to the "Normal Birth Action Plan" announced by the Ministry of Health in October by giving a timely reaction. As TJOD, we have made significant contribution to the preparation of the "Maternity Action Management Guide for Midwives" prepared by the Ministry of Health.

Since we attach great importance to scientific meetings in our country, "TJOD Webinar Series" has been put into effect and attracted great attention. Moreover, the TJOD family has undertaken the responsibility of educating our young colleagues in the light of science, and in this context, it has started the "TJOD Assistant Courses", which is a continuation of the assistant school project previously carried out by our association and the first of which will be implemented in December 2024.

As it is seen, the Turkish Society of Gynecology and Obstetrics is an organization that has a say in many important issues related to gynecology and obstetrics in our country. By committing to the progress of our scientific journal on the scientific platform in 2025, I wish the new year to bring peace, happiness, health and prosperity to our country and the world.

Best Regards
İsmail Mete Itil, Prof. MD
President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

We are together again with the December issue of TJOD, the international publication of the Turkish Society of Gynecology and Obstetrics. Following a three-month period, we are proud to bring you the articles with the highest scientific value among the many original researches, reviews and meta-analyses sent to our journal at the end of an intensive effort.

I would like to state that the recognition of our journal has increased day by day, reading rate has increased, and it has been cited by scientific journals that have a respected place in our field. Finally, I would like to thank our team who have contributed to this success and used their scientific knowledge for our journal.

Ercan Yılmaz, Prof. MD
Fatih Şendağ, Prof. MD

Clinical Investigation / Araştırma

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Fractalkine/CX3CL1 and macrophage inflammatory protein- 1β/CCL4 activity in the rat ovary with induced ovarian hyperstimulation

İndüklenmiş ovaryan hiperstimülasyonlu olan sıçan overinde fraktalkin/CX3CL1 ve makrofaj enflamatuvar protein-1β/CCL4 aktivitesinin araştırılması

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Abstract

Objective: Fractalkine (CX3CL1) and macrophage inflammatory protein-1β (MIP-1β)/CCL4 play a role in chemotactic activity, immune response, and inflammatory response. We aimed to investigate the effects of fractalkine and MIP-1β in the development of ovarian hyperstimulation syndrome (OHSS) by considering the inflammatory response during ovulation.

Materials and Methods: Two equal groups of 20 immature female rats were created. Given that one of the rats in the group died, the control group was made up of 9 rats. Group 1 (G1) (n=9): Control group; G2 (n=10): OHSS group. Rats in the G2 group were administered 10 IU FSH for 4 days and 30 IU human chorionic gonadotropin on the fifth day. At 34 days old, all rats were sacrificed, and blood and ovarian tissue samples were collected to measure CX3CL1, CX3CL1R, MIP- 1β, tumor necrosis factor-alpha (TNF-α), interleukin (IL-8), hypoxia-inducible factor (HIF-1α), and interferon-gamma (IFN-γ) levels. Immunohistochemical scoring was performed for CX3CL1 and CX3CL1R in other ovarian tissue.

Results: Rat and ovary weights and serum CX3CL1, CX3CLR1, HIF-1α, MIP-1β, TNF-α, IFN-γ and IL-8 levels were significantly higher in G2 than in G1. Tissue IL-8, TNF-α, CX3CL1, CX3CLR1, MIP-1β levels and CX3CL1 and CX3CLR1 immunoreactivity scores were significantly higher in G2 than in G1.

Conclusion: CX3CL1 and MIP-1β contribute to the pathophysiology of OHSS by playing a role in the development of inflammation.

Keywords: OHSS, fractalkine/CX3CL1, CCL4/MIP-1β, rat

Öz

Amaç: Fraktalkin (CX3CL1) ve makrofaj enflamatuvar protein-1β (MIP-1β)/CCL4, kemotaktik aktivitenin ve bağışıklık ve enflamatuvar yanıtın patofizyolojisinde rol oynar. Ovulasyondaki enflamatuvar yanıtı göz önünde bulundurarak, fraktalkin ve MIP-1β'nin over hiperstimülasyon sendromunun (OHSS) patofizyolojisindeki enflamatuvar süreçteki aktivitesini araştırmayı amaçladık.

Gereç ve Yöntemler: Yirmi adet immatür dişi sıçan 2 eşit gruba ayrıldı. Gruptaki sıçanlardan birinin ölmesi üzerine kontrol grubu 9 sıçandan oluşturuldu. Grup 1 (G1) (n=9): Kontrol grubu, G2 (n=10): OHSS grubu. G2'deki sıçanlara 4 gün boyunca 10 IU FSH ve beşinci gün 30 IU insan koryonik gonadotropin uygulandı. Otuz dördüncü günde tüm sıçanlar dekapite edildi ve biyokimyasal analizler [kan ve over doku örneklerinde CX3CL1, CX3CL1R, MIP-1β, tümör nekroz faktör-alfa (TNF-α), interlökin (IL-8), hipoksi indüklenebilir faktör (HIF-1α), gama-interferon (IFN-γ) tespit edildi] ve CX3CL1 ve CX3CL1R için immünohistokimyasal skorlama yapıldı.

PRECIS: Fractalkine/CX3CL1 and MIP-1β/CCL4 may play a role in the development of OHSS

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Bulgular: Sıçan ve over ağırlıkları ve serum CX3CL1, CX3CLR1, HIF-1α, MIP-1β, TNF-α, IFN-γ ve IL-8 düzeyleri G2'de G1'e göre anlamlı derecede yüksekti. Doku IL-8, TNF-α, CX3CL1, CX3CLR1, MIP-1β düzeyleri ve CX3CL1 ve CX3CLR1 immünoreaktivite skorları G2'de G1'e göre anlamlı derecede yüksekti.

Sonuç: CX3CL1 ve MIP-1β, enflamatuvar sürecin gelişiminde rol oynayarak OHSS patofizyolojisine katkıda bulunmaktadır.

Anahtar Kelimeler: OHSS, fraktalkine/CX3CL1, CCL4/MIP-1β, sıçan

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a serious complication that usually occurs after gonadotropin therapy to achieve ovarian stimulation during infertility treatment cycles. Mild OHSS develops in one-third of cycles, whereas moderate or severe OHSS occurs in close to 5% of cases. OHSS develops at a rate of 20% in high-risk patients[1].

In OHSS, abdominal pain due to advanced cystic growth in the ovaries and abdominal distension occurs. However, fluid leakage into the third space due to increased capillary permeability may cause pericardial and pleural effusion as well as widespread edema. Consequently, thromboembolism, adult respiratory distress syndrome, and eventually acute renal failure may occur, which can be life-threatening. These complications usually occur in severe OHSS[2].

The following stimulation of the ovaries with gonadotropin, human chorionic gonadotropin (hCG) administration increases the secretion of vascular endothelial growth factor (VEGF) in granulosa cells. In addition to VEGF, systemic and local vasoactive substances such as interleukins, histamine, prostaglandins, angiotensin II, and prolactin, as well as transforming growth factor-beta (TGF-β), are also involved both directly and indirectly in the pathogenesis of OHSS. It has also been shown to play an indirect role[1,3].

Chemokines, which are first synthesized in inflammation, play an important role in regulating leukocyte recruitment and migration[4]. Chemokines are strongly expressed in macrophages, lymphocytes, and natural killer cells, which are leukocyte populations[5]. Fractalkine (CX3CL1), a transmembrane glycoprotein with adhesion and chemotactic properties, plays a role in many pathophysiological processes, such as tissue repair, as well as the immune and inflammatory response of chemokine cells[6]. After binding to its specific receptor, CX3CL1, it plays a role in the physiological process by providing access to inflammatory response sites specific to various Inflammatory cells such as natural killer (NK) and mast cells. It achieves these effects through its chemotactic properties[7]. Tumor necrosis factor (TNF)-α, interferon-gamma (IFN)-γ, interleukin (IL)-1β, and lipopolysaccharide can initiate CX3CL1 expression in vascular endothelial cells[8]. Macrophage inflammatory protein- 1β (MIP-1β), a chemokine, plays an important role in the immune response by stimulating T-cell adhesion to the endothelial surface[9].

In this experimental pilot study, the effects of fractalkine and MIP-1β, which are proinflammatory factors associated with OHSS and inflammation, were investigated in rats with OHSS.

Materials and Methods

This experimental study was conducted after receiving approval from Fırat University Animal Experiments Local Ethics Committee (decision no: 2023/03-08, date: 27.02.2023).

In this rat experiment, 20 female Sprague-Dawley rats, aged 22-24 days, was used. In our study conducted at the Fırat University Experimental Animals Laboratory, the rats were kept in cages of five in a room with 12 hours of artificial light (08-22), 12 hours of darkness, and a temperature of 21-23°C in order to maintain their biological rhythms. Standard pellet feed and city water were used to feed the animals. Feed was placed in steel containers, and water was placed in glass bottles. The animals' cages were cleaned daily. The entire experimental procedure was performed in accordance with the guidelines (NIH Guide for the Care and Use of Laboratory Animals).

A total of 20 rats aged 22-24 days were divided into two groups: control and study groups (10 animals per group).

Group 1 (n=10): Group that did not receive any medication or surgery. Group 2 (n=10): OHSS induced group. Rats in all groups were weighed before the experimental procedure and sacrifice. Since one rat in the control group died, the control group was continued with 9 rats. To create ovarian hyperstimulation, 10 randomly selected rats were subcutaneously administered 10 IU of FSH for four consecutive days. Then, 10 rats were subcutaneously administered 30 IU hCG on the fifth day. The OHSS model was established in rats using the weight gain determination method used by Ohba et al.[10]. The weights of all animals were measured using a precision scale at 16.00 every day.

After it was shown that OHSS was induced in rats, the abdomens of the rats were opened under anesthesia by administering 40 mg/kg ketamine and 20 mg/kg xylazine on day 34. The ovaries were removed as a whole and immediately weighed on a precision scale, and the ovarian weights were recorded. Then, approximately 3-4 cc of blood was taken from the right ventricle of all rats into gel biochemistry tubes. After blood collection, rats were euthanized with high-dose anesthesia. The right ovary tissue was stored at -80°C until the day of the biochemical examination. The left ovary tissue was fixed with 10% formaldehyde for immunohistochemical examination and embedded in paraffin blocks.

Biochemical Analysis

Blood obtained from rats was placed into biochemistry tubes and centrifuged at 4000 rpm for 10 minutes at +4°C, then the serum was separated. Serum samples were divided into

portions in Eppendorf tubes and stored at -80 ºC until the day of biochemical analysis.

Right ovary tissues (1:9; w:v) were taken as a whole into tubes containing 0.01 M phosphate buffer (PBS; pH 7.4) and homogenized at 16000 rpm at 4ºC for 3 min. The obtained homogenates were centrifuged at 5000xg for 15 minutes (+4ºC) and the supernatants were separated. Protein levels in the supernatants were determined by measuring the blue complex formed by the proteins with the Folin-Phenol reagent at 650 nm in an alkaline environment. The CX3CL1, CX3CL1R, IFN-γ, IL-8, TNF-α, MIP-1β, hypoxia inducible factor (HIF-1α) levels of the supernatants were, as measured by enzyme-linked immunosorbent assay (ELISA). The values per mg of protein were used to calculate the results.

Tissue and serum CX3CL1, CX3CL1R, MIP-1β, HIF-1α, TNF-α, IFN-γ and IL-8 levels were measured using separate kits for each parameter in accordance with the manufacturer's kit procedures. Absorbance was read spectrophotometrically at 450 nm using an EPOCH 2 (BioTek Instrument, Inc, USA) microplate reader. The biochemical results obtained were expressed as ng/mg protein for CX3CL1, CX3CLR1 and HIF-1α, while they were expressed as pg/mg protein for MIP-1β, TNF-α, IFN-γ and IL-8.

The manufacturer, country of origin, catalog number, kit measurement range, and kit sensitivity for all biochemically studied parameters are shown in Table 1.

Immunohistochemical Staining

Sections of 4-6 µm thickness were obtained from paraffin blocks and placed on polylysine slides. After obtaining the CX3CL1 and CX3CR1 primary antibodies (CX3CL1 Polyclonal Antibody, bs-0811R/CX3CR1 Polyclonal Antibody, bs-1728R, Bioss, USA) were obtained, Immunohistochemical staining was performed using the avidin-biotin-peroxidase (ABC) complex method with minor modifications[11]. The preparations were examined and evaluated using a Leica DM500 microscope and photographed (Leica DFC295). The histoscore was calculated according to the extent and intensity of immunoreactive staining.

Scoring for the extent of immunostaining: Less than 25% staining =0.1; Staining between 26% and 50%=0.4; Staining between 51-75% = 0.6; Staining between 76-100% = 0.9

Scoring for the intensity of immunostaining: No staining intensity = 0; very little staining = +0.5; little staining = + 1; moderate staining = +2; severe staining = +3

Histoscore = extent × severity[11].

Statistical Analysis

For the statistical analyses of the data, the Shapiro-Wilks and Kolmogorov-Smirnov tests were used to check whether the normality assumptions were met. The Mann-Whitney U test was used to compare binary groups, and the Kruskal-Wallis test was deemed appropriate to compare multiple groups. SPSS version 22 was used for statistical analysis and the mean ± standard deviation values were examined in statistical evaluations. In the analyses, p-value <0.05 was taken as the critical value and was considered statistically significant.

Results

The mean rat weight was significantly increased in G2 compared with G1 (49.71±3.40 g vs. 64.22±5.55 g), (p<0.001), (Table 2). The ovarian weight was significantly greater in G2 than in G1 (0.021±0.003 g vs. 0.064±0.021 g), (p<0.001), (Table 2).

compared with G1, a statistically significant increase in serum CX3CL1 (p=0.001), CX3CLR1 (p=0.002), HIF-1α (p=0.003), MIP- 1β (p=0.001), TNF-α (p=0.001), IFN-γ (p=0.039) and IL8 (p=0.001) levels were observed in G2 (Table 2). Compared with G1, tissue IL-8 (p=0.004), TNF-α (p=0.001), CX3CL1 (p=0.013), CX3CLR1 (p=0.014), MIP-1β (p=0.001) levels were statistically significantly higher in G2; no statistically significant difference was detected between G1 and G2 in HIF-1α (p=0.108) and IFN-γ (p=0.166) levels (Table 3).

Compared with G1, the CX3CL1 immunoreactivity score was significantly higher in G2 (p<0.001), (Table 2), (Figure 1a, Figure 1b). When comparing G1 with G2, a statistically significant increase in the CX3CLR1 immunoreactivity score was detected in G2 (p=0.001), (Table 2), (Figure 1c, Figure 1d).

Table 1: Country, company, catalog number, kit measurement range and kit sensitivity of the ELISA kits used in the study

Parameters	Company and country	Catalog number	Measuring range	Sensitivity
TNF-α	BioTek Instrument, Inc, USA	ELK1396	15.63-1000 pg/mL	6.1 pg/mL
IL-8	BioTek Instrument, Inc, USA	E1167Ra	5-1500 ng/L	2.52 ng/L
IFN-γ	BioTek Instrument, Inc, USA	ELK1133	15.63-1000 pg/mL	5.8 pg/mL
HIF-1α	BioTek Instrument, Inc, USA	ELK1604	0.16-10 ng/mL	0.056 ng/mL
MIP-1β	BioTek Instrument, Inc, USA	ELK2545	78.13-5000 pg/ml	30 pg/mL
CX3CL1	BioTek Instrument, Inc, USA	ELK1423	0.16-10 ng/mL	0.055 ng/mL
CX3CLR1	BioTek Instrument, Inc, USA	RE2954R	1.57-100 ng/mL	0.94 ng/mL

TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1 beta, IFN-γ: Interferon-gamma, HIF-1α: Hypoxia-inducible factor-1 alpha, MIP-1β: Macrophage inflammatory protein-1 beta, CX3CL1: Fractalkine,
CX3CLR1: Fractalkine receptor-1, ELISA: Enzyme-Linked Immunosorbent Assay

Discussion

In our study, we showed that serum and tissue levels of CX3CL1, CX3CL1R, MIP-1β, IL-8 and TNF-α were significantly increased in patients with OHSS. Similarly, serum HIF-1α and IFN-γ levels were significantly higher in the OHSS group. However, tissue HIF-1α and IFN-γ levels were similar between the OHSS and control groups. Based on the results obtained from our study, we showed that fractalkine and MIP-1β may contribute to the development of OHSS because of their proinflammatory properties. CX3CL1 plays a role in the inflammatory process by interacting with inflammatory cytokines (12). We also observed that the levels of fractalkine, a proinflammatory cytokine, increased significantly in the OHSS group, along with TNF-α and IL-8 levels, in rats in which we induced OHSS. In this study, we showed that OHSS may cause a systemic inflammatory response in addition to the inflammatory response it induces in ovarian tissue. Espey[13] proposed the hypothesis that ovulation is actually an inflammatory reaction, demonstrating that the inflammatory events that occur in OHSS can also be a normal physiological process. Considering that many follicles ovulated in the OHSS group in our study, inflammatory processes may occur more severely in OHSS than in normal ovulation. We can think of this as a protective response of the ovarian tissue to tissue damage and inflammation caused by ovulation. This inflammatory response is caused by the release of chemokines and cytokines, vasodilation, immune cell infiltration, and locally produced molecular mediators to eliminate the inflammatory stimulus[14]. We believe that OHSS-induced damage to the ovaries due to ovulation triggering may also cause an inflammatory response. To understand the inflammatory process that develops as a result of normal ovulation and OHSS, it is useful to review the relationship between ovarian tissue and leukocytes. Leukocytes are localized in the periphery, interstitium, and corpus luteum of ovarian follicles. The leukocytes involved in this study secrete proteases as well as inflammatory mediators, such as cytokines and adhesion molecules, likely working in conjunction with ovarian matrix proteins[15]. In our study, we found that fractalkine, a proinflammatory cytokine, and fractalkine receptor activity were significantly increased in the ovarian tissue of patients with OHSS. In addition, we found that ovarian tissue and serum levels were significantly increased in OHSS. It has been reported that monocyte CX3CR1 mRNA and protein expression levels are increased in patients with septic shock. The results of the study revealed a significant relationship between the decrease in CX3CR1 expression and poor prognosis of the patient[16]. The similar increase in fractalkine and its receptor in our study suggests that fractalkine can be controlled in a balanced manner in the ovaries of patients with OHSS and may also have an effect on the severity of OHSS. The fact that fractalkine is present

Table 3. CX3CL1, CX3CL1R, MIP-1β, HIF-1α, TNF-α, IFN-γ and IL-8 levels in tissue of G1 and G2, values are presented as mean ± standart deviation, p<0.05 was considered to be statistically significant

Parameters	G1 (n=9)	G2 (n=10)	p
CX3CL1 (ng/mg)	0.17±0.05	0.24±0.40	0.013*
CX3CL1R (ng/mg)	2.55±1.18	5.15±2.63	0.014*
MIP-1β, pg/mg	140.83±67.04	504.10±172.64	0.001*
HIF-1α, ng/mg	0.09±0.03	3.39±5.81	0.108
TNF-α, pg/mg	116.98±53.15	260.66±54.30	0.000*
IFN-γ, ng/mg	9.47±2.89	7.90±1.78	0.166
IL-8 (pg/mg)	7.04±4.10	20.51±22.34	0.004*

G1: Control group, G2: OHSS group, HIF-1α: Hypoxia-inducible factor-1 alpha, MIP-1β: Macrophage inflammatory protein-1 beta, TNF-α: Tumor necrosis factor-alpha, IL-8: Interleukin-8, IFN-γ: Interferon-gamma, *: Compared with G1, CX3CL1: Fractalkine, CX3CLR1: Fractalkine receptor-1

Table 2. Rat and ovarian weight (mean ± standard deviation), ovarian CX3CL1 and CX3CL1R immunoreactivity scores [median (minimum-maximum)] and CX3CL1, CX3CL1R, MIP-1β, HIF-1α, TNF-α, IFN-γ and IL-8 levels in serum of G1 and G2, values are presented as mean ± standard deviation, p <0.05 was considered to be statistically significant

Parameters	G1 (n=9)	G2 (n=10)	p
Rat weight (gr)	49.71±3.40	64.22 ± 5.55	0.001*
Ovarian weight (gr)	0.021±0.003	0.064 ± 0.021	0.001*
CX3CL1	0.45 (0.30-0.60)	0.90 (0.80-1.20)[a]	0.000*
CX3CL1R	0.40 (0.20-0.60)	0.70 (0.45-1.80)[a]	0.001*
MIP-1β, pg/mL	93.95±58.15	1126.56±536.12	0.000*
HIF-1α, ng/mL	0.22±0.05	0.28±0.03	0.003*
TNF-α, pg/L	143.85±70.77	402.59±152.55	0.000*
IFN-γ, ng/L	14,830,99	18.95±4.71	0.039*
IL-8 (pg/mg)	157.62±24.88	201.74±19.33	0.000*

G1: Control group, G2: OHSS group, HIF-1α: hypoxia-inducible factor-1 alpha, MIP-1β: macrophage inflammatory protein-1 beta, TNF-α: tumor necrosis factor-alpha, IL–8: interleukin-8, IFN-γ: interferon-gamma, *: Compared with G1, CX3CL1: Fractalkine, CX3CLR1: Fractalkine receptor-1, OHSS: Ovarian hyperstimulation syndrome

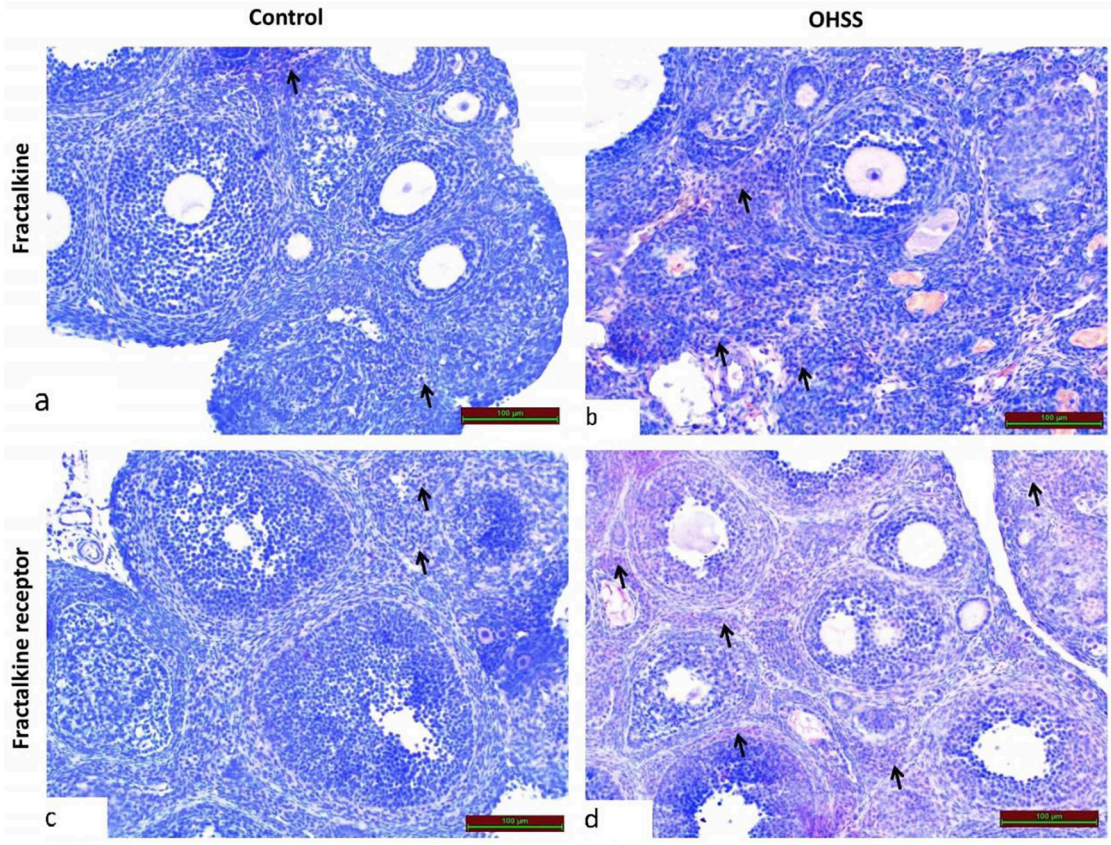

Figure 1. Immunohistochemical staining for fractalkine and fractalkine receptor in rat ovarian tissue belonging to the control and OHSS groups (a and b). Fractalkine and fractalkine receptor immunopositive cells are indicated by black arrows. A significant increase in fractalkine and fractalkine receptor immunoreactivity is observed in the OHSS group compared to the control group (c and d)

OHSS: Ovarian hyperstimulation syndrome

at higher levels in luteinizing granulosa cells (GCs) compared with GCs of the follicular phase indicates that fractalkine levels increase during the preovulatory period[17]. This increase may also explain why serum fractalkine levels are higher in women with polycystic ovary syndrome (PCOS) than in controls[18]. Fractalkine and CX3CR1 expression has been demonstrated in human ovaries, and fractalkine has been reported to stimulate progesterone biosynthesis in human luteinized granulosa cells, probably by increasing the expression of proteins associated with steroidogenesis[19]. In this context, we can compare OHSS and PCOS ovaries characterized by multifollicles. Increased fractalkine levels in women with PCOS may be associated with increased OHSS risk. As in PCOS, fractalkine and its receptor may balance steroidogenesis and the inflammatory response related to ovulation in OHSS. Fractalkine induces the migration of cytotoxic effector lymphocytes, promoting the subsequent migration of these lymphocytes to secondary chemokines such as MIP-1β/CCL4 or IL-8/CXCL8. Based on these results, fractalkine expressed in the inflamed endothelium can function as a vascular regulators of cytotoxic effector lymphocytes[20]. Skinner et al.[21] showed that MCP1, MCP2, MIP-1β and chemokine C-C motif ligand-5 (CCL5) mRNA levels increased in bovine granulosa and/or theca cells during antral follicle development. In our study, we also showed that MIP-1β levels increased significantly in the serum and tissue in the OHSS group along with an increase in fractalkine. Our findings suggest that MIP-1β may also play a role in the pathophysiology of OHSS.

HIF-1α is one of the main regulators of the cellular response to hypoxia. Hypoxia mediates several cellular responses by increasing HIF-1α and NF-κB activity[22]. In addition, HIF-1α has been shown to be associated with increased IFN-γ production[23]. In this study, we showed that serum IFN-γ and HIF-1α levels were higher in the OHSS group than in the control group. However, tissue IFN-γ and HIF-1α levels were similar between the groups. The difference between serum and tissue HIF-1α and IFN-γ levels may suggest that compensatory mechanisms in the ovary may play a role in hypoxia. In addition, serum parameters may recover later than tissue parameters[24]. Our results indicate that OHSS can cause a hypoxic environment. It has been shown that HIF-1α up-regulates CX3CR1 in hypoxic ovarian cancer cells, leading to increased sensitivity to fractalkine-induced migration and invasion. In our study, we have also shown that tissue expressions and serum levels of

fractalkine and its receptor increased along with the increase in HIF-1α. Our findings show that OHSS, similar to tumor tissue in ovarian cancer, creates a hypoxic environment in ovarian tissue, causing an increase in serum HIF-1 secretion as well as fractalkine and its receptor.

The proinflammatory role of fractalkine has been demonstrated in various animal models. In an experimental study, blockade of the CX3CL1-CX3CR1 axis was shown to improve the inflammation score of intestinal tissue by reducing leukocyte, neutrophil, and cytokine accumulation[25]. Based on this result, we can predict that CX3CL1-CX3CR1 blockade may contribute to the improvement of clinical symptoms and signs by reducing the severity of OHSS- related inflammation. We believe that further studies on this subject will be beneficial. In patients with sepsis, in whom serum fractalkine levels are much higher than in healthy controls, fractalkine levels have been reported to be positively correlated with leukocyte count, TNF-α, IL-1β, IL-6, IL-17A, and IFN-γ levels, while negatively correlated with IFN-γ and IL-10[26]. However, it has been suggested that an increase in TNF-α expression levels is a compensatory response to induce fractalkine expression and maintain its concentration at physiological levels[27]. In our study, we also found that TNF-α levels were higher in the OHSS group, along with increased fractalkine levels. However, IFN-γ levels were higher in serum, while tissue levels were similar between the OHSS and control groups. This may be due to differences between tissues and physiological homeostasis.

Basal IL-8 levels, which are low in the normal ovary, increase rapidly after LH stimulation and stimulate the infiltration of neutrophils into the ovarian tissue[28]. The significant blockade of ovulation in rabbits following the administration of IL-8 or antineutrophil antibodies suggests that chemokines play critical roles in ovulation[29]. There is currently no definitive evidence to demonstrate whether fractalkine affects the phagocytic function of neutrophils by altering IL-8. However, it has been shown that fractalkine effectively reduces IL-8 levels in rats with severe acute pancreatitis[30]. Our study showed that IL-8 levels were significantly increased in the OHSS group compared with the control group. This result revealed the role of IL-8 in the pathophysiology of OHSS.

Study Limitations

The limitations of our study are that the number of cases was limited to prevent waste of rats and that the results obtained from experimental models cannot be exactly the same as those obtained from humans due to differences between species. However, CX3CL1, CX3CL1R, and MIP-1β were studied for the first time in the inflammatory process caused by OHSS, which is the strength of our experimental study.

Conclusion

OHSS causes an increase in the levels of fractalkine and its receptors, MIP-1β, HIF- 1, TNF-α and IL-8, resulting in both an ovarian and systemic inflammatory response. The inhibition of fractalkine may be a treatment modality that can reduce the inflammatory process in OHSS and improve its clinical profile.

Ethics

Ethics Committee Approval: This experimental study was conducted after receiving approval from Fırat University Animal Experiments Local Ethics Committee (decision no: 2023/03-08, date: 27.02.2023).

Informed Consent: Not necessary.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.A., Ş.P., R.A., Concept: G.A., R.A., T.K., S.H., Design: G.A., R.A., N.İ., Data Collection or Processing: Ş.P., T.K., S.H., Analysis or Interpretation: G.A., R.A., T.K., S.H, N.İ., Literature Search: Ş.P., R.A., S.H., Writing: R.A.

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References

1. Nastri CO, Teixeira DM, Moroni RM, Leitão VM, Martins WP. Ovarian hyperstimulation syndrome: pathophysiology, staging, prediction and prevention. Ultrasound Obstet Gynecol. 2015;45:377-93.
2. Timmons D, Montrief T, Koyfman A, Long B. Ovarian hyperstimulation syndrome: A review for emergency clinicians. Am J Emerg Med. 2019;37:1577-84.
3. Palumbo A, Ávila J, Naftolin F. The Ovarian Renin-Angiotensin System (OVRAS): A major factor in ovarian function and disease. Reprod Sci. 2016;23:1644-55.
4. Baggiolini M, Dewald B, Moser B. Human chemokines: an update. Annu Rev Immunol. 1997;15:675-705.
5. Nishiyori A, Minami M, Ohtani Y, Takami S, Yamamoto J, Kawaguchi N, et al. Localization of fractalkine and CX3CR1 mRNAs in rat brain: does fractalkine play a role in signaling from neuron to microglia? FEBS Lett. 1998;429:167-72.
6. Fernández-Prieto M, Fernández-Aceñero MJ, López-Palacios N, Bodas A, Farrais S, Cuevas D, et al. CX3CL1-CX3CR1 axis: a new player in coeliac disease pathogenesis. Nutrients. 2019;11:2551.
7. Ishida Y, Hayashi T, Goto T, Kimura A, Akimoto S, Mukaida N, et al. Essential involvement of CX3CR1-mediated signals in the bactericidal host defense during septic peritonitis. J Immunol. 2008;181:4208-18.
8. Nakayama T, Watanabe Y, Oiso N, Higuchi T, Shigeta A, Mizuguchi N, et al. Eotaxin-3/CC chemokine ligand 26 is a functional ligand for CX3CR1. J Immunol. 2010;185:6472-9.
9. Tanaka Y, Hoshino-Negishi K, Kuboi Y, Tago F, Yasuda N, Imai T. Emerging role of fractalkine in the treatment of rheumatic diseases. Immunotargets Ther. 2020;9:241-53.
10. Ohba T, Ujioka T, Ishikawa K, Tanaka N, Okamura H. Ovarian hyperstimulation syndrome-model rats; the manifestation and clinical implication. Mol Cell Endocrinol. 2003;202:47-52.
11. Şanlı C, Atılgan R, Kuloğlu T, Pala Ş, Aydın Türk B, Keser HB, et al. Transient receptor potential melastatin 2 ion channel activity in

ovarian hyperstimulation syndrome physiopathology. Turk J Med Sci. 202;51:787-95.

12. Jones BA, Riegsecker S, Rahman A, Beamer M, Aboualaiwi W, Khuder SA, et al. Role of ADAM-17, p38 MAPK, cathepsins, and the proteasome pathway in the synthesis and shedding of fractalkine/CX_3 CL1 in rheumatoid arthritis. Arthritis Rheum. 2013;65:2814-25.
13. Espey LL. Ovulation as an inflammatory reaction--a hypothesis. Biol Reprod. 1980;22:73-106.
14. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454:428-35.
15. Duffy DM, Ko C, Jo M, Brannstrom M, Curry TE. Ovulation: parallels with ınflammatory processes. Endocr Rev. 2019;40:369-416.
16. Pachot A, Cazalis MA, Venet F, Turrel F, Faudot C, Voirin N, et al. Decreased expression of the fractalkine receptor CX3CR1 on circulating monocytes as new feature of sepsis-induced immunosuppression. J Immunol. 2008;180:6421-9.
17. Zhao P, De A, Hu Z, Li J, Mulders SM, Sollewijn Gelpke MD, et al. Gonadotropin stimulation of ovarian fractalkine expression and fractalkine augmentation of progesterone biosynthesis by luteinizing granulosa cells. Endocrinology. 2008;149:2782-9.
18. Demi R İ, Guler A, Alarslan P, Isil AM, Ucman O, Aslanipour B, et al. Fractalkine: an inflammatory chemokine elevated in subjects with polycystic ovary syndrome. Endocrine. 2019;65:175-83.
19. Huang S, Zhao P, Yang L, Chen Y, Yan J, Duan E, et al. Fractalkine is expressed in the human ovary and increases progesterone biosynthesis in human luteinised granulosa cells. Reprod Biol Endocrinol. 2011;9:95.
20. Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science. 2007;317:666-70.
21. Skinner MK, Schmidt M, Savenkova MI, Sadler-Riggleman I, Nilsson EE. Regulation of granulosa and theca cell transcriptomes during ovarian antral follicle development. Mol Reprod Dev. 2008;75:1457-72.
22. Xiao LJ, Chen YY, Lin P, Zou HF, Lin F, Zhao LN, et al. Hypoxia increases CX3CR1 expression via HIF-1 and NF-κB in androgen-independent prostate cancer cells. Int J Oncol. 2012;41:1827-36.
23. Smith V, Lee D, Reardon M, Shabbir R, Sahoo S, Hoskin P, et al. Hypoxia Is Associated with Increased Immune Infiltrates and Both Anti-Tumour and Immune Suppressive Signalling in Muscle-Invasive Bladder Cancer. Int J Mol Sci. 2023;24:8956.
24. Malkov MI, Lee CT, Taylor CT. Regulation of the hypoxia-ınducible factor (HIF) by pro-ınflammatory cytokines. Cells. 2021;10:2340.
25. Becker F, Holthoff C, Anthoni C, Rijcken E, Alexander JS, Gavins FN, et al. Downregulation of CX3CR1 ameliorates experimental colitis: evidence for CX3CL1-CX3CR1-mediated immune cell recruitment. Int J Colorectal Dis. 2017;32:315-24.
26. Chen X, Wei Q, Hu Y, Wang C. Role of Fractalkine in promoting inflammation in sepsis-induced multiple organ dysfunction. Infect Genet Evol. 2020;85:104569.
27. Raei Sadigh A, Darabi M, Salmassi A, Hamdi K, Farzadi L, Ghasemzadeh A, et al. Fractalkine and apoptotic/anti-apoptotic markers in granulosa cells of women with polycystic ovarian syndrome. Mol Biol Rep. 2020;47:3593-603.
28. Bukulmez O, Arici A. Leukocytes in ovarian function. Hum Reprod Update. 2000;6:1-15.
29. Ujioka T, Matsukawa A, Tanaka N, Matsuura K, Yoshinaga M, Okamura H. Interleukin-8 as an essential factor in the human chorionic gonadotropin-induced rabbit ovulatory process: interleukin-8 induces neutrophil accumulation and activation in ovulation. Biol Reprod. 1998;58:526-30.
30. Huang L, Ma J, Tang Y, Chen P, Zhang S, Zhang Y, et al. siRNA-based targeting of fractalkine overexpression suppresses inflammation development in a severe acute pancreatitis rat model. Int J Mol Med. 2012;30:514-20.

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Comparison of the effect of general anesthesia and combined epidural anesthesia on the anesthetic management of gynecological oncological surgery

Jinekolojik onkoloji cerrahisinin anestezi yönetiminde genel anestezi ile genel anesteziye eklenen epidural anestezinin etkisinin karşılaştırılması

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Abstract

Objective: To evaluate the potential advantages of combined general and epidural anesthesia for major gynecological oncological surgeries.

Materials and Methods: The data of 690 gynecological cancer were retrospectively examined, and 223 patients who met the inclusion criteria were included in the study. The patients were divided into two groups: Group G (123 patients who received general anesthesia only) and Group C (100 patients who received combined epidural and general anesthesia. The perioperative follow-up data were comparatively analyzed.

Results: Operation times in Group G were significantly lower than those in Group C (p=0.018). The blood product replacement rate was higher in Group G (p<0.05). Additionally, intraoperative bleeding rates were lower in Group C (p<0.05). Postoperatively, the analgesic requirement time of Group C was significantly later than that of Group G (p=0.0001). The first mobilization time of Group C was substantially earlier (p=0.0001). Thrombosis and cardiac complications were considerably less frequent in group C, although allergic complications were more common (p<0.05). The length of hospital stay was shorter in Group C (p<0.05).

Conclusion: Combined epidural and general anesthesia in gynecological oncological surgeries may improve postoperative outcomes, including reduced analgesic requirements, earlier patient mobilization, shorter hospitalization, and decreased rates of complications, particularly cardiovascular and thrombotic events.

Keywords: General anesthesia, epidural anesthesia, major abdominal surgery, complications, gynecological oncology

Öz

Amaç: Ameliyat sonrası komplikasyonları önlemek amacıyla majör jinekolojik onkolojik ameliyatlarda genel ve epidural anestezi kombinasyonunun avantajlarını araştırmak.

Gereç ve Yöntemler: Bu çalışmaya 690 hastanın verileri retrospektif olarak değerlendirilmiş ve araştırma kriterlerimizi karşılayan 223 hasta çalışmaya dahil edilmiştir. Hastalar, aldıkları anestezi türüne göre iki gruba ayrılmışlardır: Genel anestezi alan 123 hasta Grup G'yi; genel anestezi ile birlikte kombine epidural anestezi uygulanan 100 hasta ise Grup C'yi oluşturmuştur. Her iki grubun hastaları, ameliyat sonrası süreçleri karşılaştırmalı olarak incelenmiştir.

Bulgular: Operasyon süreleri, Grup G'de Grup C'ye kıyasla anlamlı olarak daha kısa bulunmuştur (p=0,018). Grup C'de kan basıncının daha stabil olduğu gözlemlenmiştir. Ayrıca, Grup G'de daha fazla fibrinojen transfüzyonu yapıldığı belirlenmiştir. Buna karşın, Grup C'de intraoperatif kanama oranları, Grup

PRECIS: Combining epidural anesthesia and general anesthesia in major gynecologic oncology may improve postoperative outcomes including decreased postoperative analgesic requirements, earlier mobilization, reduced length of stay and decreased risk of cardiovascular and thrombotic complications.

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G'ye göre daha düşük tespit edilmiştir. Ameliyat sonrası dönemde, Grup C'nin analjezik ihtiyacı Grup G'ye kıyasla anlamlı olarak daha uzun süreli olmuştur (p=0,0001). Aynı zamanda Grup C'de ilk mobilizasyon süresi anlamlı derecede daha kısa bulunmuştur (p=0,0001). Tromboz ve kalp komplikasyonları Grup C'de daha az sıklıkla görülürken, bu grupta hastanede ve yoğun bakım ünitesinde kalış süreleri daha kısa, alerjik komplikasyonlar ise daha sık rastlanmıştır.

Sonuç: Jinekolojik onkolojik cerrahilerde kombine epidural ve genel anestezi, ameliyat sonrası analjezik gereksinimlerinin azalması, erken mobilizasyon, kısa hastanede kalış süresi ve özellikle kardiyovasküler ve trombotik olaylar olmak üzere komplikasyonların azalması dahil postoperatif sonuçları iyileştirebilir.

Anahtar Kelimeler: Genel anestezi, epidural anestezi, majör abdominal cerrahi, komplikasyonlar, jinekolojik onkoloji

Introduction

Gynecologic cancers have many risk factors, management algorithms, and varying outcomes, and they are among the most prevalent cancers concerning women worldwide[1]. Many gynecologic tumors are managed with chemotherapy and/or radiotherapy, but some require neoadjuvant therapy. Surgical procedures are essential for some patients. On the other hand, the preferred method for the surgical treatment of gynecological cancers is radical excision surgery[2], which includes total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH + BSO)[3]. Protocols for optimal perioperative pain management in patients undergoing cytoreductive surgery for gynecologic malignancies include preemptive analgesia, neuraxial and regional techniques, local anesthetic infiltration, and multimodal analgesia[4]. The level of postoperative pain can vary widely, ranging from minor discomfort after minimally invasive cancer surgery to more severe pain following open debulking procedures. Therefore, an individualized perioperative analgesic plan, depending on the surgical approach, is critical. The administration of intravenous general anesthetics, particularly opioids, may lead to adverse effects, such as vomiting, nausea, and ileus, and can increase postoperative morbidity and mortality[5]. Epidural anesthesia combined with general anesthesia is a practicable approach to curbing the need for perioperative anesthesia, alleviating postoperative pain, and mitigating the possibility of complications[5]. Epidural anesthesia is particularly advantageous in patients with cardiovascular or pulmonary system diseases who have a high risk of deep vein thrombosis[6]. Therefore, regional anesthesia may be an effective method for reducing postoperative pain and minimizing the side effects of opioids. However, recent studies have reported contradictory outcomes in patients undergoing gynecologic oncology surgery regarding pain control and postoperative complications[6,7].

Perioperative complications related to anesthesia in gynecological surgeries include myocardial infarction (MI), arrhythmias, atelectasis, hypothermia, and blood loss[8]. In addition, pulmonary thromboembolism, thrombophlebitis, hemodynamic changes (hypotension, hypertension, bradycardia, tachycardia, MI, arrhythmias), fluid-electrolyte imbalances, and blood sugar irregularities may occur during the postoperative period. Epidural anesthesia combined with general anesthesia may reduce these complications[9]. In long-term surgeries, standard anesthetics may not be sufficient to suppress adrenergic, autonomic, and somatic responses accompanied by catastrophic complications due to surgical stimuli and intubation; therefore, additional regional anesthesia combined with general anesthesia is recommended[10,11].

The present study aimed to compare the effects of general and combined epidural anesthesia on the anesthetic management of gynecological oncological surgery.

Materials and Methods

A retrospective analysis was conducted on perioperative and postoperative complications in patients with cervical, endometrial, and ovarian cancer who underwent gynecologic oncologic surgery at Gaziantep University Şahinbey Research and Education Hospital between 01.01.2015 and 01.09.2020. Ethical approval for our study was obtained from the Gaziantep University Clinical Research Ethics Committee in the decision dated 21.10.2020 and numbered 2020/318. This study was conducted in accordance with the current guidelines of the Helsinki Declaration.

Patient Selection Criteria and Subgroups

The study included gynecological oncology patients aged 18-85 with American Society of Anesthesiologists (ASA) I-III risk classifications who underwent general or combined epidural anesthesia for TAH+BSO surgery with midline incisions. The study excluded patients with ASA IV-V risk classifications, those who were treated with other protocols or surgical options, and those with insufficient data. Retrospective data from 690 gynecological oncology patients operated on between 01.01.2015 and 01.09.2020 was reviewed, and 223 patients met the study criteria. The patients were divided into two subgroups: Those who received general anesthesia (Group G, n=123; 55.16%) and those who received combined epidural anesthesia (Group C, n=100; 44.84%).

Study Design and Principles of Anesthetic Management

General anesthesia is the standard approach for our clinic's midline surgeries performed for patients with gynecologic oncology. All patients included in the study were operated using the same algorithm used in the anesthesia procedures. The patients, whose general condition was moderate-well and cooperative, received a dose of 0.03-0.05 mg/kg intravenous (IV) midazolam 15-20 minutes before the surgery for anxiolysis and amnesia. Standard monitoring was performed in all cases (SpO_2, NIK- and IKK2-binding protein, electrocardiogram). IV fentanyl (1-2 mcg/kg) and propofol (2 mg/kg) for general anesthesia inductions, and IV rocuronium (0.4-0.5 mg/kg) for

muscle paralysis were administered to all cases. In addition, a standard cardiac protocol for general anesthesia was used in patients with limited cardiac reserve and ejection fraction below 40%. IV midazolam 0.04-0.06 mg/kg, IV fentanyl 2-3 mcg/kg, and IV propofol 1 mg/kg were administered for general anesthesia induction in these cases; moreover, IV rocuronium 0.3-0.4 mg/kg was administered for muscle paralysis. Endotracheal intubations were performed using an endotracheal tube with an internal diameter of 7.5 mm.

In the maintenance of general anesthesia, drugs were preferred, considering the patients' hemodynamic and blood pressure parameters. Sevoflurane 1.3-2 lt/min or desflurane 5-6 lt/min are preferred for inhalation anesthesia. In addition, IV remifentanil was administered at 0.1-0.5 mcg/kg/min. In addition, anesthesia maintenance was performed in the volume-controlled mode in all patients. The ventilator settings were 8-10 mL/min tidal volume, 34-41 mmHg end-tidal carbon dioxide pressure, 3.5/4 lt/min fresh gas flow amount, and 40-45% FiO_2 level. Furthermore, 10 mg IV rocuronium was administered intermittently to continue muscle paralysis.

Before the initiation of general anesthesia in Group C, the patients were seated or placed in the lateral decubitus. The area for local anesthesia was then cleaned with a skin antiseptic. Subsequently, 10-20 mg of 2% subcutaneous lidocaine was applied to the skin projections of the L3-L4 or L4-L5 intervals. Afterward, the epidural space was entered with a 17-18 gauge thick and 9-10 cm long Tuohy needle with the entry angle facing the cephalic using either the loss of resistance or the hanging drop technique. The epidural catheter was placed into 3-4 cm of the epidural interval. A 3 mL of 2% lidocaine containing adrenaline was injected into the patients as a "test dose" through an epidural catheter. Then, 30-40 mL of isobaric 0.5% bupivacaine was administered into the epidural space after assessing the vital signs. At the end of the procedure, the patients were placed in the supine position, and general anesthesia induction was started as standard.

IV 0.1-0.5 mg/kg was administered as an antiemetic at the end of the surgery. For postoperative analgesia, 1 mg/kg IV tramadol was administered at the end of the surgery in Group G. Moreover, 3-4 mg of HCL was administered through the epidural catheter approximately 1 hour before the end of the surgery in Group C. At the end of the surgery, IV sugammadex was administered 2-2.5 mg/kg in patients aged >55 years to antagonize the residual neuromuscular blockade in both groups. Furthermore, patients aged below 55 years, a total dose of 1 mg of intravenous atropine sulfate and 2.5 mg of intravenous neostigmine was administered. The patients were extubated when they reached sufficient muscle strength and then transferred to the postoperative care unit. Patients with Aldrete scores >8 at the end of postoperative care were transferred to the inpatient department[12].

Data Collection Principles

Demographic data such as age (years), body mass index (BMI), kg/m^2, ASA risk scores[13], primary malignancies, and comorbidities of the patients in both groups were recorded. Undesirable conditions such as hypotension, hypertension, bradycardia, tachycardia, MI, and arrhythmia observed during the perioperative period were documented and categorized as cardiac complications. In addition, the volume and type of IV fluid replacement (mL; crystalloid or colloid), the amount of bleeding (mL), and data on blood product transfusions, including the type [erythrocyte suspension (ES), fresh frozen plasma (FFP), fibrinogen], and amounts (units or international units), were recorded according to the data of the perioperative period. According to intraoperative records, patients with blood loss were defined as those who lost 1000 mL or more of blood during the operation or required blood product replacement. The first analgesic administration time (minutes), first mobilization time (hours), and complications, including pruritus, urinary retention, allergic reaction, and cardiac complications in the postoperative period, were scanned and recorded. The Hospital Data System documented the use of postoperative compression stockings and incidences of thrombophlebitis and thromboembolism. Finally, we recorded the patients' length of stay (days), any transfers to the intensive care unit, and the outcomes of their postoperative treatment, including discharge, transfer to another service, or death.

Statistical Analysis

All data were analyzed using Statistical Package for the Social Sciences software for Windows (v25.0; IBM, Armonk, NY, USA), MedCalc version 20.013 and R-Studio v2023.091. Individual and aggregate data were summarized using descriptive statistics, including means, standard deviations, and medians [interquartile range (IQR)]. Categorical variables are expressed as numbers of cases and percentages (%). The normality of data distribution was analyzed using the Shapiro-Wilk test. Normally distributed parametric data were compared with the Student t-test, whereas the Mann-Whitney U test was used for non-parametric data that did not show a normal distribution. P-values of <0.05 were considered statistically significant. The categorical variables were evaluated using the chi-square test.

Results

Two hundred twenty-three patients were included in the study: 123 (55.16%) in Group G and 100 (44.84%) in Group C. The mean age of participants was 59.55±10.2 years in Group G and 60.10±11.82 years in Group C, showing no significant difference between the groups (p=0.732). The ASA risk score and BMI were also similar between the groups, with no significant differences (p=0.494 and p=0.718, respectively; Table 1). In comparing the surgery duration between the groups, Group G had a median duration of 2 hours (IQR: 2-2.5), while Group C also had a median duration of 2 hours but with a more comprehensive IQR: 2-3. This difference was statistically significant (p=0.018), indicating that the surgical duration was longer in group C than in group G.

Comorbidity rates were 71.5% in Group G and 70% in Group C (p = 0.801). The distribution of primary tumor origins (ovaries,

endometrium, and cervix) was comparable between the two groups, with no statistically significant differences (p=0.850). Other comorbidities, such as hypertension, diabetes mellitus, and aortic stenosis, were also not significantly different between the groups (Table 2). However, asthma was more prevalent in Group C (18%) than in Group G (8.1%), showing statistical significance (p=0.027).

A significant difference was observed between Groups G and C regarding the use of compression stockings. In Group G, 86.2% of patients wore compression stockings, compared with 45% in Group C, a highly significant difference (p<0.001). The odds of wearing compression stockings were 7.62 times higher in Group G than in Group C [odds ratio (OR): 7.62, 95% confidence interval (CI): 3.99-14.5]. The perioperative hemodynamic stability significantly differed between the groups.

Group G had a higher rate of hypertensive patients (63.4%), whereas group C had only 14% hypertensive cases during the operation (p<0.001). The odds ratio for perioperative

Table 1. Comparison of age, ASA risk score, BMI, and surgical duration

Parameters	Group G (n=123), (55.16%)	Group C (n=100), (44.84%)	p
Age (years, mean ± SD)	59.55±10.2	60.10±11.82	0.732
ASA risk score [median (IQR)]	2 (2-3)	2 (2-3)	0.494
BMI [kg/m^2, median (IQR)]	29.3 (26.3-33)	29.6 (26.1-31.3)	0.718
Duration of surgery [hours, median (IQR)]	2(2-2.5)	2 (2-3)	**0.018***

Total number of patients: 223, n (%): Number of patients in each group and percentage
IQR: [(min) 25%-(max) 75%]
*: p<0.05 statistically significant
ASA: American Society of Anesthesiologists, BMI: Body mass index, SD: Standard deviation, IQR: Interquartile range

Table 2. Comparison of the types of comorbidities and primary tumor origins among the study groups

Parameters	Group G (n=123), (55.16%)	Group C (n=100), (44.84%)	p
Comorbidity			
Positive	88 (71.5%)	70 (70%)	0.801
Negative	35 (28.5%)	30 (30%)	
Primary tumor			
Ovary	53 (43.1%)	43 (43%)	0.850
Endometrium	44 (35.8%)	33 (33%)	
Cervix	26 (21.1%)	24 (24%)	
Hypertension			
Positive	52 (42.3%)	43 (43%)	0.913
Negative	71 (57.7%)	57 (57%)	
Diabetes mellitus			
Positive	32 (26%)	30 (30%)	0.509
Negative	91 (74%)	70 (70%)	
Hypothyroidism			
Positive	7 (5.7%)	12 (12%)	0.093
Negative	116 (94.3%)	88 (88%)	
Asthma			
Positive	10 (8.1%)	18 (18%)	**0.027***
Negative	113 (91.9%)	82 (82%)	
Hyperlipidemia			
Positive	2 (1.6%)	4 (4%)	0.508
Negative	121 (98.4%)	96 (96%)	

Table 2. Continued

Parameters	Group G (n=123), (55.16%)	Group C (n=100), (44.84%)	p
Aortal stenosis			
Positive	5 (4.1%)	3 (3%)	0.669
Negative	118 (95.9%)	97 (97%)	
Heart failure			
Positive	7 (5.7%)	7 (7%)	0.689
Negative	116 (94.3%)	93 (93%)	

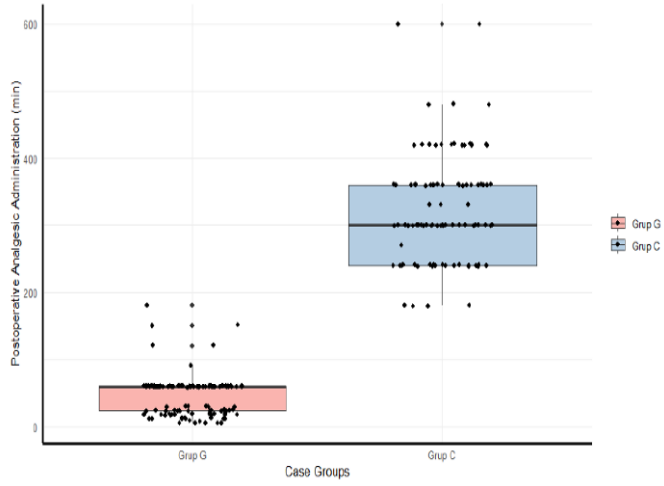
*: p<0.05 statistically significant

hypertension was 10.64 in Group G compared with Group C (OR: 10.64, 95% CI: 5.43-20.8). Regarding intraoperative bleeding, 67.5% of patients in Group G experienced bleeding, compared with 40% in Group C, which was statistically significant (p <0.001). The odds of bleeding were approximately three times higher in Group G than in Group C (OR: 3.11, 95% CI: 1.79-5.39).

Group G had a significantly higher mean FFP replacement (2.36±1.28 units) compared with Group C (2.15±0.98 units) (p=0.006). Similarly, ES replacement was higher in Group G (1.86±1.04 units) than in Group C (1.59±0.54 units) (p<0.001). The amount of Ringer's lactate solution administered was lower in Group G (1300 mL) than in Group C (1400 mL), with statistical significance (p=0.002). Furthermore, the postoperative analgesic administration period was significantly shorter in Group G (46±29.53 minutes) than in Group C (324.60±81.39 minutes) (p<0.001, Graph 1). The first mobilization time was earlier in Group C [5 (4.7-6) hours] than in Group G [9 (8-10) hours]; additionally, the length of hospital stay was shorter in Group C (5.14±1.81 days) than in Group G (8.11±3.57 days), both of which were statistically significant (p<0.001; Table 3).

Postoperative thrombosis occurred in 6.5% of patients in Group G, whereas no cases were reported in Group C (p=0.009). Additionally, postoperative cardiac complications were more frequent in Group G (17.1%) compared with Group C (0%), showing high statistical significance (p<0.001; Table 4).

Anesthetic-related side effects were significantly more frequent in Group C. Itching, urinary retention, and allergic reactions were observed only in Group C (p<0.001 for all comparisons). None of these side effects occurred in Group G, leading to statistically significant differences between the two groups (Table 5).



Graph 1. Period of postoperative analgesic administration in each case group

Discussion

Stress response and pain in patients with gynecological oncology due to major surgical trauma cause delayed mobilization, extended hospitalization, and excessive need for additional analgesics, thus delaying the healing process and recovery. It is also known that surgical stress suppresses the cell-mediated immune (CMI) system by increasing the levels of anti-CMI cytokines and catecholamines and has adverse effects on the healing process. Similarly, general anesthesia and systemic opioids also suppress CMI[14]. The perioperative epidural procedure is thought to blunt the surgical stress response by causing pro-tumorigenic cytokine and catecholamine release and counteract surgery-induced CMI inhibition by reducing the need for general anesthesia plus systemic opioids[15]. The stress response and pain that occur in patients after major surgical trauma cause delayed mobilization, prolonged hospital stays, and the need for additional analgesics, thus delaying the treatment and recovery processes[16].

A retrospective study by Guay[17] found that epidural anesthesia combined with general anesthesia reduced pain during movement and rest, reduced the amount of postoperative analgesic need, and prolonged the duration of analgesic need, resulting in more effective analgesia. Accordingly, the authors found a decrease in the incidence of arrhythmias that may occur with pain.

In our cases in which combined epidural injection was performed, the beneficial effects desired from the anesthetic drug

Table 3. Comparison of perioperative administration and hospitalization duration between groups G and C

Parameters	Group G (n=123), (55.16%)	Group C (n=100), (44.84%)	p
The unit of FFP replacement (units, mean ± SD)	2.36±1.28 n=86 (69.9%)	2.15±0.98 n=53 (53%)	**0.006***
Unite of ES replacement (units, mean ± SD)	1.86±1.04 n=84 (68.3%)	1.59±0.54 n=41 (41%)	**0.000***
Unite of fibrinogen replacement (units, mean ± SD)	1.88±1.44 n=96 (78%)	1.78±1.03 n=65 (65%)	0.075
RL level [mL, median (IQR)]	1300 (1000-1500) n=113 (91.9%)	1400 (1200-1800) n=97 (97%)	**0.002***
Period of postoperative analgesic therapy (minimum, mean ± SD)	46±29.53 n=123 (100%)	324.60±81.39 n=100 (100%)	**0.000***
The first mobilization time (hours, median (IQR))	9 (8-10) n=123 (100%)	5 (4.7-6) n=100 (100%)	**0.000***
Hospitalization period (day, mean ± SD)	8.11±3.57 n=123 (100%)	5.14±1.81 n=100 (100%)	**0.000***
Intensive care unit (day, mean ± SD)	0.58±1.72 n=123 (100%)	0.27±1.07 n=100 (100%)	0.138
Drained acid content (mL, mean ± SD)	1625±1333.41 n=16 (13%)	1430±2109.99 n=15 (15%)	0.722
Postoperative albumin infusion (flacon, mean ± SD)	2.69±1.40 n=16 (13%)	2.60±1.88 n=15 (15%)	0.717

First mobilization time: represents the time of the patient walking for the first time during the postoperative period.
*: p<0.05 statistically significant
FFP: Fresh frozen plasma, ES: Erythrocyte suspension, PSS: Physiological saline solution, RL: Ringer lactate, SD: Standard deviation

Table 4. Comparison of thrombosis and cardiac complications between Groups G and C

	Group G (n=123), (55.16%)	Group C (n=100), (44.84%)	p
Post-op thrombosis			
Positive	8 (6.5%)	0 (0%)	**0.009***
Negative	115 (93.5%)	100 (100%)	
Postoperative cardiac complications			
Positive	21 (17.1%)	0 (0%)	**0.000***
Negative	102 (82.9%)	100 (100%)	

*: p<0.05 statistically significant

Table 5. Comparison of the postoperative side effects of anesthetic administration between groups G and C

	Group G (n=123), (55.16%)	Group C (n=100), (44.84%)	p
Itching			
Positive	0, (0%)	17, (17%)	**0.000***
Negative	123, (100%)	83, (83%)	
Urinary retention			
Positive	0, (0%)	14, (14%)	**0.000***
Negative	123, (100%)	86, (86%)	
The allergic reaction			
Positive	1, (0.8%)	17, (17%)	**0.000***
Negative	122, (99.2%)	83, (83%)	

*: p<0.05 statistically significant

used were as follows: Rapid effect, high toxic drug dose limit, minimal impact on hemodynamics, and long anesthetic and analgesic effect duration. In our clinical practice, bupivacaine is widely used because of its long-acting properties, short onset of action, long duration of anesthesia and analgesia, and dissociative blockade. Based on our clinical study, Group C had a significantly prolonged duration of analgesic requirement and pain onset. This indicates that patients in Group C experienced more effective postoperative analgesia than those who received standard general anesthesia. Patients in Group C are expected to experience better postoperative pain relief than those receiving standard general anesthesia. This is because an epidural block is performed before surgery, and morphine is administered through an epidural catheter at the end of the procedure.

A study by Warta et al.[18] reported that spinal anesthesia performed before laparoscopic hysterectomy reduces postoperative pain and opioid use. Upon analyzing the parameters measured in both groups during our study, it was observed that the operation times in Group C were longer. It is a well-known fact that older patients are at a higher likelihood of experiencing comorbid conditions. Moreover, exposure to general anesthetics during prolonged anesthesia periods can lead to complications and longer postoperative recovery times in this age group.

In our study, we compared the first mobilization times of the patients in two groups: Group C and Group G. Patients in Group C were mobilized earlier than those in Group G. A study by Liu et al.[19] found that adding epidural anesthesia to general anesthesia provided better pain control and analgesia, early mobilization, and a shorter hospitalization period. Similar results were observed in Group C of our study.

In a study by Motamed et al.[20],54 patients were divided into four groups. The first group received epidural morphine and bupivacaine, the second group received only morphine, the third group received only bupivacaine, and the fourth group received patient-controlled analgesia. Results showed that the first group had balanced and stable analgesia, shorter hospital stays, and fewer cases of hypotension. However, allergic reactions like itching were more common in the morphine-only group, and hypotension was more frequent in the bupivacaine-only group[20]. In our study, no situation was detected that disrupted the hemodynamic stability of the patients because bupivacaine was administered epidurally and morphine was distributed for analgesic purposes near the end of surgery. Again, in parallel to the study conducted by Motamed et al.[20],allergic reactions due to postoperative itching were higher. It is believed that the higher incidence of urinary retention in Group C may be due to the removal of urinary catheters. This is supported by the fact that 14 patients in Group C experienced urinary retention after surgery. In addition, bladder atony caused by epidural anesthesia may have also contributed to this issue. Similarly, Shir et al.[21] found that combining epidural neuraxial blockade with general anesthesia for central lower abdominal surgery reduced postoperative analgesic use and improved pain control. Ladjevic et al.[22] compared two groups of patients who underwent radical prostatectomy as lower abdominal surgery. One group received general anesthesia alone, while the other received combined epidural and general anesthesia. The researchers found that the group that received combined epidural anesthesia had better pain control and less perioperative bleeding than the group that received general anesthesia only. Likewise, our study found that patients in Group C had less perioperative bleeding, resulting in a lower need for blood products. Patients with coagulation problems were evaluated in group G because epidural catheters were not placed in this group. Because patients with coagulation problems are more likely to experience bleeding, this may have caused a relative increase in the favor of Group G. Moreover, the combination of the epidural catheter and spinal epidural may have resulted in less blood loss due to sympathetic denervation occurring distal to the level where regional anesthesia was applied, compared to patients who only received general anesthesia.

Study Limitations

We declare that greater standardization is necessary when comparing the two groups because the study's retrospective design and the data based on archival records constitute essential limitations. Therefore, these findings should be confirmed in prospective randomized controlled trials.

Conclusions

Combined epidural and general anesthesia in major gynecological oncological surgeries may improve postoperative outcomes. These advantages include decreased postoperative analgesic requirements, earlier mobilization, reduced hospitalization duration, and reduced risk of cardiovascular and thrombotic complications. This study emphasizes the potential efficacy of combined anesthesia as an anesthetic technique in this specific patient demographic. Furthermore, it underscores the significance of personalized perioperative care and the necessity for tailored strategies to optimize outcomes for diverse patient cohorts.

Ethics

Ethics Committee Approval: Ethical approval for our study was obtained from the Gaziantep University Clinical Research Ethics Committee in the decision dated 21.10.2020 and numbered 2020/318.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.M., B.K.U., Concept: İ.M., B.K.U., Design: İ.M., B.K.U., Data Collection or Processing: İ.T., Analysis or Interpretation: F.Ç., İ.T., M.C., S.G., M.G.U., Literature Search: F.Ç., İ.T., M.C., Writing: İ.M., B.K.U., F.Ç.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424. Erratum in: CA Cancer J Clin. 2020;70:313.
2. Krawczyk P, Trojnarska D, Baran R, Lonc T, Swistek R, Tyszecki P, et al. Postoperative gynecologic oncology admissions to intensive care unit in the tertiary care center: an eight-year retrospective study. Ginekol Pol. 2023;94:599-604.
3. Bentivegna E, Maulard A, Miailhe G, Gouy S, Morice P. Gynaecologic cancer surgery and preservation of fertility. J Visc Surg. 2018;155 Suppl 1:S23-9.
4. Patel K, Shergill S, Vadivelu N, Rajput K. Analgesia for Gynecologic Oncologic Surgeries: a narrative review. Curr Pain Headache Rep. 2022;26:1-13.
5. Zhong S, Zhong X, Zhong X, Liu Y. Comparison between the effect of epidural anesthesia combined with epidural analgesia and general anesthesia combined with intravenous analgesia on prognosis of ovarian cancer patients. Oncol Lett. 2019;17:5662-8.
6. Chen LM, Weinberg VK, Chen C, Powell CB, Chen LL, Chan JK, et al. Perioperative outcomes comparing patient controlled epidural versus intravenous analgesia in gynecologic oncology surgery. Gynecol Oncol. 2009;115:357-61.
7. Huepenbecker SP, Cusworth SE, Kuroki LM, Lu P, Samen CDK, Woolfolk C, et al. Continuous epidural infusion in gynecologic oncology patients undergoing exploratory laparotomy: the new standard for decreased postoperative pain and opioid use. Gynecol Oncol. 2019;153:356-61.
8. Reynolds L, Beckmann J, Kurz A. Perioperative complications of hypothermia. Best Pract Res Clin Anaesthesiol. 2008;22:645-57.
9. Ackroyd SA, Hernandez E, Roberts ME, Chu C, Rubin S, Mantia-Smaldone G, et al. Postoperative complications of epidural analgesia at hysterectomy for gynecologic malignancies: an analysis of the National Surgical Quality Improvement Program. Int J Gynecol Cancer. 2020;30:1203-9.
10. Kabon B, Fleischmann E, Treschan T, Taguchi A, Kapral S, Kurz A. Thoracic epidural anesthesia increases tissue oxygenation during major abdominal surgery. Anesth Analg. 2003;97:1812-7.
11. Vail EA, Feng R, Sieber F, Carson JL, Ellenberg SS, Magaziner J, et al; REGAIN (Regional versus General Anesthesia for Promoting Independence after Hip Fracture) Investigators. Long-term outcomes with spinal versus general anesthesia for hip fracture surgery: a randomized trial. Anesthesiology. 2024;140:375-86.
12. Aldrete JA. The post-anesthesia recovery score revisited. J Clin Anesth. 1995;7:89-91.
13. Daabiss M. American Society of Anaesthesiologists physical status classification. Indian J Anaesth. 2011;55:111-5.
14. Ben-Eliyahu S. The promotion of tumor metastasis by surgery and stress: immunological basis and implications for psychoneuroimmunology. Brain Behav Immun. 2003;17 Suppl 1:S27-36.
15. Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol. 200;2:293-9.
16. Chitnis SS, Tang R, Mariano ER. The role of regional analgesia in personalized postoperative pain management. Korean J Anesthesiol. 2020;73:363-71.
17. Guay J. The benefits of adding epidural analgesia to general anesthesia: a metaanalysis. J Anesth. 2006;20:335-40.
18. Warta KA, Lu X, Nguyen TD, Shakar RM, Beste TM. Spinal anesthesia prior to laparoscopic hysterectomy resulted in decreased postoperative pain and opioid use. JSLS. 2023;27:e2023.00050.
19. Liu F, Zhang J, Zeng XQ, Zhao YQ, Zuo YX. [Application of general anesthesia combined with epidural anesthesia/analgesia in rehabilitation after gastric cancer resection]. Zhonghua Yi Xue Za Zhi. 2017;97:1089-92. Chinese.
20. Motamed C, Spencer A, Farhat F, Bourgain JL, Lasser P, Jayr C. Postoperative hypoxaemia: continuous extradural infusion of bupivacaine and morphine vs patient-controlled analgesia with intravenous morphine. Br J Anaesth. 1998;80:742-7. Erratum in: Br J Anaesth. 1998;81:826-7.
21. Shir Y, Raja SN, Frank SM. The effect of epidural versus general anesthesia on postoperative pain and analgesic requirements in patients undergoing radical prostatectomy. Anesthesiology. 1994;80:49-56.
22. Ladjevic N, Likic-Ladjevic I, Dzamic Z, Acimovic M, Dragicevic D, Durutovic O. Combined general and epidural anaesthesia versus general anaesthesia for radical cystectomy. Acta Chir Iugosl. 2007;54:89-91.

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Maternal serum apelin-13 levels in early- and late-onset preeclampsia

Erken ve geç başlangıçlı preeklampside maternal serum apelin-13 düzeyleri

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Abstract

Objective: To assess whether alterations in maternal serum apelin-13 levels differ between early-onset preeclampsia (EO-PE) and late-onset preeclampsia (LO-PE).

Materials and Methods: A prospective case-control study included 90 preeclamptic cases and 90 normotensive healthy pregnant women as controls. Preeclampsia cases were subclassified as EO-PE and LO-PE. Blood samples were collected, centrifuged, and the separated serum was stored at -80°C for further testing. Ethylenediamine tetraacetic acid blood was used for complete blood count. Serum sample was used for analysis of biochemical parameters. Maternal serum apelin-13 concentrations were measured using ELISA. Demographic details and fetal outcomes were recorded.

Results: Results indicated significantly lower gestational age at sampling and delivery in preeclampsia cases. Blood pressure (systolic, diastolic, and mean arterial pressure) was elevated in preeclampsia. Maternal serum apelin-13 levels (261.7±110.6 pg/mL) were significantly reduced in preeclamptic cases compared to controls (575.3±164.7 pg/mL). Adverse fetal outcomes were more prevalent in preeclampsia. Regarding EO-PE and LO-PE, gestational age at sampling and delivery was lower in EO-PE compared to LO-PE. Maternal serum apelin-13 levels (371.3±116.0 pg/mL) were higher in EO-PE. A 40.9% reduction in apelin-13 levels was observed in LO-PE compared to EO-PE, indicating a gradual reduction in apelin-13 levels in preeclampsia. Adverse fetal outcomes, such as birth weight (1.8±0.5 kg), were lower, and other adverse outcomes were higher in EO-PE compared to LO-PE.

Conclusion: Circulating serum apelin-13 concentration was reduced in preeclampsia and was higher in EO-PE than in LO-PE. Apelin-13 serves as a potential indicator for discriminating early-onset preeclampsia.

Keywords: Apelin-13, adverse fetal outcomes, early-onset preeclampsia, late-onset preeclampsia, vasoconstriction

Öz

Amaç: Erken başlangıçlı preeklampsi (EO-PE) ve geç başlangıçlı preeklampsi (LO-PE) arasında maternal serum apelin-13 düzeylerindeki değişikliklerin farklılık gösterip göstermediğini araştırmak planlanmıştır.

Gereç ve Yöntemler: Bu çalışmaya 90 preeklamptik olgu ve 90 normotansif sağlıklı gebe kontrol olarak dahil edildi. Preeklampsi olguları EO-PE ve LO-PE olarak alt sınıflara ayrıldı. Maternal kan örnekleri toplandı, santrifüj edildi ve serum örnekleri daha ileri testler için -80°C'de saklandı. Tam kan sayımı için etilendiamin tetraasetik asitli kan örnekleri kullanıldı. Serum örneği biyokimyasal parametrelerin analizi için kullanıldı. Maternal serum apelin-13 düzeyleri ELISA yöntemi ile ölçüldü. Demografik bilgiler ve fetal sonuçlar kaydedildi.

Bulgular: Preeklampside örnekleme ve doğum sırasında anlamlı olarak daha düşük gebelik yaşı tespit edildi. Kan basıncı (sistolik, diyastolik ve ortalama arter basıncı) preeklampside anlamlı olarak yüksekti. Maternal serum apelin-13 (261,7±110,6 pg/mL) düzeyleri preeklamptik olgularda anlamlı olarak düşüktü (575,3±164,7 pg/mL). Olumsuz fetal sonuçlar preeklampside daha fazlaydı. EO-PE ve LO-PE ile ilgili olarak, örnekleme ve doğum anındaki

PRECIS: Circulating maternal serum apelin-13 concentrations were low in preeclampsia. The levels of early-onset preeclampsia were higher, indicating that apelin-13 serves as an indicator for discriminating early-onset preeclampsia.

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gebelik yaşı EO-PE'de LO-PE'den anlamlı olarak düşüktü. Maternal serum apelin-13 (371,3±116,0 pg/mL) düzeyleri EO-PE'de LO-PE'den anlamlı olarak yüksekti. Başka bir deyişle, LO-PE'de EO-PE'ye göre apelin-13 düzeylerinde %40,9 azalma gözlendi, bu da preeklampside apelin-13 düzeylerinin kademeli olarak azaldığını göstermektedir. Doğum ağırlığı (1,8±0,5 kg) EO-PE'de LO-PE'den düşüktü, diğer olumsuz fetal sonuçlar ise EO-PE'de LO-PE'den fazlaydı.

Sonuç: Dolaşımdaki maternal serum apelin-13 düzeyleri preeklampside düşüktü. Düzeyler EO-PE'de LO-PE'den anlamlı olarak daha yüksekti. Apelin-13'ün erken başlangıçlı preeklampsinin ayrımında belirteç görevi gördüğü kanıtlanmıştır.

Anahtar Kelimeler: Apelin-13, olumsuz fetal sonuçlar, erken başlangıçlı preeklampsi, geç başlangıçlı preeklampsi, vazokonstriksiyon

Introduction

Preeclampsia (PE) is a pregnancy-specific complication associated with the onset of hypertension and proteinuria after 20 weeks of gestational age[1]. The prevalence of PE is around 2-8% globally and 10.3% in India[2].

Based on the time of onset or delivery, PE has been sub-classified into early-onset PE (EO-PE), which requires delivery ≤34 weeks of gestational age, and late-onset PE (LO-PE), with delivery ≥34 weeks of gestational age. The EO-PE and LO-PE have different etiopathogenesis and outcomes. Hence, PE can be treated in two different forms. However, there are some uncertainties regarding pregnancy outcomes. EO-PE is mainly linked to improper placentation and abnormal remodeling of spiral arteries, which usually occurs during the first half of pregnancy. Placental dysfunction results in increased secretion of inflammatory and antiangiogenic factors into the circulation, causing PE. Whereas in fetuses, this will result in utero-placental circulatory issues, intrauterine fetal growth restriction (IUGR), or intrauterine death (IUD), posing increased risk to mother and fetus, whereas LO-PE may be due to predisposing maternal factors[1,3]. It was reported that EO-PE is linked to adverse maternal/perinatal outcomes, whereas LO-PE is usually not as severe. The incidence of abnormal placentation, overall mortality, and IUGR has been shown to be associated with PE severity and its duration[4,5]. This novel concept provides better knowledge of the PE etiopathogenesis mechanism. Understanding this concept will increase awareness of disease severity and facilitate better maternal and fetal outcomes.

Despite the considerable maternal and fetal complications, the exact mechanism of PE remains unclear. However, PE is a multisystem disorder with possible underlying pathophysiological mechanisms like abnormal placentation, shallow remodeling of spiral arteries, systemic inflammation, endothelial dysfunction, and hemodynamic alterations during pregnancy[3,5].

Studies have reported that Apelin is an angiogenic molecule essential for blood vessel growth and endothelial cell proliferation[6,7]. In humans, the *APLN* gene, located on chromosome Xq25-26.1, contains 3 exons and 1 intron, and it produces short fragments of apelin peptides. In the biosynthesis of apelin, the precursor preproapelin is sequentially cleaved to generate short peptides such as apelin-36, apelin-17, apelin-13, and (Pyr1) apelin-13. All of these short peptides show agonistic activity on the apelin receptor (APJ/APLNR receptor). Among these, apelin-13 is biologically more active and causes vasodilation[8].

Apelinergic system role in cardiovascular diseases is reported [9]. Whereas, apelinrole in PE has not been explored much. Expression of apelin and APJ/APLNR are expressed in human tissues, with increased concentrations observed in placenta[10,11]. In addition, the apelinergic system is associated with the regulation of vascular bore size and integrity[7]. Apelin induces nitric oxide (NO)-dependent vasodilation, apoptosis, and decreases vascular inflammation[7,12].

A few studies have reported that apelin and the APJ/APLNR receptor are targeted for the treatment of cardiovascular diseases and increased blood pressure[13,14]. In addition, studies have reported an association between apelin and pregnancy diseases like PE, gestational diabetes and IUGR[15-17].

Abnormal placentation is a key pathophysiological mechanism in PE development, especially EO-PE. Therefore, there is a need to establish markers for the early detection of PE. To our knowledge, this is the first report to assess maternal serum apelin-13 (a biologically active peptide) concentration in EO-PE and LO-PE in PE patients in Southern India. We aimed to investigate whether changes in maternal serum apelin-13 levels differ between EO-PE and LO-PE.

Materials and Methods

This prospective case-control study was conducted at the biochemistry department in association with the obstetrics and gynecology department of RL Jalappa Hospital and Research Center, Sri Devaraj Urs Medical College, a constituent institute of Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India. The sample size was calculated with 80% power and a 95% confidence interval. A total of 180 participants were recruited after obtaining ethics committee approval Sri Devaraj Urs Academy of Higher Education and Research Central Ethics Comnittee (decision no: SDUAHER/KLR/CEC/34/2018-2019, date: 14.05.2018) and informed consent. The study subjects were divided into 90 preeclamptic cases and 90 healthy (normotensive) pregnant controls. Furthermore, the PE cases were sub-classified into EO-PE (n=45), requiring delivery ≤34 weeks of gestation, and LO-PE (n=45), with delivery ≥34 weeks of gestation. PE was diagnosed based on ACOG guidelines[18].

Results

Table 1 presents demographic, hematological, and biochemical parameters. The results showed a lower gestational age at sampling (34.1±2.2 weeks) and at delivery (35.2±2.1 weeks) in PE compared to normotensive healthy pregnant

Table 1. Demographic details, haematological and biochemical parameters of preeclampsia and normotensive healthy pregnant women

Parameters	Preeclampsia (n=90) (mean ± SD)	Normotensive healthy pregnant women (n=90) (mean ± SD)	p
Demographic details			
Age (years)	23.0±3.5	23.4±3.2	0.290
Primigravida (n, %)	70 (77.7%)	66 (73.3 %)	-
Multigravida (n, %)	20 (22.2%)	24 (26.6%)	-
Gestational age at sampling (weeks)	34.1±2.2	38.1±1.2	0.000*
Gestational age at delivery (weeks)	35.2±2.1	38.2±1.1	0.000*
SBP (mmHg)	159.1±16.6	116.4±7.8	0.000*
DBP (mmHg)	102.4±11.5	74.7±6.3	0.000*
MAP (mmHg)	120.9±11.7	88.5±6.2	0.000*
Presence of proteinuria (n, %)	90 (100%)	-	-
Haematological parameters			
Hb (g%)	11.1±2.0	11.3±1.4	0.784
WBC (10^3/L)	12.7±3.3	13.4±4.2	0.503
Platelet count x (10^9/L)	221.2±75.5	242.8±60.2	0.147
Biochemical parameters			
RBS (mg/dL)	84.1±21.2	83.6±16.9	0.549
Serum urea (mg/dL)	17.1±10.4	14.5±4.6	0.185
Serum creatinine (mg/dL)	0.57±0.22	0.47±0.11	0.000*
Serum uric acid (mg/dL)	5.9±1.8	4.7±1.4	0.000*
Serum AST (IU/L)	27.9±17.7	20.5±7.9	0.000*
Serum ALT (IU/L)	20.1±12.2	13.7±6.8	0.000*
Maternal serum apelin-13 (pg/mL)	261.7±110.6	575.3±164.7	0.000*

*: Statistically significant, SD: Standard deviation, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean arterial pressure, Hb: Haemoglobin, WBC: White blood cells, RBS: Random blood sugar, AST: Aspartate transaminase, ALT: Alanine transaminase

women. Systolic blood pressure (SBP) (159.1±16.6 mmHg), diastolic blood pressure (102.4±11.5 mmHg), mean arterial pressure (120.9±11.7 mmHg), serum creatinine (0.57±0.22 mg/dL), serum uric acid (5.9±1.8 mg/dL), serum aspartate aminotransferase (27.9±17.7 IU/L), and serum alanine aminotransferase (20.1±12.2 IU/L) levels were significantly elevated in PE compared to healthy pregnant women. Proteinuria was observed in all preeclamptic subjects. Serum apelin-13 (261.7±110.6 pg/mL) levels were significantly lower in PE cases than in healthy pregnant women.

Table 2 depicts the adverse fetal outcomes, which were higher in babies born to preeclamptic mothers, including low birth weight (2.2±0.6 kg), babies requiring neonatal intensive care unit (NICU) admission (42, 46.6%), respiratory distress syndrome (RDS) (29, 32.2%), low birth weight (LBW) (19, 21.1%), and IUD (11, 12.2%).

Table 3 describes the demographic details, hematological, and biochemical parameters of EO-PE and LO-PE. Maternal age was significantly higher in EO-PE (23.9±3.5 years) compared to LO-PE. Gestational age at sampling (31.5±1.7 weeks) and at delivery (32.6±1.2 weeks) was significantly lower in EO-PE (31.0±3.0 weeks) compared to LO-PE. Serum urea (19.8±12.9 mg/dL), serum creatinine (0.62±0.25 mg/dL), and serum uric acid (6.2±1.8 mg/dL) levels were higher in early-onset PE than in late-onset PE. In the subgroup analysis, serum apelin-13 (371.3±116.0 pg/mL) levels were higher in EO-PE than in LO-PE. A 40.9% reduction in apelin-13 levels was observed in LO-PE compared to EO-PE, indicating a gradual reduction in apelin-13 concentration in PE.

Table 4 presents the adverse fetal outcomes in babies born to mothers with EO-PE and LO-PE. Birth weight (1.8±0.5 kg) was lower in early-onset PE than in late-onset PE. Other adverse outcomes, such as babies requiring NICU admission (32, 71.1%), RDS (20, 44.4%), LBW (18, 40%), and IUD (8, 17.7%), were more common in EO-PE.

Table 2. Comparison of birth weight and adverse foetal outcomes in babies born to mother with preeclampsia and normotensive healthy pregnant women

Parameters (n, %)	Preeclampsia (n=90)	Normotensive healthy pregnant women (n=90)
Birth weight (kg)	2.2±0.6	2.8±0.4a*
Respiratory distress syndrome	29 (32.2%)	6 (6.6%)
Low birth weight	19 (21.1%)	5 (5.5%)
New borns requiring NICU Admission	42 (46.6%)	16 (17.7%)
Intrauterine death	11 (12.2%)	-

[a]: Mean±SD ($p<0.05$), *: Statistically significant, NICU: Neonatal intensive care unit

Table 3. Demographic details, haematological and biochemical parameters of EO-PE and LO-PE

Parameters	EO-PE (n=45), mean ± SD	LO-PE (n=45), mean ± SD	p
Demographic details			
Age (years)	23.9±3.5	22.1±3.3	0.003*
Primigravida (n, %)	31 (68.8%)	39 (86.6%)	-
Multigravida (n, %)	14 (31.1%)	6 (13.3%)	-
Gestational age at sampling (weeks)	31.5±1.7	36.0±1.3	0.000*
Gestational age at delivery (weeks)	32.6±1.2	36.2±1.1	0.000*
SBP (mmHg)	162.0±16.8	156.2±15.9	0.094
DBP (mmHg)	103.3±12.0	101.5±11.0	0.520
MAP (mmHg)	122.0±11.8	119.9±11.6	0.292
Presence of proteinuria (n, %)	45 (100%)	45 (100%)	-
Pulse rate (bpm)	88.4±6.0	88.1±6.1	0.549
Haematological parameters			
Hb (g%)	10.9±2.0	11.2±1.9	0.675
WBC (10^3/L)	12.1±3.5	13.4±3.0	0.030*
Platelet count x (10^9/L)	205.1±88.1	237.2±56.8	0.125
Biochemical parameters			
RBS (mg/dL)	86.2±25.6	82.0±15.6	0.732
Serum urea (mg/dL)	19.8±12.9	14.4±6.2	0.024*
Serum creatinine (mg/dL)	0.62±0.25	0.51±0.18	0.006*
Serum uric acid (mg/dL)	6.2±1.8	5.6±1.8	0.045*
Serum AST (IU/L)	30.0±15.8	25.8±19.4	0.065
Serum ALT (IU/L)	21.2±13.6	19.0±10.7	0.523
Maternal serum apelin-13 (pg/mL)	371.3±116.0	152.2±47.9	0.000*

*: Statistically significant, SD: Standard deviation, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean arterial pressure, Hb: Haemoglobin, WBC: White blood cells, RBS: Random blood sugar, AST: Aspartate transaminase, ALT: Alanine transaminase, EO-PE: Early-onset preeclampsia, LO-PE: Late-onset preeclampsia

Table 4. Comparison of birth weight and adverse foetal outcomes in babies born to mother with EO-PE and LO-PE

Parameters (n, %)	EO-PE (n=45)	LO-PE (n=45)
Birth weight (kg)	1.8±0.5	2.6±0.5[a*]
Respiratory distress syndrome	20 (44.4%)	9 (20%)
Low birth weight	18 (40%)	1 (2.2%)
Newborns requiring NICU admission	32 (71.1%)	10 (22.2%)
Intrauterine death	8 (17.7%)	3 (6.6%)

[a]: Mean ± SD, ($p<0.05$), *:Statistically significant, NICU: Neonatal intensive care unit EO-PE: Early-onset preeclampsia, LO-PE: Late-onset preeclampsia

Discussion

PE is a pregnancy-specific and life-threatening disorder. The placenta plays a crucial role in the pathophysiology of such diseases and is regarded as the source of inflammation. It secretes vasoconstrictor molecules into the maternal circulation, initiating endothelial cell dysfunction and vasospasm[19].

In this study, serum apelin-13 concentration was measured to evaluate its usefulness as a discriminative biomarker to differentiate between early-onset PE (EO-PE) and late-onset PE (LO-PE). The results indicated reduced apelin-13 levels in PE compared to healthy pregnant women. In the subgroup analysis, apelin-13 concentrations were higher in EO-PE than in LO-PE. In other words, a 40.9% reduction in apelin-13 levels was observed in LO-PE compared to EO-PE, suggesting a gradual reduction in serum apelin-13 levels from EO-PE to LO- PE. In line with our previous studies, we reported significantly lower serum apelin-13 levels in PE compared to healthy pregnant women[15,19].

Similar to our findings, a study by Deniz et al.[20] demonstrated that apelin and NO levels were lower in PE compared to healthy pregnant women. Another study by Sattar Taha et al.[21] reported decreased apelin levels in PE compared to healthy pregnant women. Inzukua[22] found reduced mRNA expression of apelin in the placenta of preeclamptic women and decreased immune-histochemical signals for the apelin/APJ receptor.

Regarding EO-PE and LO-PE. Kucur et al.[3] reported increased circulating levels of apelin in EO-PE compared with LO-PE. However, the authors reported total apelin concentration rather than bioactive short peptides/fragments[23]. In this study, we observed significantly elevated serum apelin-13 (biologically active peptide) levels in EO-PE compared with LO-PE. Therefore, this reduced bioactive apelin-13 concentration may affect the trophoblast invasion of spiral arteries. These abnormalities in the remodeling of spiral arteries cause high-resistance utero-placental circulation, as seen in PE[24].

Although apelin peptides in PE have been recently studied, the possible discriminative role of apelin-13 in EO-PE and LO-PE has not yet been established. The human placenta is a tissue where angiogenesis, blood pressure, and flow are essential for promoting embryonic development and fetal growth. It has been reported that, in normal pregnancy, placental expression of apelin is higher, indicating its role in placentation[25]. Apelin favors angiogenesis, stimulates blood vessel growth and differentiation, and regulates blood pressure and flow[5].

Based on the current study findings, maternal serum apelin-13 may serve as a discriminative marker between EO-PE and LO-PE. EO-PE is usually linked with improper placentation and subsequent hypoxic placenta, which causes the activation of a cascade of events, such as an imbalance between angiogenic and anti-angiogenic factors, increased oxidative stress, dysfunctional endothelium, and immunological dysregulation, ultimately leading to the clinical manifestation and complications of PE[26-29]. LO-PE is linked with normal placentation and utero-placental perfusion, resulting in better perinatal outcomes[27].

In this study, adverse fetal outcomes were higher in EO-PE compared to LO-PE, including decreased birth weight, babies requiring NICU admission, RDS, and low birth weight. In accordance with our findings, Akbar et al.[27] reported that EO-PE is associated with poor maternal and perinatal outcomes.

It is well known that an imbalance between angiogenic and anti-angiogenic markers is associated with PE complications[30-32]. Numerous pro-angiogenic and anti-angiogenic markers play significant roles in the development of the placental vascular bed, especially vascular endothelial growth factor (VEGF). The levels of VEGF have been shown to be reduced in EO-PE[33,34]. It has been reported that the apelinergic system promotes the expression of VEGF. Therefore, the reduced apelin-13 concentration in PE may result in reduced VEGF levels, playing a significant role in the development of abnormal placentation associated with EO-PE[23].

Study Limitations

The main limitation of this study was the sample size, screening for placental expression of apelin and other confounding factors, such as lifestyle parameters and genetic and epigenetic factors.

Conclusion

The current study may conclude that circulating maternal serum apelin-13 concentrations were lower in PE than in normotensive pregnant women. The levels were higher in EO-PE than in LO-PE, indicating the role of apelin in discriminating EO-PE. Further studies with larger sample sizes are recommended to investigate its precise role in EO-PE and LO-PE, treatment strategies for PE management.

Ethics

Ethics Committee Approval: The study was approved by the Sri Devaraj Urs Academy of Higher Education and Research Central Ethics Committee (decision no: SDUAHER/KLR/CEC/34/2018-2019, date: 14.05.2018)

Informed Consent: All participants provided informed consent before entering the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.S.R, Concept R.G., D.C.D., S.S.R, Design R.G., D.C.D., S.S.R, Data Collection or Processing: R.G., D.C.D., S.S.R, Analysis or Interpretation R.G., D.C.D., S.S.R, Literature Search: R.G., D.C.D., S.S.R, Writing: R.G., D.C.D., S.S.R.

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References

1. ACOG Practice Bulletin No. 202: Gestational hypertension and preeclampsia. Obstet Gynecol. 2019;133:1.
2. Magee LA, Sharma S, Nathan HL, Adetoro OO, Bellad MB, Goudar S, et al. The incidence of pregnancy hypertension in India, Pakistan, Mozambique, and Nigeria: A prospective population-level analysis. PLoS Med. 2019;16:e1002783.
3. Kucur M, Tuten A, Oncul M, Acikgoz AS, Yuksel MA, Imamoglu M, et al. Maternal serum apelin and YKL-40 levels in early and late-onset pre-eclampsia. Hypertens Pregnancy. 2014;33:467-75.
4. Wadhwani P, Saha PK, Kalra JK, Gainder S, Sundaram V. A study to compare maternal and perinatal outcome in early vs. late onset preeclampsia. Obstet Gynecol Sci. 2020;63:270-7.
5. Gandham R, Dayanand CD, Sheela SR, Kiranmayee P. Maternal serum Apelin 13 and APLN gene promoter variant -1860T>C in preeclampsia. J Matern Fetal Neonatal Med. 2022;35:5008-16.
6. Mlyczyńska E, Kurowska P, Drwal E, Opydo-Chanek M, Tworzydło W, Kotula-Balak M, et al. Apelin and apelin receptor in human placenta: expression, signalling pathway and regulation of trophoblast JEG-3 and BeWo cells proliferation and cell cycle. Int J Mol Med. 2020;45:691-702.
7. Kidoya H, Naito H, Takakura N. Apelin induces enlarged and nonleaky blood vessels for functional recovery from ischemia. Blood. 2010 Apr;115:3166-74.
8. Gandham R, Sumathi ME, Dayanand CD, Sheela SR, Kiranmayee P. Apelin and its receptor: an overview. J Clin Diagn Res. 2019;13:12930.
9. Japp AG, Cruden NL, Barnes G, van Gemeren N, Mathews J, Adamson J, et al. Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. Circulation. 2010;121:1818-27.
10. Furuya M, Okuda M, Usui H, Takenouchi T, Kami D, Nozawa A, et al. Expression of angiotensin II receptor-like 1 in the placentas of pregnancy-induced hypertension. Int J Gynecol Pathol. 2012;31:227-35.
11. Kasai A, Shintani N, Oda M, Kakuda M, Hashimoto H, Matsuda T, et al. Apelin is a novel angiogenic factor in retinal endothelial cells. Biochem Biophys Res Commun. 2004;325:395-400.
12. Leeper NJ, Tedesco MM, Kojima Y, Schultz GM, Kundu RK, Ashley EA, et al. Apelin prevents aortic aneurysm formation by inhibiting macrophage inflammation. Am J Physiol Heart Circ Physiol. 2009;296:H1329-35.
13. Bortoff KD, Qiu C, Runyon S, Williams MA, Maitra R. Decreased maternal plasma apelin concentrations in preeclampsia. Hypertens Pregnancy. 2012;31:398-404.
14. Cox CM, D'Agostino SL, Miller MK, Heimark RL, Krieg PA. Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. Dev Biol. 2006;296:177-89.
15. Gandham R, Dayanand CD, Sheela SR. Apelin 13 and blood pressure: is there any association in pre-eclampsia? A case-control study. J Clin Diagn Res. 2021;15:BC01-BC04.
16. Seely EW, Solomon CG. Insulin resistance and its potential role in pregnancy-induced hypertension. J Clin Endocrinol Metab. 2003;88:2393-8.
17. Van Mieghem T, Doherty A, Baczyk D, Drewlo S, Baud D, Carvalho J, et al. Apelin in normal pregnancy and pregnancies complicated by placental ınsufficiency. Reprod Sci. 2016;23:1037-43.
18. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. Obstet Gynecol. 2013;122:1122-31.
19. Gandham R, Dayanand CD, Sheela SR, Kiranmayee P. Impact of oxidative stress on maternal serum apelin 13 and endothelial nitric oxide synthase in preeclampsia. Biomed Pharmacol J. 2020;13:2041-8.
20. Deniz R, Baykus Y, Ustebay S, Ugur K, Yavuzkir Ş, Aydin S. Evaluation of elabela, apelin and nitric oxide findings in maternal blood of normal pregnant women, pregnant women with pre-eclampsia, severe pre-eclampsia and umbilical arteries and venules of newborns. J Obstet Gynaecol. 2019;39:907-12.
21. Sattar Taha A, Zahraei Z, Al-Hakeim HK. Serum apelin and galectin-3 in preeclampsia in Iraq. Hypertens Pregnancy. 2020;39:379-86.
22. Inuzuka H, Nishizawa H, Inagaki A, Suzuki M, Ota S, Miyamura H, Miyazaki J, et al. Decreased expression of apelin in placentas from severe pre-eclampsia patients. Hypertens Pregnancy. 2013;32:410-21.
23. Wójtowicz A, Zembala-Szczerba M, Babczyk D, Kołodziejczyk-Pietruszka M, Lewaczyńska O, Huras H. Early- and late-onset preeclampsia: a comprehensive cohort study of laboratory and clinical findings according to the new ISHHP criteria. Int J Hypertens. 2019;2019:4108271.
24. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod. 2003;69:1-7.
25. Cobellis L, De Falco M, Mastrogiacomo A, Giraldi D, Dattilo D, Scaffa C, Colacurci N, et al. Modulation of apelin and APJ receptor in normal and preeclampsia-complicated placentas. Histol Histopathol. 2007;22:1-8.
26. Huppertz B. Placental origins of preeclampsia: challenging the current hypothesis. Hypertension. 2008;51:970-5.
27. Akbar MIA, Kinanti H, Ernawati EE, Lestar P. Maternal and perinatal outcomes of early-onset and late-onset preeclampsia at a tertiary center hospital. J South Asian Feder Obst Gynae. 2021;13:338-42.
28. Pinheiro CC, Rayol P, Gozzani L, Reis LM, Zampieri G, Dias CB, et al. The relationship of angiogenic factors to maternal and neonatal manifestations of early-onset and late-onset preeclampsia. Prenat Diagn. 2014;34:1084-92.
29. Gathiram P, Moodley J. Pre-eclampsia: its pathogenesis and pathophysiolgy. Cardiovasc J Afr. 2016;27:71-8.
30. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med. 2006;12:642-9. Erratum in: Nat Med. 2006;12:862.

31. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004;350:672-83.

32. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006;355:992-1005.

33. Kidoya H, Takakura N. Biology of the apelin-APJ axis in vascular formation. J Biochem. 2012;152(2):125-31.

34. Raymond D, Peterson E. A critical review of early-onset and late-onset preeclampsia. Obstet Gynecol Surv. 2011;66:497-506.

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Comprehensive analysis of selenoprotein gene expression and prognostic value in ovarian cancer

Selenoprotein gen ekspresyonunun ve yumurtalık kanserinde prognostik değerinin kapsamlı analizi

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Abstract

Objective: To comprehensively analyze the expression and prognostic value of selenoprotein in ovarian cancer (OV).

Materials and Methods: GEPIA and cBioPortal were used to analyze selenoprotein expression and mutations and copy number variations. Kaplan-Meier plotter and the tumor immune estimation resource were used to evaluate the impact of these genes on clinical prognosis and their correlation with tumor immune infiltration.

Results: Compared with normal tissues, the expression of iodothyronine deiodinase 3 (DIO3), glutathione peroxidase 4, SECISBP2, SELM, and SELP was decreased in the four gynecological malignancies. In OV, selenoprotein had the highest number of mutations (309) and mutation frequency (52.91%), whereas the lowest was observed in endometrial cancer (29.72%). DIO3, selenoprotein O (SELO), and selenoprotein T (SELT) are significantly related to the prognosis of OV. Immune infiltration analysis showed that DIO3 was associated with tumor-associated macrophages, SELO with $CD4^+$ T-cells and monocytes, and SELT with T-cells. Enrichment analysis revealed that DIO3 is mainly involved in inflammatory immune responses and the Ras signaling pathway, SELO is primarily related to innate immune responses, and SELT is closely associated with mitochondrial oxidative phosphorylation.

Conclusion: This study explored the expression characteristics of 25 selenoprotein in patients with gynecological malignancies and found that DIO3, SELO, and SELT were significantly associated with the prognosis and clinical features of OV, which are potential therapeutic targets.

Keywords: Ovarian cancer, selenoproteins, prognosis, immune infiltration

Öz

Amaç: Yumurtalık kanserinde (YK) selenoproteinlerin ekspresyonunu ve prognostik değerini kapsamlı bir şekilde analiz etmek amaçlanmıştır.

Gereç ve Yöntemler: Selenoprotein ekspresyonunu, mutasyonlarını ve kopya sayısı varyasyonlarını analiz etmek için GEPIA ve cBioPortal kullanıldı. Bu genlerin klinik prognoz üzerindeki etkisini ve tümör immün infiltrasyonu ile korelasyonunu değerlendirmek için Kaplan-Meier plotter ve TIMER kullanıldı.

Bulgular: Normal dokularla karşılaştırıldığında, DIO3, GPX3, SECISBP2, SELM ve SELP ekspresyonları dört jinekolojik malignitede azalmıştır. Yumurtalık kanserinde selenoproteinler en yüksek mutasyon sayısına (309) ve mutasyon sıklığına (%52,91) sahipken, endometriyal kanserde (%29,72) en düşük mutasyon sayısına ve sıklığına sahip idi. DIO3, SELO ve SELT, YK prognozuyla anlamlı olarak ilişkili bulunmuştur. İmmün infiltrasyon analizi, DIO3'ün tümörle ilişkili makrofajlarla, SELO'nun $CD4^+$ T-hücreleri ve monositlerle ve SELT'nin T-hücreleriyle ilişkili olduğunu göstermiştir. Zenginleştirme analizi, DIO3'ün esas olarak enflamatuvar immün yanıtlarda ve Ras sinyal yolunda yer aldığını, SELO'nun esas olarak doğal bağışıklık yanıtlarıyla ilişkili olduğunu ve SELT'in mitokondriyal oksidatif fosforilasyonla yakından ilişkili olduğunu ortaya koymuştur.

Sonuç: Bu çalışmada, jinekolojik malignitelerde 25 selenoproteinin ekspresyon özellikleri araştırılmıştır ve DIO3, SELO ve SELT'in potansiyel terapötik hedef olan YK'nin prognozu ve klinik özellikleriyle önemli ölçüde ilişkili olduğunu bulunmuştur.

Anahtar Kelimeler: Yumurtalık kanseri, selenoproteinler, prognoz, immün infiltrasyon

PRECIS: Iodothyronine deiodinase 3, selenoprotein O, and selenoprotein T were significantly dysregulated in ovarian cancer and associated with the prognosis and clinical features of ovarian cancer, which were potential therapeutic target.

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Introduction

Gynecological malignancies, such as ovarian cancer (OV), cervical cancer, endometrial cancer, and uterine carcinosarcoma, pose significant threats to women's health. With changes in lifestyle and an aging population, the incidence of these tumors has been steadily increasing in China. Among them, cervical cancer has the highest morbidity rate, whereas OV has the highest mortality[1]. However, compared with cervical cancer, effective screening methods for ovarian and endometrial cancers remain inadequate[2]. The challenges of early diagnosis, along with limited treatment options in advanced stages, contribute to the highest mortality rate among all gynecological malignancies. The etiology of gynecological malignancies involves multiple factors, including reproductive history, hormone, genetics, environment, and lifestyle. Therefore, identifying prognostic factors and predictive biomarkers and investigating their underlying mechanisms are crucial for developing more effective diagnostic and therapeutic strategies.

Selenium is a trace element crucial for the biological functions of human cells, particularly in the synthesis of selenoprotein, which possess anti-inflammatory and antioxidant properties[3]. Multiple studies have demonstrated that selenium levels are generally low in most patients with gynecological malignancies and are closely associated with poor prognosis. Additionally, selenium supplementation has been shown to reduce the risk of OV in women[4-8]. A Phase I clinical trial found that using selenium alongside carboplatin and paclitaxel was safe and well tolerated in patients with advanced gynecological malignancies[9]. Mechanistically, higher selenium levels trigger ferroptosis in OV cells by downregulating glutathione peroxidase 4 (GPX4), thereby exerting a therapeutic effect[10]. Despite the potential antitumor effects of selenium, recent epidemiological data indicate that high levels of selenium exposure are associated with an increased incidence of certain cancers[11,12]. 25 selenoprotein have been identified, but their functions have only been partially understood[13]. The hierarchical regulation of selenoprotein in the body and the sex-specific effects of selenium may explain the inconsistent results regarding the effectiveness of selenium supplementation in cancer prevention[14]. Therefore, it is essential to conduct an in-depth exploration of the roles of different selenoprotein in gynecological malignancies, particularly to understand their potential mechanisms and expression patterns.

In this study, we conducted a comprehensive analysis of the expression, mutations, and copy number variations of 25 selenoprotein in patients with gynecological malignancies. Specifically, we focused on OV by performing a prognostic analysis of differentially expressed selenoprotein and further exploring their associations with the clinicopathological characteristics of patients with OV. Through multi-omics data analysis, we identified that iodothyronine deiodinase 3 (DIO3), selenoprotein O (SELO), and selenoprotein T (SELT) are significantly dysregulated in OV and are associated with poor prognosis. Additionally, we conducted immune infiltration analysis and gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses on these selenoprotein-related genes to explore their potential biological functions and mechanisms in OV. Therefore, this study enhances our understanding of the potential roles of selenoprotein in the initiation and progression of OV.

Materials and Methods

Analysis of selenoprotein mRNA expression

The Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) database was used to analyze the difference in the mRNA expression of selenoprotein between tumor and normal tissues and to investigate the correlation between selenoprotein gene expression and immune cell marker genes in OV[15].

Analysis of selenoprotein mutations and copy number variations

The cancer genome atlas (TCGA)-OV, cervical cancer, endometrial cancer, and uterine carcinosarcoma datasets from the cBioPortal (http://www.cbioportal.org/) database were used to perform mutation and copy number variation analyses of selenoprotein, as well as prognosis analysis before and after gene mutations[16,17].

Analysis of Kaplan-Meier Plotter database

Kaplan-Meier plotter (http://kmplot.com/analysis/) database was used to analyze the correlation between selenoprotein expression and the survival of OV patients[18].

Protein expression analysis of selenoprotein

Immunohistochemical images of SELO and SELT were obtained from the Human protein mapping (HPA, https://www.proteinatlas.org/) database. The Universal Analysis of Cancer (UALCAN, http://ualcan.path.uab.edu/) database was used to obtain expression data for DIO3, SELO, and SELT based on various clinical characteristics of OV[19].

Immune infiltration analysis

The tumor immune estimation resource (TIMER, https://cistrome.shinyapps.io/timer/) database was used to assess the association between DIO3, SELO, and SELT with tumor-infiltrating immune cells and immune cell marker genes[20].

Gene correlation and enrichment analysis

WebGestalt (https://www.webgestalt.org/) was used to perform GO and KEGG pathway enrichment analyses of genes correlated with DIO3, SLEO, and selenoprotein T (SLET), which were obtained from LinkedOmics (http://linkedomics.org/login.php)[21,22].

Statistical Analysis

Survival curves were generated using the Kaplan-Meier plotter, and the results are presented as hazard ratios and p-values

derived from the logrank test. Spearman's exact test was used to analyze the correlation between gene expression. The bubble map is plotted using the R ggplot package. P<0.05 was considered statistically significant.

Results

mRNA expression of selenoprotein in different types of gynecological malignancies

First, the results from the GEPIA database showed that in four gynecological malignancies, the expression levels of DIO3, GPX3, SECISBP2, SELM, and SELP were generally lower than those in normal tissues (Figure 1). However, DIO1, EEFSEC, SELI, SELK, SELV, SELW, SEPHS1, TXNRD1, and TXNRD2 were not dysregulated (Supplementary Figure 1). Additionally, some selenoprotein exhibit significant changes in expression in specific tumor types. Compared with normal tissues, GPX1, SLET, and SEPHS2 expression was significantly increased in OV, whereas SEPSECS, TXNRD3, and SELO expression was significantly downregulated. In cervical cancer, GPX2 and MSRB1 were markedly upregulated, whereas SELENBP1 was significantly downregulated. In endometrial cancer, the expression of DIO2, GPX1, GPX4, and SEPHS2 was notably increased, whereas SEPSECS showed a significant decrease in uterine carcinosarcoma (Supplementary Figure 2, 3).

Mutations and copy number variations of selenoprotein in TCGA-OV, cervical cancer, endometrial cancer, and uterine carcinosarcoma datasets

Next, we found that OV had the highest number of selenoprotein gene mutations (309) and the highest mutation frequency (52.91%), whereas endometrial cancer had the fewest mutations (162) and a frequency of 29.72% (Figure 2A). Additionally, patients with OV mutations showed better overall survival compared with those without mutations (p=0.0347), but there was no significant difference in disease-free survival (p=0.734) (Figure 2B, C). In contrast, compared with the non-mutated group, patients with mutations in endometrial cancer had worse overall survival (p=0.151) and disease-free survival (p=0.0902). For cervical cancer and uterine carcinosarcoma, there were no significant differences in OS and disease-free survival between the mutated and non-mutated groups

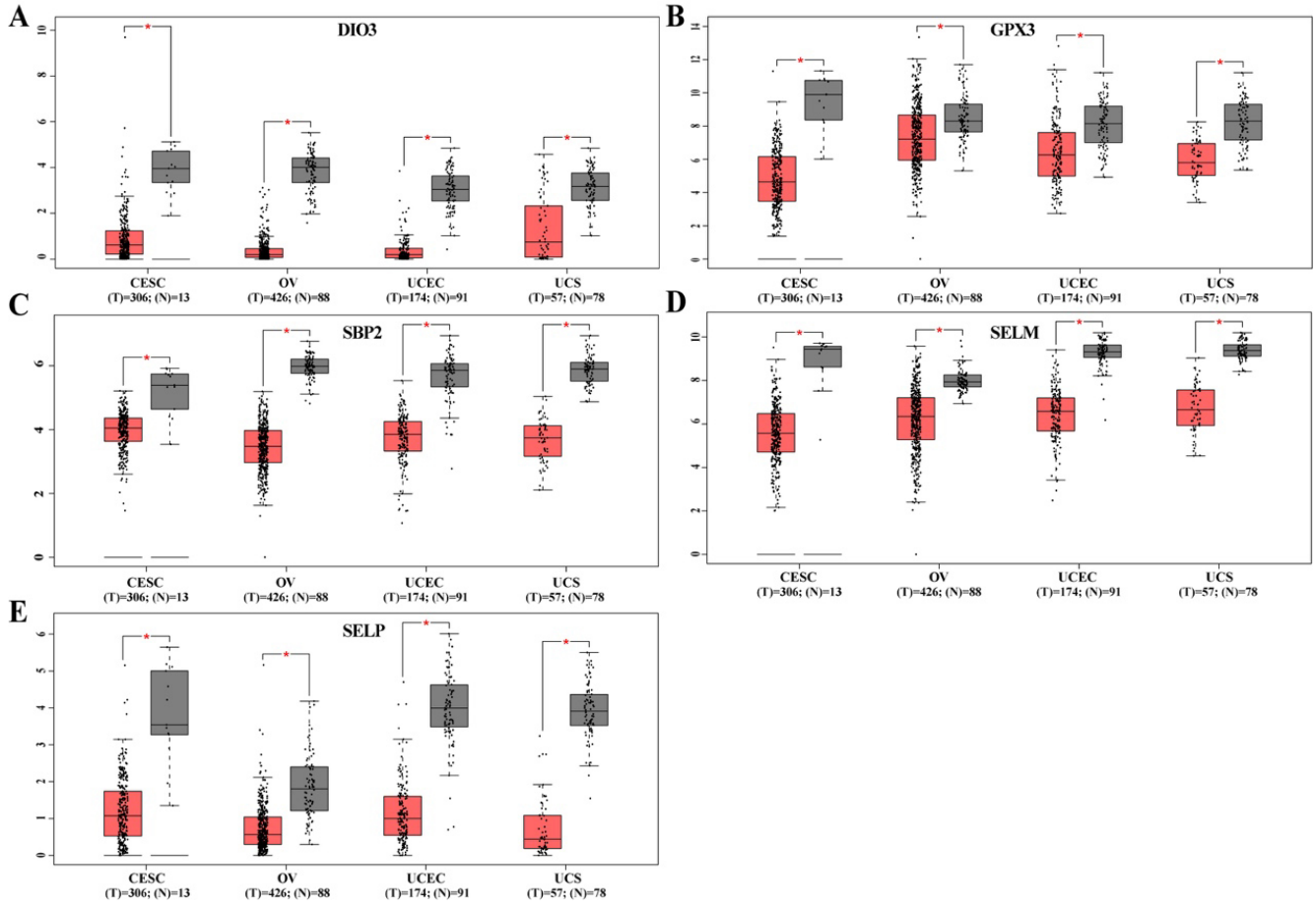

Figure 1. The mRNA expression of selenoprotein in four gynecological malignancies (GEPIA). A-E) The expression of five consistently downregulated selenoprotein in tumor and normal tissues [DIO3 (A), GPX3 (B), SECISBP2 (C), SELM (D), and SELP (E)]

The red asterisks (*) indicating significant differences (p<0.05), CESC: Cell carcinoma and endocervical adenocarcinoma, OV: Ovarian cancer, UCEC: Uterine corpus endometrial carcinoma

(Supplementary Figure 4A-C). Moreover, SELT (15%), SELV (12%), and SELENBP1 (12%) had the highest mutation rates in OV; SELT (8%) and SELP (7%) had the highest mutation rates in cervical cancer; SELENBP1 (10%) and SELT (5%) were the most frequently mutated in endometrial cancer; SELT (21%) and SELENBP1 (13%) showed the highest mutation rates in uterine carcinosarcoma (Figure 2D and Supplementary Figure 4D-F). Based on these results, we selected OV as the focus of our subsequent research.

Prognostic value of selenoprotein in OV

For the prognostic value of differentially expressed selenoprotein in OV, we found that low expression of DIO3, SECISBP2, and SELO, as well as high expression of GPX3, SELM, and SELP, were associated with poorer overall survival (Figure 3A). Additionally, low expression of DIO3, SELO, and SEPHS2 and high expression of GPX3, SECISBP2, SELM, and SELT were associated with worse progression-free survival (Figure 3B). Based on the expression differences and clinical significance of these genes, we selected DIO3, SELO, and SELT as the primary molecules for further research.

Association between DIO3, SELO, and SELT expression and clinicopathological features in patients with OV

By analysis in the HPA, UALCAN, and Kaplan-Meier plotter databases, we found that DIO3, SELO, and SELT were not significantly correlated with the clinical stages or tumor grades of OV. However, as the tumor grade increased (indicating lower

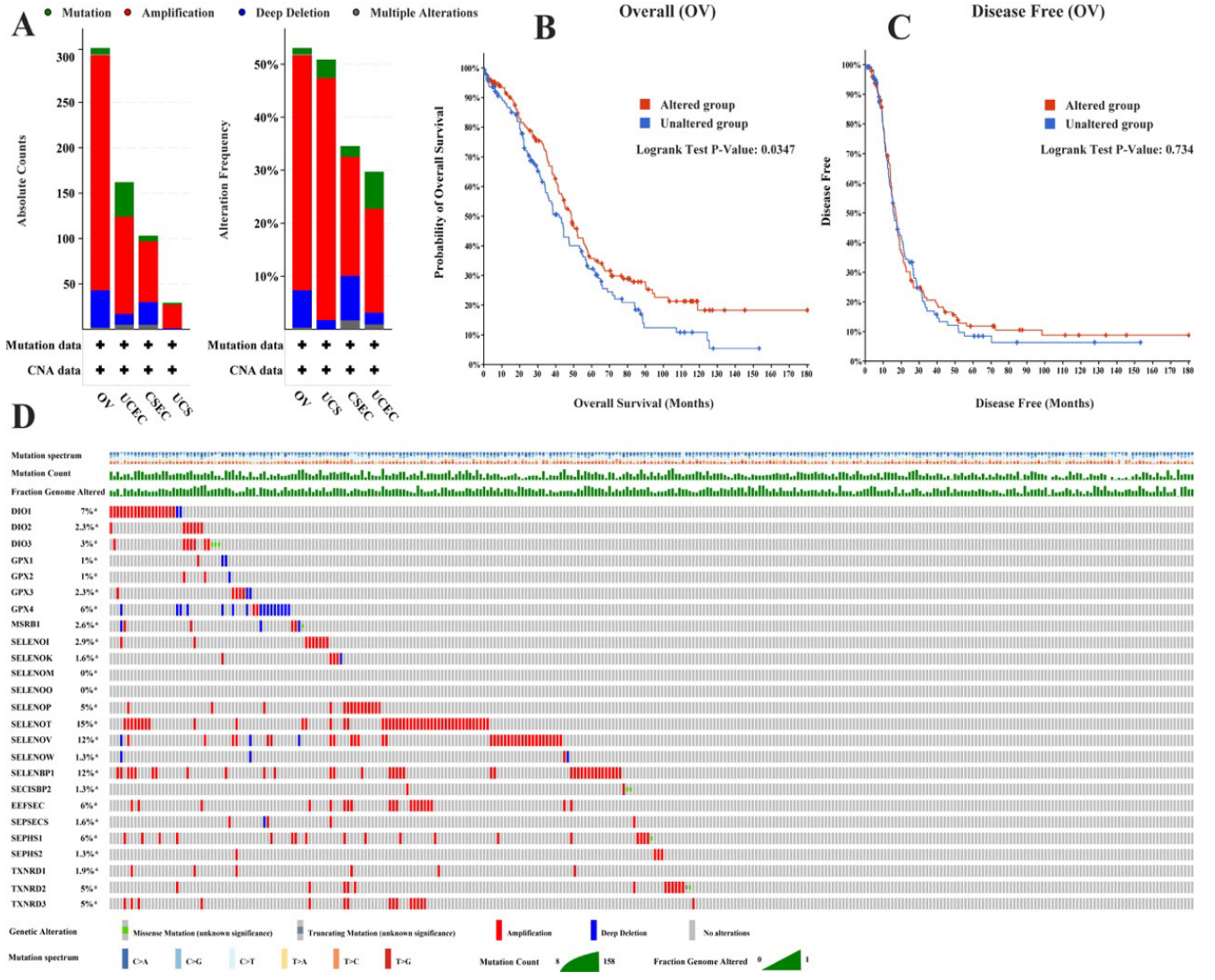

Figure 2. Mutations and copy number variations of selenoprotein (cBioPortal). Numbers and frequencies of selenoprotein mutations in four gynecological malignancies. B, C. Comparison of overall survival (B) and disease-free survival (C) between the selenoprotein gene-mutated and non-mutated groups in patients with OV. Relationship between mutations and copy number variations of 25 selenioproteins and OV

OV: Ovarian cancer, Logrank p<0.05 indicates statistical significance, CESC: Cell carcinoma and endocervical adenocarcinoma, UCEC: Uterine corpus endometrial carcinoma

differentiation), the expression of DIO3 tended to decrease gradually (Figure 4A, B). Regarding the TP53 mutation status, unlike DIO3 and SELO, SELT expression was significantly increased in patients with TP53 mutations (Figure 4C). At the protein level (data on DIO3 is lacking), immunohistochemistry and total protein analysis revealed that SELO expression was significantly reduced, whereas SELT expression was significantly increased in OV. SELO protein levels decreased with advancing tumor stage and grade, whereas SELT protein levels increased in patients with higher stages (stages 2 and 3) and grades (grades 2 and 3) (Figure 4D-G). Low DIO3 expression was significantly associated with poorer overall survival and progression-free survival in OV patients with the following characteristics: CA125 levels below the lower quartile, optimal or suboptimal debulk, and receiving platinum-based chemotherapy (Supplementary Table 1, p<0.05). Additionally, low expression of SLEO was also significantly associated with poorer overall survival and progression-free survival in serous and grade 2-3 OV patients, which may also exhibit average CA125 levels below the lower quartile and optimal debulk (Supplementary Table 2, p<0.05). High SELT expression was significantly associated with poorer progression-free survival in the following patient groups: Serous OV, stage 3-4, grade 3, P53 mutation, optimal debulk, and receiving platinum-based or gemcitabine chemotherapy (Supplementary Table 3, p<0.05).

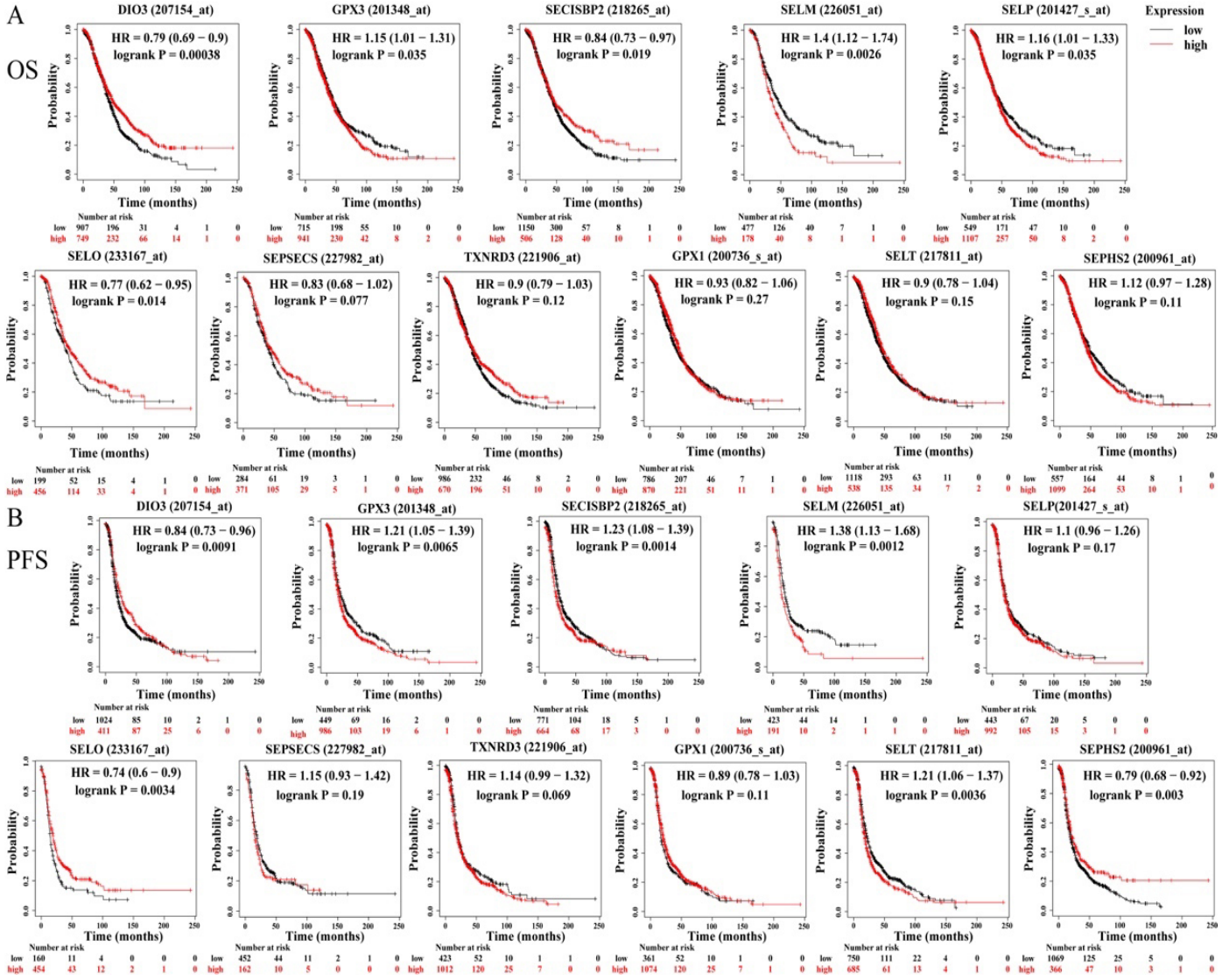

Figure 3. Prognostic value of selenoprotein differentially expressed in OVs (Kaplan-Meier plotter). A-B. Comparison of overall survival (A) and progression-free survival (B) between the high and low selenoprotein expression groups in patients with ovarian cancer

OV: Ovarian cancer, DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T, HR: Hazard ratio. Logrank p<0.05 indicates statistical significance

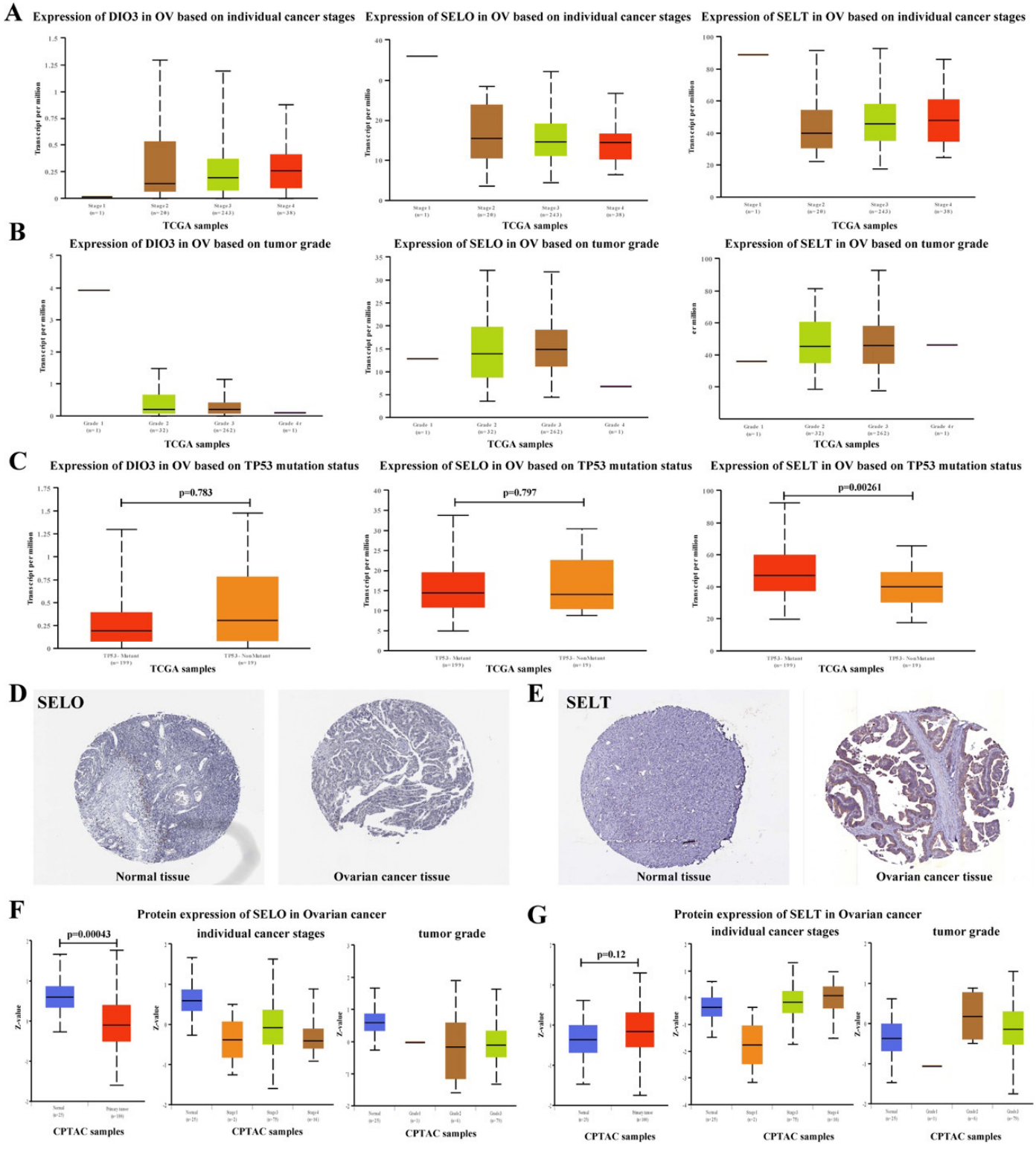

Figure 4. Association between DIO3, SELO, and SELT expression and clinicopathological features in patients with OV (UALCAN and HPA). A-C) DIO3, SELO, and SELT expression in OV based on tumor stage (A), tumor grade (B), and TP53 mutation (C). D, E) Representative immunohistochemical images of SELO (D) and SELT € in normal and OV tissues. Protein levels of SLEO (F) and SELT (G) in normal and ovarian cancer tissues based on tumor stage and grade

OV: Ovarian cancer, DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T, UALCAN: Universal Analysis of Cancer, HPA: Human protein mapping, TCGA: Cancer genome atlas, CPTAC: Clinical proteomic tumor analysis consortium. P<0.05 was considered statistically significant

Association between DIO3, SELO, and SELT expression and immune infiltration in OV

Tumor-infiltrating lymphocytes are independent predictors of cancer survival. We found that DIO3 was negatively associated with macrophage infiltration (r=-0.165, p=2.89e^{-4}) (Figure 5A); SELO was negatively correlated with tumor purity (r=-0.159, p=0.0117), while positively correlated with CD4$^+$ T-cells (r=0.168, p=0.0084) (Figure 5B); SELT was negatively correlated with tumor purity (r=-0.158, p=4.78e^{-4}), while positively associated with CD8$^+$ T-cells (r=0.15, p=9.73e^{-4}), CD4$^+$T-cells (r=0.094, p=0.0403), macrophages (r=0.277, p=7.04e^{-10}), neutrophils (r=0.285, p=1.99e^{-10}) and dendritic cells (r=0.201, p=9.35e^{-6}) (Figure 5C).

Correlation analysis of DIO3, SELO, and SELT expression with immune cell marker genes

The analyses in the TIMER and GEPIA databases revealed that DIO3 was significantly correlated with tumor-associated macrophage (TAM) marker genes and some marker genes of different T-cell subsets in OVs. SELO was mainly significantly associated with the marker genes of CD4$^+$ T-cells and monocytes. Moreover, SELT was significantly correlated with the marker genes of total T-cell, CD8$^+$ T-cell, Th1 cell, and exhausted T-cell (Supplementary Table 4, 5). Specifically, *CCL2, CD68*, and *IL10* (TAMs marker genes) were significantly correlated with DIO3 (Figure 5D). *CD4* (CD4$^+$ T-cell marker gene) and *CD86* and CSF1R (monocyte marker genes) were significantly correlated with SELO (Figure 5E). *CD2, CD3D* (total T-cell marker genes), *CD8B* (CD8$^+$ T-cell marker genes), *STAT1, STAT4, IFNG, TNF* (Th1 cell marker genes), *PDCD1, CTLA4, LAG3,* and *HAVCR2* (exhausted T-cell marker genes) were significantly correlated with SELT (Figure 5F).

Biological functions and signaling pathways of DIO3, SELO, and SELT in OV

Significantly correlated genes with DIO3, SELO, and SELT in OV were identified by LinkedOmics database. The expression patterns of the top 50 positively and negatively correlated genes are presented as heatmaps (Supplementary Figure 5A-C). GO enrichment analysis indicated that DIO3 was positively associated with inflammatory and immune responses and Ras activity. In contrast, it was mainly negatively correlated with cilium assembly and microtubule movement (Supplementary Figure 6A). SELO was mainly involved in NF-κB signaling pathway and MAP kinase activity, while negatively regulating chromatin and histone modification (Supplementary Figure 6B). SELT was associated with mitochondria-related biological activities and also showed a negative correlation with the regulation of chromatin and histones (Supplementary Figure 6C). Additionally, KEGG enrichment analysis revealed that DIO3 was primarily involved in Ras and chemokine signaling pathways (Figure 6A). SELO was mainly associated with the NOD-like receptor, toll-like receptor, and TNF signaling pathways (Figure 6B). Consistent with the GO enrichment analysis, SELT was closely related to oxidative phosphorylation (Figure 6C).

Discussion

Limited treatment options, resistance to existing chemotherapeutic drugs, and tumor recurrence are the primary obstacles to extending the survival of patients with gynecological malignancies. Selenium, an essential trace element, has significant antiviral properties and antitumor effects. Although clinical trials on selenium supplementation for the prevention of endometrial and cervical cancers have yielded mixed results, studies suggest that selenium may reduce the risk of developing OV[5,23,24]. Additionally, selenium supplements have been shown to significantly alleviate the toxic side effects associated with chemotherapy or radiotherapy, thereby improving the quality of life of patients[24-26]. Selenium exerts its effects in the body primarily through the synthesis of selenoprotein, which have anti-inflammatory and antioxidant properties. However, the specific functions and mechanisms of most selenoprotein remain unclear. Therefore, this study comprehensively analyzed the expression patterns of 25 selenoprotein in gynecological malignancies and their potential prognostic value in OV, aiming to provide a scientific basis for the application of selenoprotein in cancer therapy.

Several selenoprotein, such as DIO3, GPX3, SECISBP2, SELM, and SELP, are significantly downregulated in gynecological malignancies, which may be related to lower serum selenium levels in patients with cancer[27,28]. By analyzing the selenoprotein mutations, we observed these genes exhibit the highest number and frequency of mutations in OV. Moreover, patients with OV with mutations in these genes had a significantly higher tumor mutational burden (TMB) compared with those without mutations, which is often associated with better overall survival. It has been reported that TMB levels are significantly positively correlated with the effectiveness of PD-1 inhibitors, and patients with tumors with high TMB levels lived longer[29]. This may be because a higher number of mutated genes leads to the production of more abnormal proteins, thereby enhancing the recognition and activation of the immune system, which in turn improves the effectiveness of immunotherapy and chemotherapy. Through prognostic and immune infiltration analyses, we found that the expression of DIO3, SELO, and SELT was significantly associated with the prognosis and clinical characteristics of patients undergoing OV. These genes are also involved in regulating the infiltration of immune cells into the tumor microenvironment. These findings revealed the important roles of DIO3, SELO, and SELT in the pathogenesis of OV and may provide new targets for future therapeutic strategies.

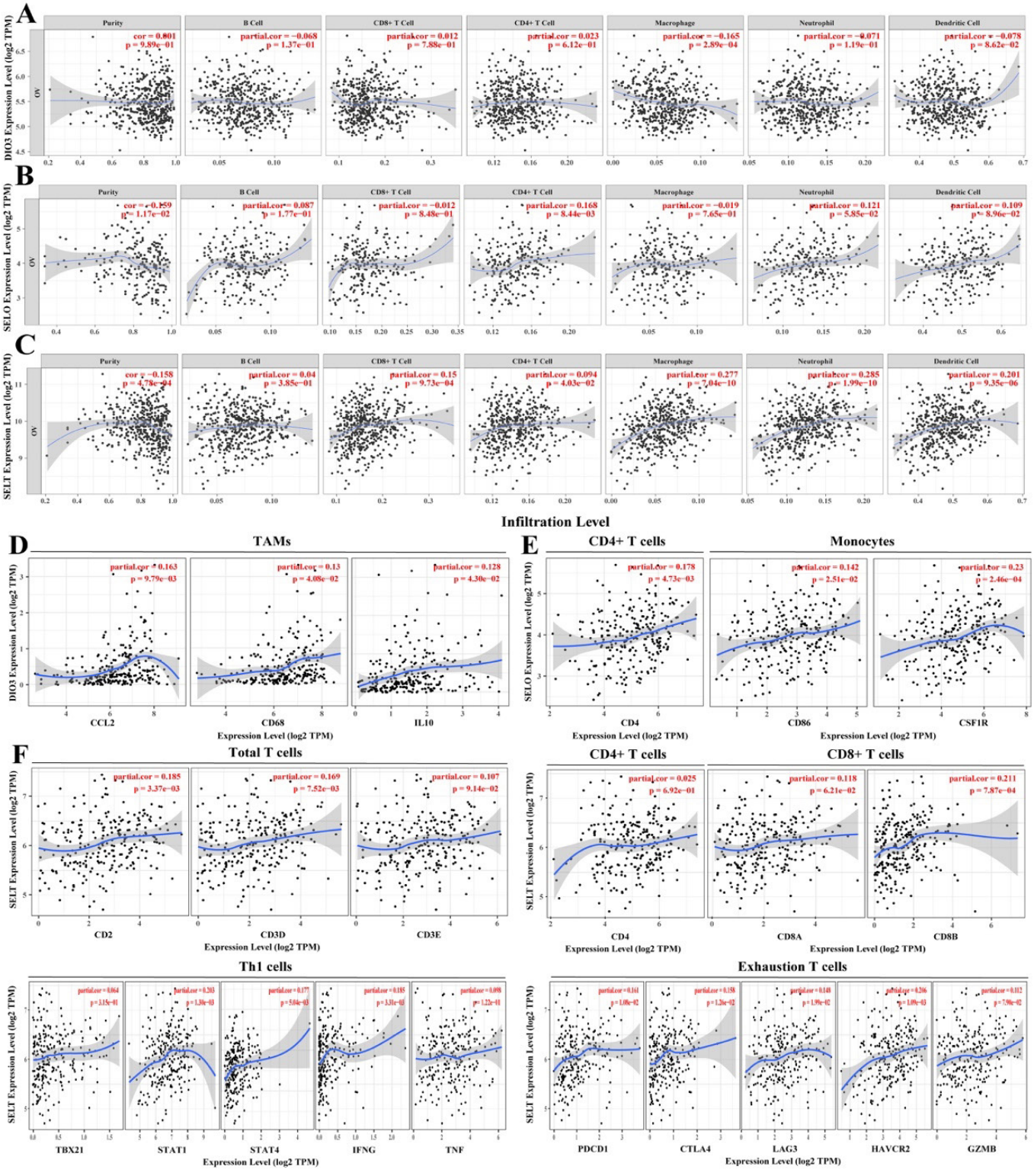

Figure 5. Correlation between the expression of DIO3, SELO, and SELT and immune infiltration in ovarian cancer (TIMER). Correlation between the expression of DIO3 (A), SELO (B), and SELT (C) and tumor purity, as well as the infiltration of B-cells, CD8+ T-cells, CD4+ T-cells, macrophages, neutrophils, and dendritic cells. Correlations between DIO3 expression, CCL2CD68 expression, and IL10. E. Correlations between SELO expression and CD4, CD86, and CSF1R expression. Correlation between SELT expression and CD2CD3D, CD3E, CD8A, CD8B, TBX21, STAT1, STAT4, IFNG, TNF, PDCD1, CTLA4, LAG3, HAVCR2, GZMB. P<0.05 was considered statistically significant

DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T, TNF: Tumor necrosis factor, TIMER: Tumor immune estimation resource

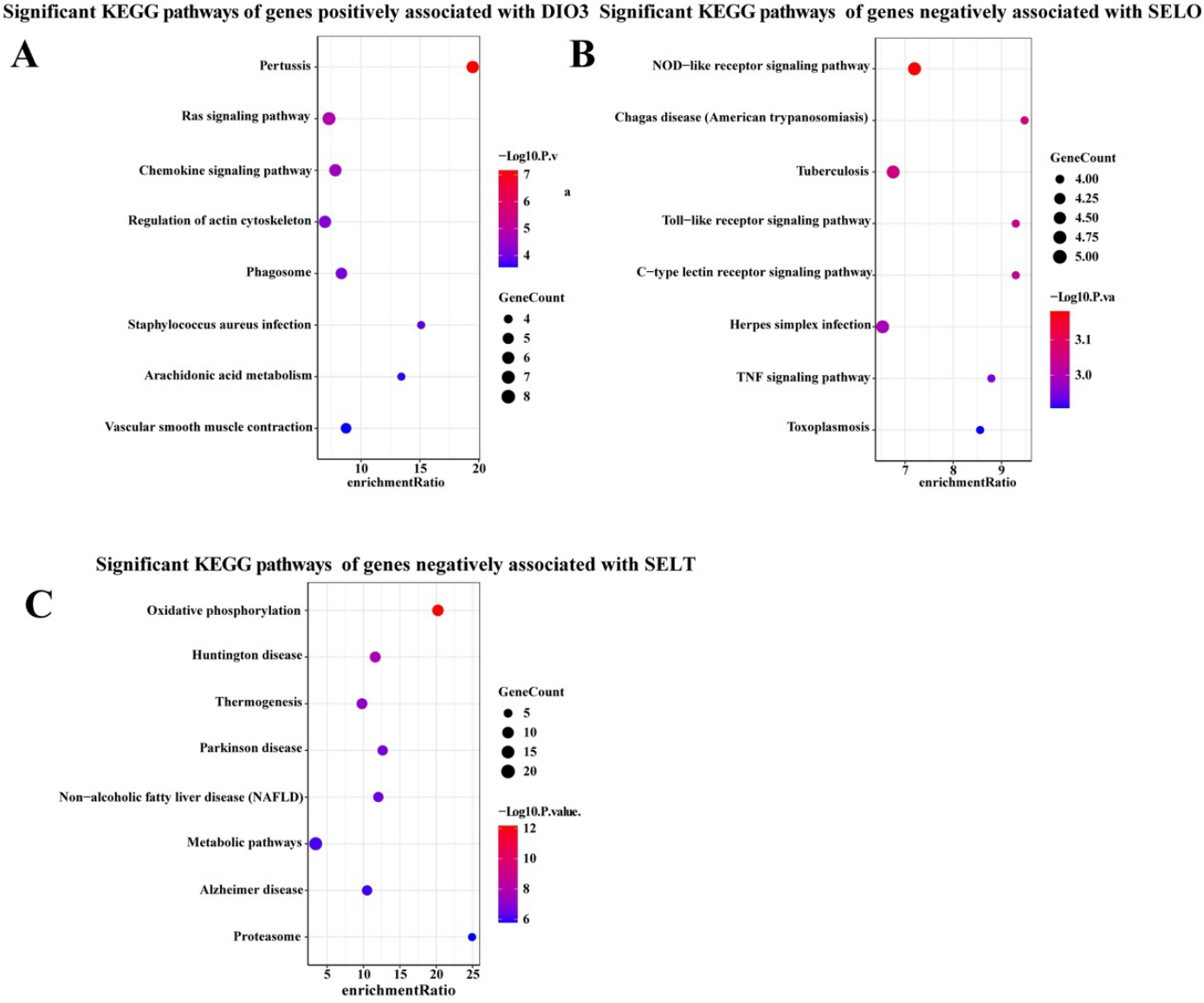

Figure 6. KEGG enrichment analysis of genes associated with DIO3, SELO, and SELT in ovarian cancer (LinkedOmics and WebGestalt). A-C. Bubble plot showing the KEGG enrichment analysis results for the top 100 genes with significant positive/negative associations with DIO3 (A), SELO (B), and SELT (C)

KEGG: Kyoto Encyclopedia of Genes and Genomes, DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T, p<0.05 was considered statistically significant

DIO3 contains a selenocysteine (Sec) active site that is capable of inactivating thyroid hormone T3. In various tumor types, abnormal DIO3 expression is closely associated with tumor proliferation and differentiation[30]. Our findings suggest that DIO3 is a potential biomarker and therapeutic target of OV. In contrast, Moskovich et al.[31,32] found that increased DIO3 expression promoted tumor development and metabolic reprograming by modulating T3 in high-grade serous OV. They further found that a small-molecule inhibitor targeting DIO3 was effective in inhibiting tumor growth[31,32]. However, low DIO3 expression was negatively associated with overall survival and progression-free survival in low-grade (grade 1) OV. In high-grade (grade 2+3 and grade 3) OV, low DIO3 expression was still significantly negatively associated with overall survival but positively associated with progression-free survival (Supplementary Table 1). These results suggest that DIO3 may play a very different role in different types, stages, and grades of OV. In addition to regulating the deactivation of T3, DIO3 may also be involved in epigenetic regulation through genomic imprinted regions co-formed with DLK1[33]. Frequent interactions between different regulatory pathways may contribute to contradictory findings. Consistent with our

immune infiltration and enrichment analyses, Zhang et al.[34] demonstrated that the DLK1-DIO3 locus is closely linked to Ras-induced hepatocarcinogenesis. Additionally, the DLK1-DIO3 region has been associated with alterations in immune cell and inflammatory cytokine levels in various diseases[35-37]. However, reports on DLK1-DIO3 in OV are extremely limited, indicating the urgent need for further research to explore the potential role of DIO3 in OV.

SELO is a mitochondrial protein with redox activity involved in ATP amidation[38,39]. Previous studies have shown that SELO is downregulated in gastric and liver cancers, and this downregulation is associated with poor prognosis in patients[40,41]. Similarly, SELO was significantly downregulated in four gynecological malignancies. The multi-omics analysis further revealed that low SELO expression is associated with poor prognosis in patients undergoing OV. This effect may be mediated through the regulation of innate immune response pathways, which influence the dynamics of tumor burden and the infiltration of $CD4^+$ T-cells. However, no studies have investigated the involvement of SELO in tumor pathogenesis or immune regulation processes. Therefore, further investigation into SELO's regulatory role in OV, particularly through adaptive immune response pathways and its redox activity, represents a novel and significant research direction.

SELT is an endoplasmic reticulum membrane protein with thioredoxin reductase activity[42]. Studies have shown that SELT expression is significantly increased in breast cancer, and it contributes to the prevention of apoptosis in cancer cells[43]. Additionally, SELT protects the heart from ischemia-reperfusion injury by inhibiting apoptosis and oxidative stress[44]. In this study, we found that SELT expression was significantly increased in Ovs and was closely associated with poor patient prognosis and resistance to platinum-based chemotherapy. This may be because SELT protected OV cells against apoptosis by inhibiting oxidative stress responses and calcium ion flux, thereby promoting tumor growth. Furthermore, our results suggest that SELT plays a critical role in T-cell differentiation and homeostasis regulation through oxidative phosphorylation. The differentiation of T-cells is closely linked to changes in energy metabolism: Naive and memory T-cells maintain high levels of oxidative phosphorylation, whereas effector T-cells rely on aerobic glycolysis. In contrast, continuous tumor antigen stimulation could impair the oxidative phosphorylation pathway in activated T-cells, leading to their transition into hypometabolic exhausted T-cells, which suppresses both mitochondrial respiration and glycolytic function[45]. Although no direct studies have linked SELT to T-cell differentiation, SELT-regulated oxidative phosphorylation and mitochondrial respiration may play critical roles in the remodeling of the tumor immune microenvironment in OV. Future research should focus on the relationship between SELT-regulated tumor immune microenvironment and OV progression.

Study Limitations

This study utilized multiple databases to validate the reliability of the findings. However, there are some limitations. Further fundamental experiments are essential to elucidate the molecular mechanisms of selenoprotein in the progression of OV.

Conclusion

In conclusion, through comprehensive bioinformatics analysis, this study revealed an association between dysregulated expression of DIO3, SELO, and SELT and poor prognosis in OV. We further explored the functions and pathways involved in these three selenoprotein to elucidate their roles in disease development in OV. Our findings not only provide new insights into the possible regulatory pathways of DIO3, SELO, and SELT but also provide new perspectives on the role of these selenoprotein in OV.

Ethics

Ethics Committee Approval: The Yuhang Third People's Hospital Ethics Committee has confirmed that no ethics approval was required, and this study was performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000.

Informed Consent:

Acknowledgments

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Footnotes

Authorship Contributions

Concept: Y.H., H.D., Design: Y.H., H.D., Data Collection or Processing: Y.H., H.S., H.D., Analysis or Interpretation: Y.H., H.D., Literature Search: Y.H., H.S., H.D., Writing: Y.H.

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

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References

1. Xia C, Dong X, Li H, Cao M, Sun D, He S, et al. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J (Engl). 2022;135:584-90.
2. Zhou Z, Li W, Zhang F, Hu K. The value of squamous cell carcinoma antigen (SCCa) to determine the lymph nodal metastasis in cervical cancer: A meta-analysis and literature review. PLoS One. 2017;12:e018616.
3. Rayman MP. Selenium and human health. Lancet. 2012;379:1256-68.
4. Sundström H, Ylikorkala O, Kauppila A. Serum selenium and thromboxane levels in patients with gynecological cancer. Carcinogenesis. 1986;7:1051-2.
5. Terry PD, Qin B, Camacho F, Moorman PG, Alberg AJ, Barnholtz-Sloan JS, et al. Supplemental selenium can decrease ovarian cancer risk in African-American women. J Nutr. 2017;147:621-7.

6. Gifkins D, Olson SH, Paddock L, King M, Demissie K, Lu SE, et al. Total and individual antioxidant intake and risk of epithelial ovarian cancer. BMC Cancer. 2012;12:211.
7. Janowska M, Potocka N, Paszek S, Skrzypa M, Wróbel A, Kluz M, et al. Serum selenium concentration in women with endometrial cancer. Nutrients. 2022;14:958.
8. Qi L, Wang Y, Wang R, Wang M, Jablonska E, Zhou H, et al. Association between plasma selenium and its untargeted metabolomic profiling and cervical cancer prognosis. Biol Trace Elem Res . 2023;201:4637-48.
9. Song M, Kumaran MN, Gounder M, Gibbon DG, Nieves-Neira W, Vaidya A, et al. Phase I trial of selenium plus chemotherapy in gynecologic cancers. Gynecol Oncol. 2018;150:478-86.
10. Choi JA, Lee EH, Cho H, Kim JH. High-dose selenium induces ferroptotic cell death in ovarian cancer. Int J Mol Sci. 2023;24:1918.
11. Vinceti M, Vicentini M, Wise LA, Sacchettini C, Malagoli C, Ballotari P, et al. Cancer incidence following long-term consumption of water with high inorganic selenium content. Sci Total Environ. 2018;635:390-6.
12. Narod SA, Huzarski T, Jakubowska A, Gronwald J, Cybulski C, Oszurek O, et al. Serum selenium levels and cancer risk: a nested case–control study. Hered Cancer Clin Pract. 2019;17:33.
13. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigó R, et al. Characterization of mammalian selenoproteomes. Science. 2003;300:1439-43.
14. Schomburg L, Schweizer U. Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium. Biochim Biophys Acta. 2009;1790:1453-62.
15. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45:W98-102.
16. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401-4. Erratum in: Cancer Discov. 2012;2:960.
17. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6:pl1.
18. Lánczky A, Győrffy B. Web-based survival analysis tool tailored for medical research (KMplot): Development and implementation. J Med Internet Res. 2021;23:e27633.
19. Chandrashekar DS, Karthikeyan SK, Korla PK, Patel H, Shovon AR, Athar M, et al. UALCAN: An update to the integrated cancer data analysis platform. Neoplasia. 2022;25:18-27.
20. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108-e10.
21. Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analysis of multi-omics data within and across 32 cancer types. Nucleic Acids Res. 2018;46:D956-63.
22. Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. Nucleic Acids Res. 2019;47:W199-205.
23. 23. Kho PF, Glubb DM, Thompson DJ, Spurdle AB, O'Mara TA. The Role of Selenium in endometrial cancer risk: A mendelian randomization study. Front Oncol. 2019;9:182.
24. Muecke R, Micke O, Schomburg L, Glatzel M, Reichl B, Kisters K, et al. Multicenter, phase III trial comparing selenium supplementation with observation in gynecologic radiation oncology: follow-up analysis of the survival data 6 years after cessation of randomization. Integr Cancer Ther. 2014;13:463-7.
25. Muecke R, Schomburg L, Glatzel M, Berndt-Skorka R, Baaske D, Reichl B, et al. Multicenter, phase 3 trial comparing selenium supplementation with observation in gynecologic radiation oncology. Int J Radiat Oncol Biol Phys. 2010;78:828-35.
26. Sieja K, Talerczyk M. Selenium as an element in the treatment of ovarian cancer in women receiving chemotherapy. Gynecol Oncol. 2004;93:320-7.
27. Kluza M, Paszek S, Kluza K, Januszek S, Potocka N, Skrzypa M, et al. Serum selenium concentration in women with ovarian cancer. Nutrients. 2023;15:850.
28. He D, Wang Z, Huang C, Fang X, Chen D. Serum selenium levels and cervical cancer: A systematic review and meta-analysis. Biol Trace Elem Res. 2017;179:195-202.
29. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 2019;30:44-56.
30. Luongo C, Dentice M, Salvatore D. Deiodinases and their intricate role in thyroid hormone homeostasis. Nat Rev Endocrinol. 2019;15:479-88.
31. Moskovich D, Alfandari A, Finkelshtein Y, Weisz A, Katzav A, Kidron D, et al. DIO3, the thyroid hormone inactivating enzyme, promotes tumorigenesis and metabolic reprograming in high-grade serous ovarian cancer. Cancer Lett. 2021;501:224-233. Erratum in: Cancer Lett. 2023;563:216196.
32. Moskovich D, Finkelshtein Y, Alfandari A, Rosemarin A, Lifschytz T, Weisz A, et al. Targeting the DIO3 enzyme using first-in-class inhibitors effectively suppresses tumor growth: a new paradigm in ovarian cancer treatment. Oncogene. 2021;40:6248-57. Erratum in: Oncogene. 2023;42:1508.
33. Glazov EA, McWilliam S, Barris WC, Dalrymple BP. Origin, evolution, and biological role of miRNA cluster in DLK-DIO3 genomic region in placental mammals. Mol Biol Evol. 2008;25:939-48.
34. Zhang J, Li H, Dong J, Zhang N, Liu Y, Luo X, et al. Omics-based identification of shared and gender disparity routes in *Hras12V*-induced hepatocarcinogenesis: An important role for Dlk1-Dio3 genomic imprinting region. Front Genet. 2021;12:620594.
35. Groeneveld S, Faget J, Zangger N, Meylan E. Snail mediates repression of the Dlk1-Dio3 locus in lung tumor-infiltrating immune cells. Oncotarget. 2018;9:32331-45.
36. Al-Harbi S, Choudhary GS, Ebron JS, Hill BT, Vivekanathan N, Ting AH, et al. miR-377-dependent BCL-xL regulation drives chemotherapeutic resistance in B-cell lymphoid malignancies. Mol Cancer. 2015;14:185.
37. Dai R, Lu R, Ahmed SA. The upregulation of genomically imprinted DLK1-Dio3 miRNAs in Murine Lupus Is Associated with Global DNA Hypomethylation. PLoS One. 2016;11:e0153509.
38. Han SJ, Lee BC, Yim SH, Gladyshev VN, Lee SR. Characterization of mammalian selenoprotein o: a redox-active mitochondrial protein. PLoS One. 2014;9:e95518.
39. Sreelatha A, Yee SS, Lopez VA, Park BC, Kinch LN, Pilch S, et al. Protein AMPylation by an Evolutionarily Conserved Pseudokinase. Cell. 2018;175:809-21.e19.

40. Lan X, Xing J, Gao H, Li S, Quan L, Jiang Y, et al. Decreased selenoprotein expression as a poor prognosticator of gastric cancer in humans. Biol Trace Elem Res. 2017;178:22-8.

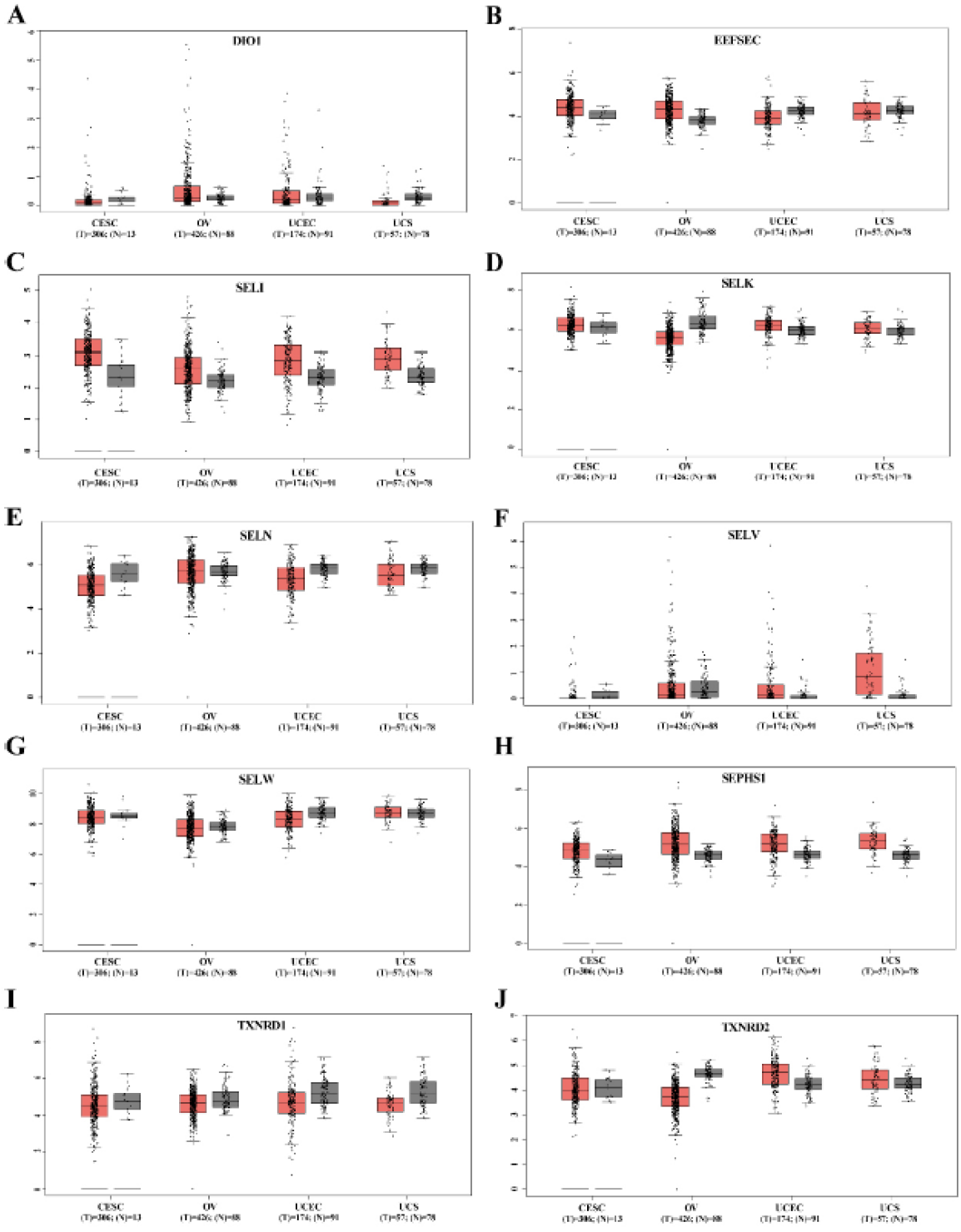
41. Zhao H, Tang J, Xu J, Cao L, Jia G, Long D, et al. Selenoprotein genes exhibit differential expression patterns between hepatoma HepG2 and normal hepatocyte LO2 cell lines. Biol Trace Elem Res. 2015;167:236-41.

42. Pothion H, Jehan C, Tostivint H, Cartier D, Bucharles C, Falluel-Morel A, et al. Selenoprotein T: essential oxidoreductase for endoplasmic reticulum homeostasis. Antioxid Redox Signal. 2020;33:1257-75.

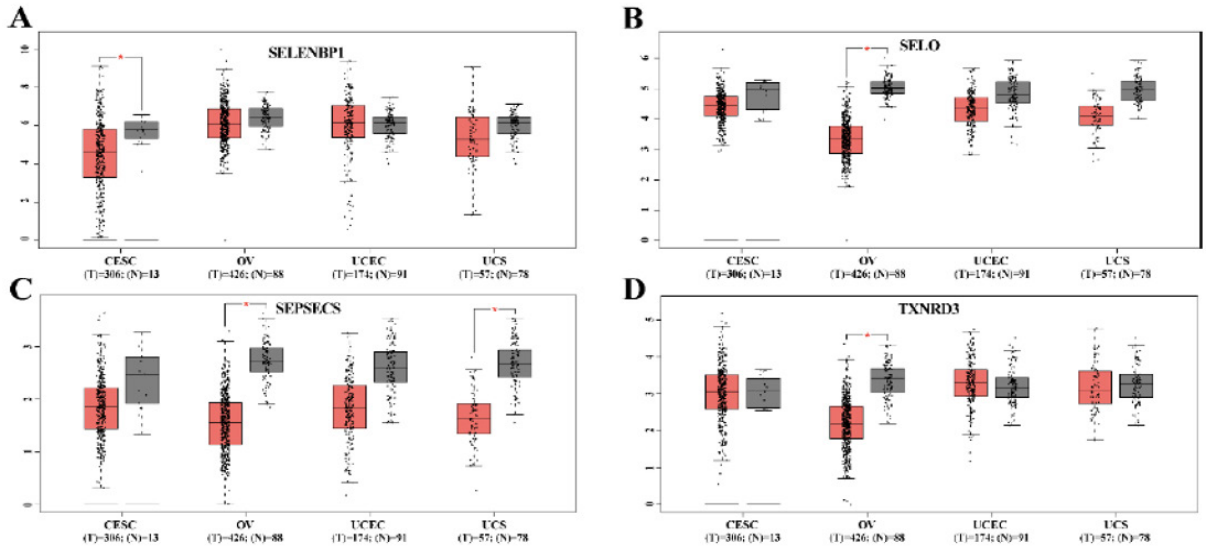
43. Zhuang W, Liu J, Li W. hsa-miR-33-5p as a therapeutic target promotes apoptosis of breast cancer cells *via* selenoprotein T. Front Med (Lausanne). 2021;8:651473.

44. Rocca C, Boukhzar L, Granieri MC, Alsharif I, Mazza R, Lefranc B, et al. A selenoprotein T-derived peptide protects the heart against ischemia/reperfusion injury by inhibiting apoptosis and oxidative stress. Acta Physiol (Oxf). 2018;223:e13067.

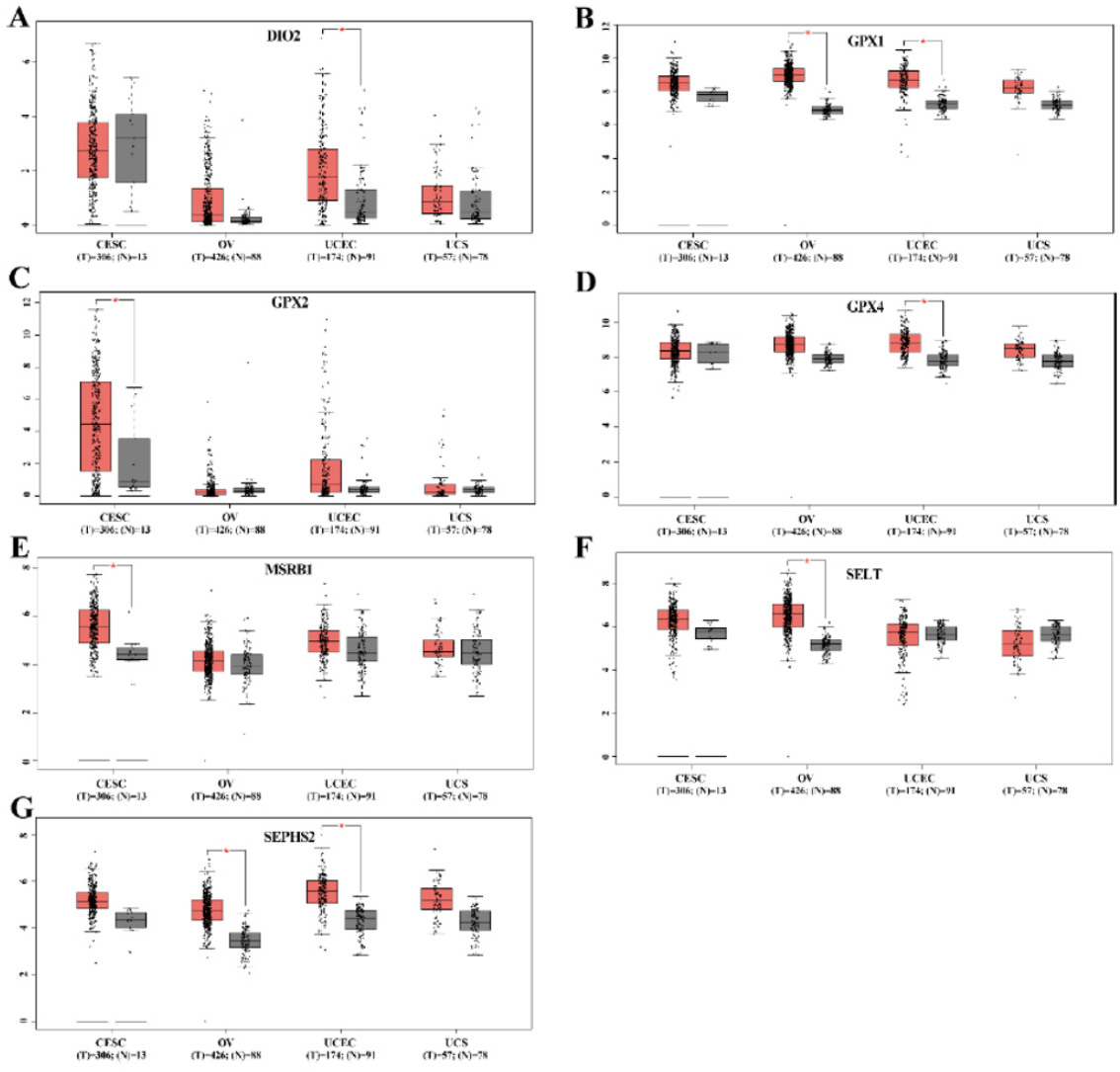
45. Vardhana SA, Hwee MA, Berisa M, Wells DK, Yost KE, King B, et al. Impaired mitochondrial oxidative phosphorylation limits T-cell self-renewal following exposure to persistent antigen. Nat Immunol. 2020;21:1022-33.



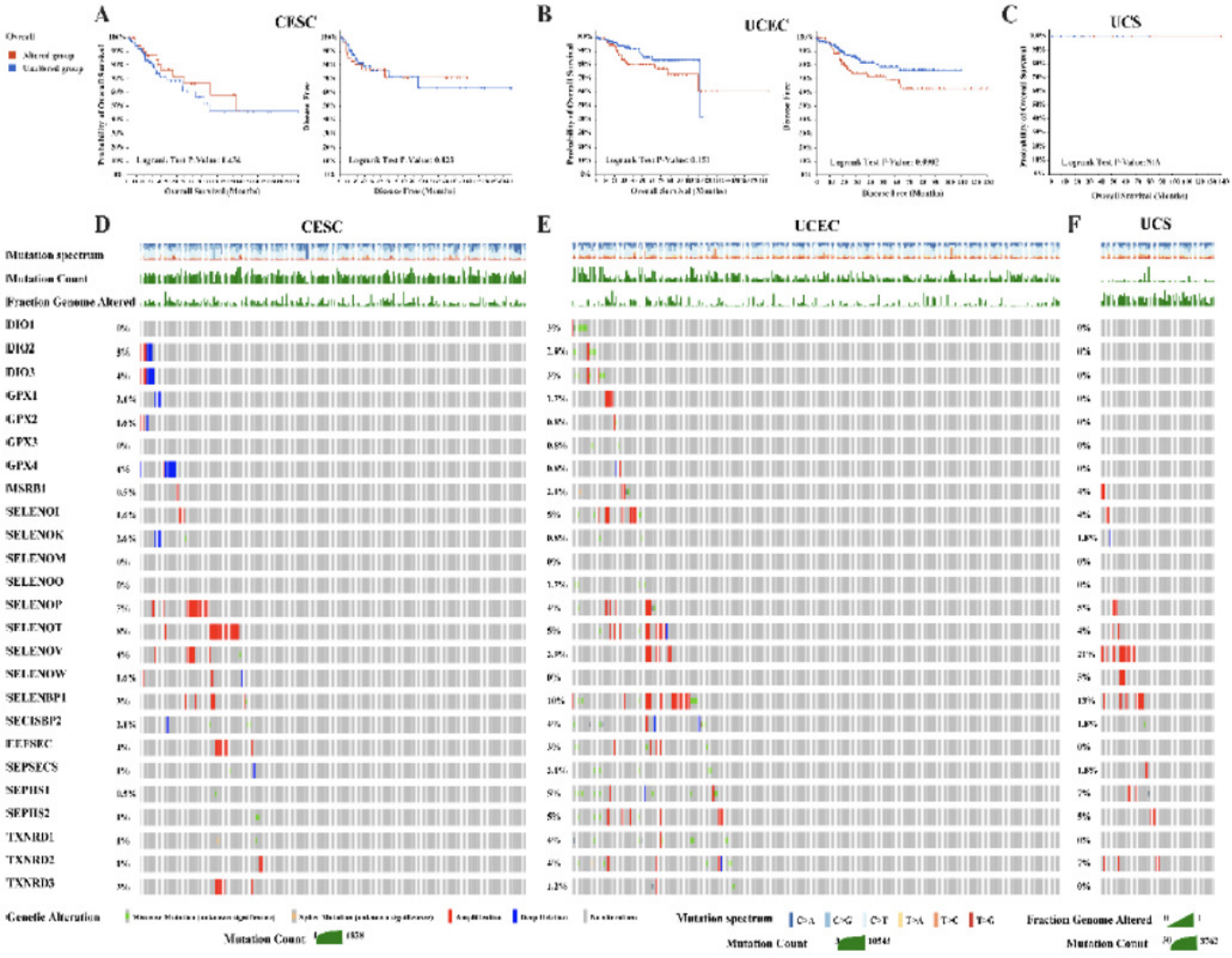
Supplementary Figure 1. mRNA expression of non-differential selenoprotein in patients with gynecological malignancies



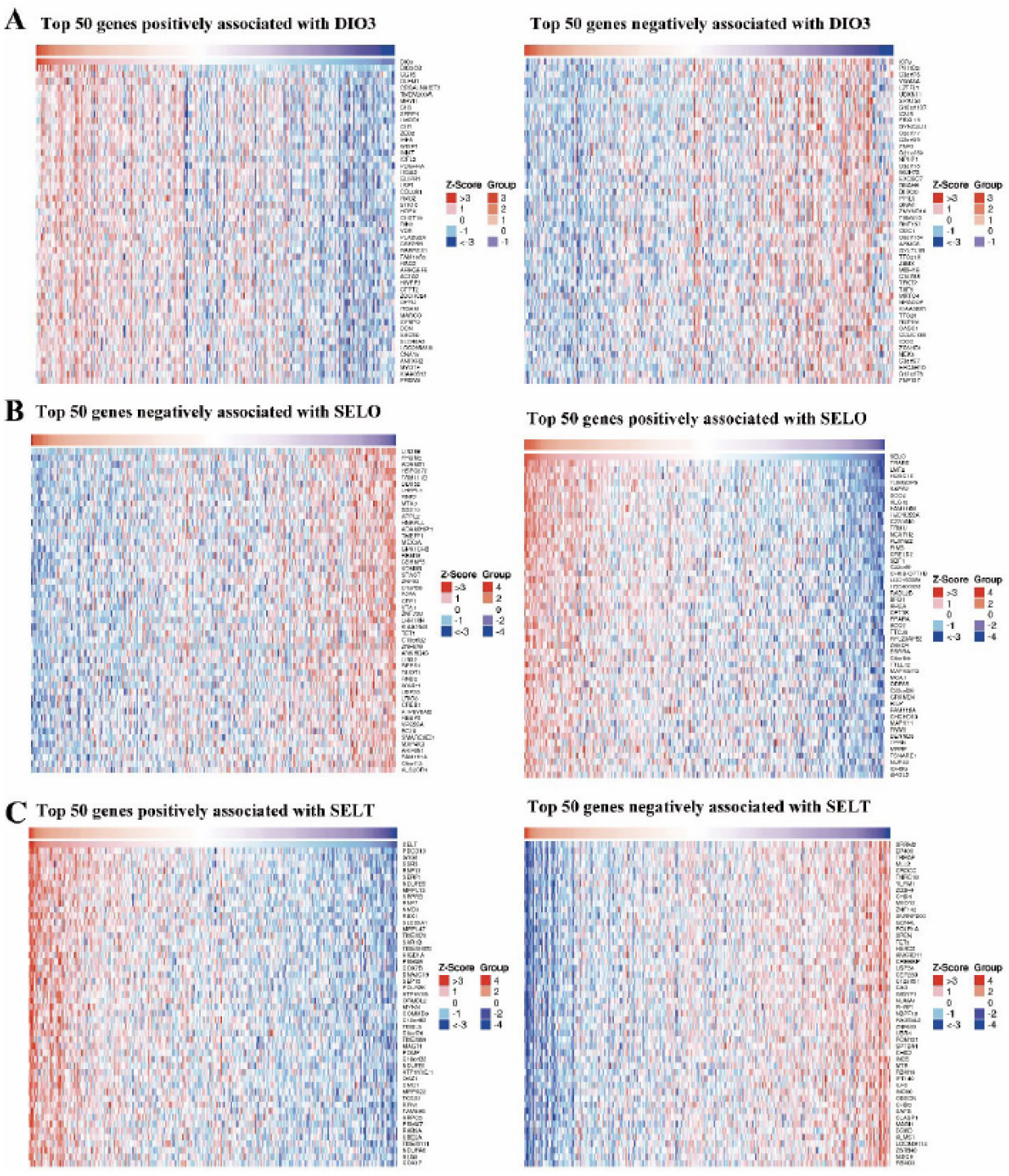
Supplementary Figure 2. Expression of downregulated selenoprotein in patients with gynecological malignancies



Supplementary Figure 3. Expression of upregulated selenoprotein in patients with gynecological malignancies

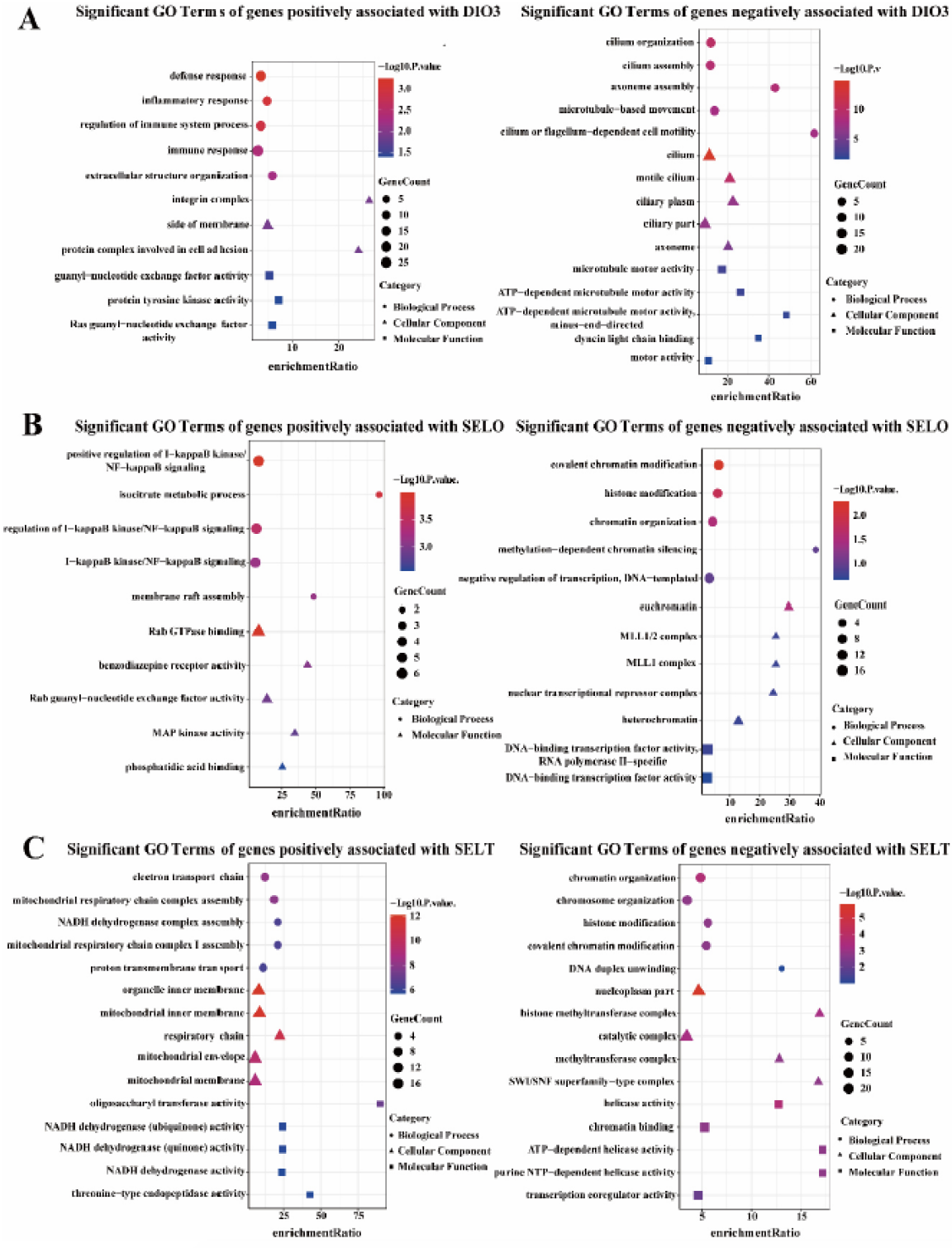


Supplementary Figure 4. Mutations and copy number variations of selenoprotein in cervical cancer, endometrial cancer and uterine carcinosarcoma (cBioPortal)



Supplementary Figure 5. Significant genes associated with DIO3, SELO, and SELT in ovarian cancer (LinkedOmics)

DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T



Supplementary Figure 6. Gene ontology enrichment analysis of genes associated with DIO3, SELO, and SELT in ovarian cancer (WebGestalt)

DIO3: Iodothyronine deiodinase 3, SELO: Selenoprotein O, SELT: Selenoprotein T

Supplementary Table 1. DIO3 in ovary cancer in Kaplan-Meier plotter

Clinicopathological feature	Overall survival (n=1657)			Progression- free survival (n=1436)		
	n	Hazard ratio	p	n	Hazard ratio	p
Histology						
Serous	1207	0.78 (0.67-0.92)	0.003	1104	1.26 (1.08-1.46)	0.003
Endometrioid	37	2.46 (0.41-14.75)	0.31	51	2.37 (0.93-6.03)	0.061
Stage						
1	74	0.27 (0.09-0.83)	0.015	96	2.03 (0.7-5.87)	0.18
1+2	135	0.31 (0.14-0.69)	0.0025	163	0.54 (0.3-0.96)	0.033
2	61	0.37 (0.12-1.14)	0.071	67	0.6 (0.3-1.18)	0.13
2+3	1105	0.76 (0.65-0.9)	0.0013	986	1.26 (1.08-1.46)	0.003
2+3+4	1281	0.77 (0.67-0.9)	0.00084	1148	1.29 (1.08-1.46)	7e-04
3	1044	0.75 (0.64-0.89)	0.00081	919	1.31 (1.11-1.49)	0.0011
3+4	1220	0.77 (0.66-0.89)	0.00059	1081	1.36 (1.16-1.58)	7.7e-05
4	176	1.47 (0.99-2.16)	0.052	162	1.75 (1.18-2.58)	0.0046
Grade						
1	56	0.39 (0.14-1.06)	0.055	37	0.43 (0.14-1.31)	0.13
1+2	380	0.77 (0.57-1.02)	0.071	293	1.3 (0.96-1.77)	0.092
2	324	0.8 (0.59-1.08)	0.15	256	1.47 (1.05-2.08)	0.026
2+3	1339	0.79 (0.68-0.92)	0.0019	1093	1.22 (1.05-1.42)	0.011
3	1015	0.78 (0.66-0.93)	0.0061	837	1.18 (0.99-1.39)	0.057
4	20	-	-	19	-	-
TP53 mutation						
Mutated	506	1.33 (1-1.78)	0.051	483	1.49 (1.19-1.86)	0.00041
Wild type	94	1.72 (0.91-3.23)	0.089	84	1.34 (0.79-2.26)	0.28
Average CA-125						
Below lower quartile	395	0.66 (0.5-0.87)	0.0025	326	0.61 (0.45-0.82)	0.0011
Debulk						
Optimal	801	0.61 (0.5-0.75)	1.7e-06	696	0.69 (0.57-0.84)	0.00014
Suboptimal	536	0.81 (0.64-1.01)	0.066	459	0.7 (0.56-0.88)	0.0023
Chemotherapy						
Platin	1409	0.84 (0.72-0.98)	0.025	1259	0.76 (0.66-0.88)	0.00012
Taxol	793	0.77 (0.64-0.93)	0.0064	715	1.2 (1-1.45)	0.053
Taxol+platin	776	0.76 (0.63-0.92)	0.0057	698	1.19 (0.99-1.44)	0.063
Avastin	50	0.25 (0.06-1.1)	0.048	50	0.63 (0.32-1.24)	0.18
Docetaxel	108	0.66 (0.37-1.16)	0.14	106	0.69 (0.42-1.13)	0.14
Gemcitabine	135	0.7 (0.44-1.12)	0.13	131	0.82 (0.55-1.2)	0.31
Paclitaxel	220	0.52(0.32-0.87)	0.011	229	1.17 (0.82-1.68)	0.38
Topotecan	119	1.52(0.99-2.31)	0.052	118	1.19 (0.81-1.77)	0.38

p<0.05 indicates statistical significance, CA-125: Cancer antigen-125

Supplementary Table 2. SELO in ovary cancer in Kaplan-Meier plotter

Clinicopathological feature	Overall survival (n=1657)			Progression- free survival (n=1436)		
	n	Hazard ratio	p	n	Hazard ratio	p
Histology						
Serous	523	0.8 (0.64-1)	0.054	483	0.77 (0.62-0.95)	0.016
Endometrioid	30	3.01 (0.42-21.42)	0.25	44	0.55 (0.17-1.77)	0.31
Stage						
1	51	0.27 (0.07-1.17)	0.064	74	0.26 (0.07-1)	0.036
1+2	83	0.6 (0.21-1.69)	0.33	115	2.01 (0.96-4.17)	0.057
2	32	4.82 (1.04-22.27)	0.028	41	3.02 (1.23-7.39)	0.011
2+3	458	0.83 (0.64-1.07)	0.15	465	0.84 (0.67-1.06)	0.14
2+3+4	519	0.86 (0.68-1.09)	0.22	535	0.87 (0.7-1.07)	0.17
3	426	0.81 (0.63-1.04)	0.1	424	0.85 (0.67-1.07)	0.16
3+4	487	0.85 (0.67-1.07)	0.17	494	0.87 (0.7-1.07)	0.19
4	61	1.41 (0.76-2.61)	0.27	70	0.61 (0.36-1.03)	0.064
Grade						
1	41	0.53 (0.18-1.56)	0.24	28	0.16 (0.04-0.62)	0.0026
1+2	203	0.7 (0.45-1.09)	0.12	189	0.57 (0.37-0.89)	0.011
2	162	0.81 (0.51-1.28)	0.31	161	0.69 (0.47-1.02)	0.063
2+3	554	0.79 (0.63-0.98)	0.034	476	0.79 (0.63-0.99)	0.04
3	392	0.7 (0.54-0.92)	0.01	315	0.71 (0.54-0.93)	0.011
4	18	0.32 (0.09-1.13)	0.063	19	-	-
TP53 mutation						
Mutated	124	1.29 (0.86-1.94)	0.21	124	1.51 (0.99-2.28)	0.053
Wild type	19	0.36 (0.11-1.21)	0.086	19	0.51 (0.19-1.38)	0.17
Average CA-125						
Below lower quartile	106	0.53 (0.32-0.89)	0.015	59	0.51 (0.28-0.93)	0.026
Debulk						
Optimal	243	0.63 (0.41-0.96)	0.032	240	0.7 (0.5-0.97)	0.031
Suboptimal	235	0.75 (0.56-1.01)	0.059	234	0.72 (0.55-0.95)	0.019
Chemotherapy						
Platin	478	0.86 (0.67-1.1)	0.22	502	0.86 (0.69-1.06)	0.16
Taxol	357	0.84 (0.61-1.16)	0.29	381	1.23 (0.96-1.59)	0.11
Taxol+platin	356	0.84 (0.62-1.16)	0.29	380	1.24 (0.96-1.59)	0.1
Avastin	-	-	-	-	-	-
Docetaxel	-	-	-	-	-	-
Gemcitabine	-	-	-	-	-	-
Paclitaxel	-	-	-	28	1.45 (0.62-3.4)	0.39
Topotecan	-	-	-	-	-	-

p<0.05 indicates statistical significance, CA-125: Cancer antigen-125

Supplementary Table 3. SELT in ovary cancer in Kaplan-Meier plotter

Clinicopathological feature	Overall survival (n=1657)			Progression- free survival (n=1436)		
	n	Hazard ratio	p	n	Hazard ratio	p
Histology						
Serous	1207	0.89 (0.76-1.04)	0.15	1104	1.26 (1.09-1.45)	0.002
Endometrioid	37	0.35 (0.06-2.09)	0.23	51	0.6 (0.2-1.82)	0.36
Stage						
1	74	1.96 (0.53-7.34)	0.31	96	0.37 (0.1-1.32)	0.11
1+2	135	0.4 (0.12-1.33)	0.12	163	0.59 (0.28-1.26)	0.17
2	61	0.17 (0.02-1.27)	0.048	67	0.44 (0.18-1.05)	0.058
2+3	1105	1.16 (0.98-1.36)	0.087	986	1.17 (1-1.36)	0.045
2+3+4	1281	0.92 (0.79-1.07)	0.27	1148	1.2 (1.04-1.39)	0.011
3	1044	1.19 (1-1.4)	0.047	919	1.2 (1.02-1.4)	0.025
3+4	1220	1.1 (0.94-1.28)	0.22	1081	1.24 (1.07-1.43)	0.0044
4	176	0.63 (0.43-0.92)	0.016	162	1.88 (1.17-3)	0.0075
Grade						
1	56	0.41 (0.13-1.26)	0.11	37	0.51 (0.17-1.52)	0.22
1+2	380	1.47 (1.06-2.02)	0.019	293	1.32 (0.94-1.84)	0.1
2	324	1.46 (1.03-2.06)	0.033	256	1.28 (0.92-1.8)	0.14
2+3	1339	0.84 (0.72-0.98)	0.024	1093	1.27 (1.06-1.43)	0.007
3	1015	0.78 (0.66-0.94)	0.0083	837	1.29 (1.08-1.54)	0.0047
4	20	-	-	19	-	-
TP53 mutation						
Mutated	506	0.87 (0.69-1.09)	0.23	483	1.36 (1.09-1.7)	0.007
Wild type	94	0.65 (0.33-1.29)	0.22	84	0.63 (0.35-1.14)	0.12
Average CA-125						
Below lower quartile	395	1.41 (1.06-1.88)	0.016	326	1.29 (0.98-1.7)	0.07
Debulk						
Optimal	801	1.17 (0.95-1.45)	0.14	696	1.31 (1.08-1.59)	0.0051
Suboptimal	536	0.72 (0.58-0.89)	0.0029	459	1.19 (0.96-1.48)	0.1
Chemotherapy						
Platin	1409	0.89 (0.77-1.03)	0.12	1259	1.2 (1.06-1.37)	0.0051
Taxol	793	0.87 (0.71-1.05)	0.15	715	1.18 (0.99-1.4)	0.061
Taxol+Platin	776	0.86 (0.7-1.04)	0.12	698	1.19 (0.99-1.41)	0.058
Avastin	50	0.53(0.18-1.61)	0.26	50	0.74 (0.37-1.47)	0.38
Docetaxel	108	0.41 (0.23-0.72)	0.0015	106	0.68 (0.38-1.22)	0.2
Gemcitabine	135	1.32 (0.87-2.02)	0.19	131	1.72 (1.14-2.6)	0.0086
Paclitaxel	220	0.65(0.41-1.05)	0.074	229	0.85 (0.6-1.18)	0.33
Topotecan	119	0.7 (0.47-1.04)	0.073	118	1.45 (0.98-2.14)	0.063

p<0.05 indicates statistical significance, CA-125: Cancer antigen-125

Supplementary Table 4. Correlation analysis between DIO3, SELO, SELT and relate genes and markers of immune cells in TIMER

Description	Gene markers	DIO3 none		Purity		SELO none		Purity		SELT none		Purity	
		Cor	p	Cor	p	Cor	p	Cor	p	Cor	p	Cor	p
T-cell	CD3D	0.292	****	0.079	0.22	0.065	0.26	0	0.99	0.261	****	0.169	**
	CD3E	0.312	****	0.102	0.11	0.147	*	0.098	0.11	0.21	***	0.107	0.09
	CD2	0.308	****	0.101	0.11	0.115	*	0.067	0.3	0.265	****	0.185	**
$CD8^+$ T-cell	CD8A	0.274	****	0.084	0.19	0.065	0.26	0.014	0.83	0.234	****	0.118	0.06
	CD8B	0.21	***	0.062	0.33	0.016	0.78	-0.045	0.48	0.299	****	0.211	***
$CD4^+$ T-cell	CD4	0.297	****	0.116	0.07	0.251	****	0.178	**	0.103	0.07	0.025	0.7
Th 1	TBX21	0.291	****	0.08	0.21	0.175	**	0.114	0.07	0.159	**	0.064	0.32
	STAT1	0.026	0.66	0.017	0.79	0.067	0.24	0.067	0.29	0.186	**	0.203	**
	STAT4	0.278	****	0.134	*	0.145	*	0.122	0.05	0.206	***	0.177	**
	IFNG	0.17	**	0.003	0.965	0.062	0.28	0.011	0.86	0.26	****	0.185	**
	TNF	0.214	***	0.099	0.12	0.236	****	0.26	****	0.186	**	0.098	0.12
Th 2	GATA3	0.315	****	0.204	**	0.116	*	0.027	0.67	0.134	*	0.077	0.23
	STAT6	0.08	0.16	0.085	0.18	0.108	0.06	0.045	0.48	-0.147	*	-0.092	0.15
	STAT5A	0.154	**	0.1	0.115	0.209	***	0.12	0.058	-0.089	0.12	-0.111	0.08
	IL13	0.145	*	0.197	**	0.065	0.261	0.08	0.208	0.01	0.856	0.025	0.691
Tfh	BCL6	0.146	*	0.226	***	0.286	****	0.259	****	-0.024	0.675	0.008	0.894
	IL21	0.017	0.762	0.013	0.837	0.066	0.256	0.118	0.062	0.183	**	0.134	*
Th 17	STAT3	0.252	****	0.179	**	0.208	***	0.16	*	0.073	0.205	0.062	0.332
	IL17A	0.12	*	0.08	0.206	0.053	0.36	0.022	0.728	0.126	*	0.107	0.093
Treg	FOXP3	0.267	****	0.101	0.11	0.164	**	0.09	0.156	0.207	***	0.143	*
	CCR8	0.215	***	0.106	0.095	0.028	0.626	-0.03	0.64	0.142	*	0.09	0.157
	STAT5B	0.173	**	0.143	*	0.172	**	-0.091	0.154	-0.13	*	-0.11	0.083
	TGFB1	0.333	****	0.126	*	0.201	***	0.081	0.204	0.191	***	0.159	*
T-cell exhaustion	PDCD1	0.25	****	0.116	0.067	0.134	*	0.071	0.261	0.222	****	0.161	*
	CTLA4	0.272	****	0.083	0.189	0.107	0.062	0.042	0.51	0.255	****	0.158	*
	LAG3	0.199	***	0.079	0.213	0.094	0.101	0.052	0.414	0.185	**	0.148	*
	HAVCR2	0.336	****	0.094	0.141	0.205	***	0.146	*	0.295	****	0.206	**
	GZMB	0.233	****	0.056	0.375	0.068	0.24	0.032	0.62	0.218	***	0.112	0.07
B-cell	CD19	0.046	0.426	-0.009	0.888	-0.015	0.801	-0.007	0.908	0.048	0.403	0.016	0.807
	CD79A	0.259	****	0.139	*	0.053	0.361	-0.003	0.962	0.097	0.091	-0.005	0.94
Monocyte	CD86	0.304	****	0.072	0.259	0.19	***	0.142	*	0.269	****	0.18	**
	CSF1R	0.342	****	0.117	0.066	0.295	****	0.23	***	0.117	*	0.003	0.96
TAM	CCL2	0.332	****	0.163	**	0.111	0.053	0.078	0.221	0.152	**	0.037	0.558
	CD68	0.35	****	0.13	*	0.204	***	0.146	*	0.261	****	0.185	**
	IL10	0.276	****	0.128	*	0.01	0.862	-0.046	0.472	0.205	***	0.108	0.09

Supplementary Table 4. continued

Description	Gene markers	DIO3 none		Purity		SELO none		Purity		SELT none		Purity	
M1 macrophage	NOS2	0.088	0.125	0.045	0.479	0.015	0.798	-0.036	0.571	0.057	0.319	0.072	0.258
	IRF5	0.148	**	0.071	0.264	0.268	****	0.242	***	0.136	*	0.132	*
	PTGS2	0.185	**	0.088	0.166	-0.039	0.498	-0.11	0.083	0.039	0.498	0.034	0.589
M2 macrophage	CD163	0.315	****	0.111	0.081	0.209	***	0.143	*	0.161	**	0.058	0.359
	VSIG4	0.297	****	0.066	0.299	0.129	*	0.071	0.267	0.221	***	0.097	0.129
	MS4A4A	0.3	****	0.078	0.218	0.115	*	0.045	0.476	0.26	****	0.167	**
Neutrophils	CEACAM	0.033	0.566	0.084	0.189	0.141	*	0.153	*	-0.141	*	-0.059	0.356
	ITGAM	0.378	****	0.168	**	0.281	****	0.2	**	0.149	**	0.081	0.202
	CCR7	0.283	****	0.121	0.057	0.137	*	0.051	0.421	0.148	**	0.086	0.174
Natural killer cell	KIR2DL1	0.128	*	0.079	0.216	0.049	0.393	0.033	0.603	0.074	0.244	0.066	0.253
	KIR2DL3	0.148	**	0.054	0.398	0.174	**	0.143	*	-0.002	0.978	-0.058	0.359
	KIR2DL4	0.202	***	0.042	0.507	0.12	*	0.061	0.337	0.122	*	0.044	0.485
	KIR3DL1	0.122	*	0.006	0.928	0.059	0.309	0.032	0.62	0.098	0.088	0.069	0.278
	KIR3DL2	0.164	**	0.063	0.324	0.089	0.124	0.041	0.522	0.079	0.172	0.051	0.42
	KIR3DL3	0.051	0.379	0.003	0.967	0.018	0.751	0.001	0.954	0.025	0.666	-0.006	0.929
	KIR2DS4	0.157	**	0.076	0.234	0.065	0.262	0.021	0.743	0.084	0.145	0.053	0.402
Dendritic cell	HLA-	0.281	****	0.056	0.376	0.221	***	0.2	**	0.186	**	0.051	0.427
	DPB1	-	-	-	-	-	-	-	-	-	-	-	-
	HLA-	0.18	**	0	0.996	0.096	0.096	0.054	0.4	0.137	*	0.021	0.741
	DQB1	-	-	-	-	-	-	-	-	-	-	-	-
	HLA-	0.254	****	0.085	0.181	0.136	*	0.118	0.062	0.241	****	0.116	0.066
	DRA												
	HLA-	0.266	****	0.062	0.328	0.173	**	0.141	*	0.193	***	0.067	0.29
	DPA1	-	-	-	-	-	-	-	-	-	-	-	-
	CD1C	0.333	****	0.157	*	0.127	*	0.067	0.295	0.061	0.293	-0.025	0.697
	NRP1	0.229	****	0.039	0.543	0.2	***	0.144	*	0.182	**	0.133	*
	ITGAX	0.36	****	0.166	**	0.355	****	0.327	****	0.16	**	0.08	0.207

*: p<0.05, **: p<0.01; ***: p<0.001, ****: p<0.000, Th: T helper cell, TAM: Tumor-associated macrophage

Supplementary Table 5. Correlation analysis between DIO3. SELO. SELT and relate genes and markers of immune cells in GEPIA

Description	Gene markers	DIO3				SELO				SELT			
		Tumor		Normal		Tumor		Normal		Tumor		Normal	
		Cor	p	Cor	p	Cor	p	Cor	P	Cor	p	Cor	p
T-cell	CD3D	0.25	****	-0.01	0.92	0.028	0.57	-0.12	0.28	0.22	****	0.04	0.74
	CD3E	0.29	****	0.06	0.56	0.15	**	-0.1	0.33	0.22	****	0.12	0.27
	CD2	0.31	****	0.06	0.58	0.12	*	-0.1	0.38	0.31	****	0.18	0.1
$CD8^+$ T-cell	CD8A	0.28	****	0.04	0.73	0.062	0.2	-0.11	0.33	0.29	****	0.19	0.08
	CD8B	0.21	****	-0.1	0.37	-0.03	0.54	-0.1	0.36	0.31	****	0.12	0.28
$CD4^+$ T-cell	CD4	0.34	****	0.34	**	0.25	****	0.003	0.97	0.28	****	0.32	**
Th 1	TBX21	0.34	****	-0.05	0.68	0.19	****	-0.07	0.53	0.27	****	-0.06	0.56
	STAT1	0.12	*	-0.11	0.31	0.2	****	-0.18	0.094	0.41	****	0.48	****
	STAT4	0.28	****	-0.33	**	0.19	****	0.15	0.17	0.28	****	-0.03	0.78
	IFNG	0.17	***	-0.33	**	0.058	0.23	-0.03	0.76	0.26	****	-0.16	0.13
	TNF	0.24	****	-0.05	0.65	0.24	****	-0.12	0.25	0.29	****	0.1	0.34
Th 2	GATA3	0.28	****	-0.04	0.73	0.097	*	-0.11	0.31	0.14	**	-0.04	0.71
	STAT6	0.13	**	-0.26	*	0.33	****	0.42	****	0.14	**	-0.26	*
	STAT5A	0.24	****	0.43	****	0.3	****	-0.05	0.67	0.23	****	0.27	*
	IL13	0.12	*	0.07	0.55	0.17	***	0.03	0.77	-0.016	0.74	-0.09	0.4
Tfh	BCL6	0.23	****	0.05	0.63	0.39	****	-0.06	0.56	0.22	****	-0.01	0.91
	IL21	0.1	*	0.18	0.087	0.015	0.75	0.05	0.63	0.14	**	0.11	0.3
Th17	STAT3	0.31	****	0.27	**	0.31	****	-0.16	0.13	0.39	****	0.3	**
	IL17A	0.027	0.58	-0.13	0.21	0.027	0.58	-0.14	0.19	0.065	0.18	0.25	*
Treg	FOXP3	0.28	****	-0.07	0.5	0.2	****	0.02	0.89	0.3	****	0.05	0.63
	CCR8	0.23	****	-0.06	0.61	0.12	0.017	-0.2	0.056	0.31	****	0.1	0.34
	STAT5B	0.24	****	0.18	0.098	0.28	****	0.23	*	0.25	****	0.19	0.08
	TGFB1	0.37	****	0.12	0.25	0.27	****	-0.18	0.1	0.38	****	0.28	**
T-cell exhaustion	PDCD1	0.26	****	0.2	0.067	0.17	***	-0.09	0.4	0.28	****	0.22	*
	CTLA4	0.28	****	-0.06	0.58	0.13	**	-0.26	*	0.28	****	0.2	0.06
	LAG3	0.2	****	0.17	0.1	0.13	**	0.34	**	0.18	***	-0.15	0.15
	HAVCR2	0.36	****	0.34	**	0.22	****	-0.29	**	0.43	****	0.47	****
	GZMB	0.22	****	0.05	0.63	0.12	*	0.03	0.78	0.23	****	-0.01	0.94
B-cell	CD19	0.085	0.078	-0.37	***	0.07	0.15	0.26	*	0.095	*	-0.24	*
	CD79A	0.22	****	0.14	0.21	0.028	0.56	-0.26	*	0.11	*	0.08	0.45
Monocyte	CD86	0.35	****	0.25	*	0.18	***	-0.38	***	0.4	****	0.46	****
	CSF1R	0.37	****	0.25	*	0.31	****	-0.16	0.13	0.32	****	0.45	****
TAM	CCL2	0.34	****	-0.06	0.58	0.13	0.008	-0.23	*	0.25	****	0.25	*
	CD68	0.38	****	0.14	0.21	0.24	****	-0.44	****	0.43	****	0.56	****
	IL10	0.32	****	0.24	*	0.11	*	-0.23	*	0.41	****	0.37	***

Supplementary Table 5. continued

Description	Gene markers	DIO3				SELO				SELT			
		Tumor		Normal		Tumor		Normal		Tumor		Normal	
M1 Macrophage	NOS2	0.18	***	-0.19	0.075	0.16	***	0.13	0.23	0.22	****	-0.01	0.95
	IRF5	0.2	****	0.21	*	0.34	****	-0.12	0.28	0.34	****	0.38	****
	PTGS2	0.26	****	-0.06	0.6	0.0082	0.87	-0.1	0.33	0.22	****	-0.06	0.57
M2 Macrophage	CD163	0.29	****	0.52	****	0.18	***	-0.32	**	0.28	****	0.46	****
	VSIG4	0.35	****	0.46	****	0.15	**	-0.27	*	0.36	****	0.5	****
	MS4A4A	0.34	****	0.36	***	0.14	**	-0.29	**	0.4	****	0.43	****
Neutrophils	CEACAM8	0.09	0.064	-0.15	0.16	0.14	**	-0.04	0.69	-0.034	0.48	-0.07	0.53
	ITGAM	0.41	****	0.32	**	0.31	****	-0.25	*	0.36	****	0.44	****
	CCR7	0.28	****	0.21	*	0.2	****	-0.16	0.14	0.29	****	0.19	0.07
Natural killer cell	KIR2DL1	0.18	***	0.13	0.23	0.14	**	-0.05	0.62	0.19	****	-0.03	0.77
	KIR2DL3	0.25	****	0.01	0.95	0.17	****	-0.16	0.15	0.2	****	0.02	0.82
	KIR2DL4	0.24	****	0.05	0.65	0.19	****	-0.15	0.17	0.23	****	0.1	0.37
	KIR3DL1	0.19	****	0.05	0.65	0.11	*	0.03	0.81	0.18	***	0.01	0.93
	KIR3DL2	0.21	****	0.05	0.68	0.21	****	-0.05	0.66	0.19	****	-0.02	0.85
	KIR3DL3	0.09	0.064	0.14	0.19	0.12	*	0	1	0.069	0.16	0.08	0.44
	KIR2DS4	0.13	**	0.1	0.36	0.14	**	0.07	0.49	0.2	****	-0.11	0.33
Dendritic cell	HLA-DPB1	0.33	****	-0.13	0.24	0.19	****	-0.25	*	0.23	****	0.17	0.12
	HLA-DQB1	0.16	**	-0.15	0.16	0.06	0.21	-0.12	0.26	0.14	**	0.1	0.34
	HLA-DRA	0.3	****	0.01	0.9	0.13	**	-0.32	**	0.29	****	0.35	****
	HLA-DPA1	0.32	****	-0.01	0.92	0.16	***	-0.21	*	0.27	****	0.27	*
	CD1C	0.32	****	0.15	0.16	0.12	*	0.002	0.98	0.15	**	0.19	0.08
	NRP1	0.27	****	-0.15	0.15	0.23	****	-0.23	*	0.34	****	0.43	****
	ITGAX	0.35	****	-0.1	0.36	0.39	****	-0.05	0.63	0.31	****	-0.02	0.88

*: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001, Th: T helper cell, TAM: Tumor-associated macrophage

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The effect of gonadotropin gap for non-growing follicles in poor ovarian response: Might this be a new strategy?

Zayıf over yanıtında büyümeyen foliküller için gonadotropinlere ara vermenin etkisi: Yeni bir strateji olabilir mi?

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Abstract

Objective: Cessation of gonadotropin stimulation might affect follicular growth in patients in POSEIDON groups 3 and 4, which are unresponsive to high-dose stimulation.

Materials and Methods: In this retrospective study, data were extracted from the medical records of patients treated at the Acıbadem Maslak Hospital Assisted Reproductive Technologies Unit between November 2010 and December 2020. Eighty-five patients who fulfilled the inclusion criteria were included in the study. Gonadotropin stimulation was discontinued if the follicle diameter increased by 2 mm within 7 days after the initiation of stimulation in patients in groups 3 and 4. The outcomes of the treatment strategy and pregnancy were recorded.

Results: Follicular growth was observed in 40% (34/85) of patients, of whom 52.9% (18/34) had 2pn embryos. Ten of the 85 patients (11.8%) underwent embryo transfer, resulting in biochemical pregnancy for two patients and healthy live birth for one patient.

Conclusion: When high-dose stimulation is ineffective, discontinuing gonadotropin administration during ovarian stimulation may provide patients with the opportunity to conceive using their own biological oocytes. To the best of our knowledge, this is the first study to report a live birth rate using this strategy.

Keywords: Poor ovarian response, diminished ovarian reserve, failure to ovarian stimulation, gonadotropin gap, in vitro fertilization

Öz

Amaç: Yüksek doz stimülasyona yanıt vermeyen POSEIDON grup 3 ve 4 hastalarda gonadotropin stimülasyonunun kesilmesinin folikül büyümesi üzerine etkisini araştırmayı amaçladık.

Gereç ve Yöntemler: Bu retrospektif çalışmada, Kasım 2010-Aralık 2020 tarihleri arasında Acıbadem Maslak Hastanesi Yardımcı Üreme Teknikleri Ünitesinde tedavi gören hastaların tıbbi kayıtlarından veriler elde edilmiştir. Dahil edilme kriterlerini karşılayan 85 hasta çalışmaya alınmıştır. POSEIDON grup 3 ve 4 hastalarda stimülasyon başlangıcından itibaren 7 gün içinde folikül çapı 2 mm'den az arttığında gonadotropin stimülasyonu kesilmiştir. Tedavi stratejisi ve gebelik sonuçları kaydedilmiştir.

Bulgular: Hastaların %40'ında (34/85) folikül büyümesi gözlemlenmiş ve bu hastaların %52,9'unda (18/34) 2pn embriyolar elde edilmiştir. Seksen beş hastadan on tanesine (%11,8) embriyo transferi yapılmış ve iki hastada biyokimyasal gebelik, bir hastada sağlıklı canlı doğum elde edilmiştir.

PRECIS: Discontinuing gonadotropin stimulation in poor ovarian responders may enable follicular growth and conception, with the potential for live birth using biological oocytes.

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Sonuç: Yüksek doz stimülasyonun etkisiz olduğu durumlarda, over stimülasyonu sırasında gonadotropin uygulamasının kesilmesi, hastalara kendi biyolojik oositlerini kullanarak hamile kalma şansı sağlayabilir. Bildiğimiz kadarıyla, çalışmamız bu stratejiyle canlı doğum oranını bildiren ilk çalışmadır.

Anahtar Kelimeler: Zayıf over yanıtı, azalmış over rezervi, over stimülasyonunda başarısızlık, gonadotropinin kesilmesi, in vitro fertilizasyon

Introduction

Poor ovarian response (POR) is a common problem encountered during in vitro fertilization (IVF) cycles and is associated with low success rates and high treatment costs[1]. Although the definition of POR varies in the literature, the POSEIDON classification system is widely accepted for categorizing patients according to their ovarian reserve status[2]. This system takes into account factors such as age, ovarian reserve markers [anti-Müllerian hormone (AMH), antral follicle coun (AFC)], and the number of oocytes retrieved in previous cycles of conventional ovarian stimulation to divide patients with poor prognosis into four groups. Group 1 consisted of patients under the age of 35 years who demonstrated sufficient ovarian reserve parameters (AFC ≥5, AMH ≥1.2 ng/mL), and those with unexpected poor or suboptimal ovarian response. Group 2 included patients aged >35 years with similar ovarian reserve parameters. Patients younger than 35 years old with poor ovarian reserve parameters (AFC <5, AMH <1.2 ng/mL) are categorized in group 3, whereas group 4 consisted of patients older than 35 years old with poor ovarian reserve parameters[3]. Documentation of at least two POR episodes is necessary to define a patient as a poor responder[4].

Patients with diminished ovarian reserve or expected POR are classified into POSEIDON groups 3 and 4, which comprise 10% and 55% of all IVF patients, respectively[1]. Due to the smaller number of oocytes and fewer embryos produced, patients classified as POSEIDON have a lower chance of achieving a cumulative live birth per cycle compared with non-POSEIDON patients[5]. Although several protocols have been developed to improve ovarian stimulation and outcomes in these patients, their effectiveness remains a topic of debate.

In this study, we aimed to evaluate the efficacy and outcomes of cessation of gonadotropin during ovarian stimulation in patients in POSEIDON groups 3 and 4, which are unresponsive to high-dose stimulation.

Material and Methods

Study Population

The medical records of patients treated at Acıbadem Maslak Hospital Assisted Reproductive Technologies Unit between November 2010 and December 2020 were scanned, and 85 eligible patients were included in the study. Patients in approximately 27-47 years of age who met the POSEIDON criteria groups 3 or 4 were included in the study. Patients with an increase in follicle diameter of 2 mm within 7 days after the initiation of gonadotropin treatment were recruited in the study. Patients with severe male characteristics were excluded. The study was approved by the Acıbadem Mehmet Ali Aydınlar University Medical Research Ethics Committee (decision no: ATADEK-2021-04/31, date: 24.02.2021).

Ovarian Stimulation Protocol

Patients diagnosed with POR according to the POSEIDON criteria underwent transvaginal ultrasound (TVUSG) on the 2nd or 3rd days of the menstrual cycle. A flowchart of the ovarian stimulation protocol is presented in Figure 1. Briefly, ovarian stimulation was started with 300 IU follicle-stimulating hormone (FSH) (Gonal F, Serono) and 300 IU hMG (Merional, IBSA, Switzerland) when at least 1 antral follicle was detected. On the 5th days of stimulation, TVUSG was repeated, and if an increase of at least 2 mm in dominant follicle size diameter was observed, gonadotropin treatment was continued at the same dose. If the increase in follicle diameter was 2 mm, ovarian stimulation was continued for 2 more days, and follicular growth was restored. If the increase was still less than 2 mm in

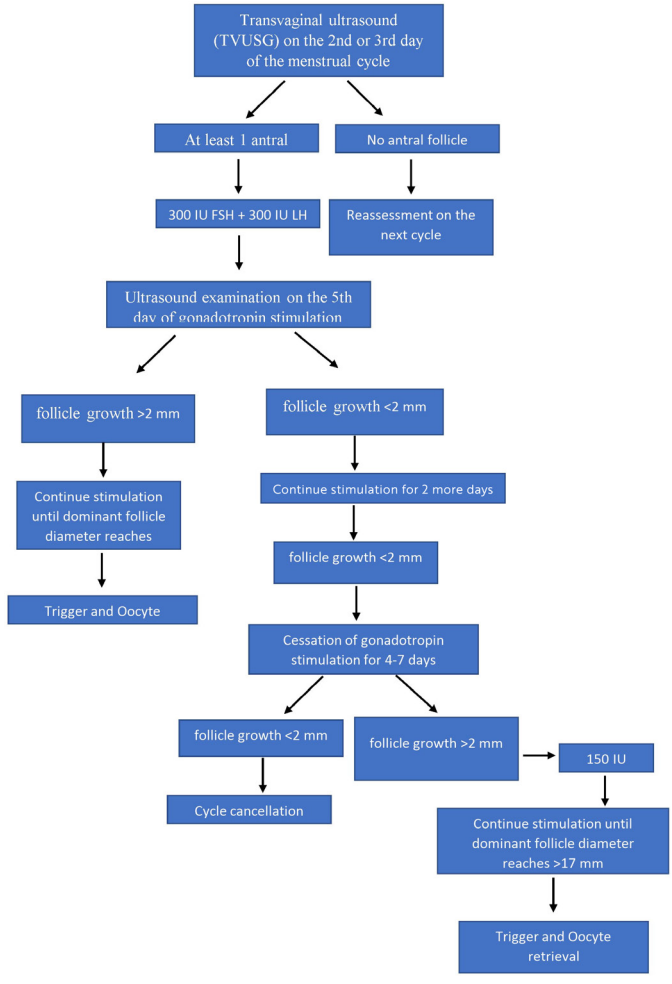

Figure 1. Flowchart of the ovarian stimulation protocol

the third control, the gonadotropin stimulation was suspended for 4-7 days. After 4-7 days without gonadotropin stimulation, follicles were checked with TVUSG again, and in case of no change in dominant follicle size compared to the last ultrasound examination, the cycle was canceled. If the follicle size increased by more than 2 mm, then 150 IU HMG (FSH+LH) was initiated. When the leading follicle diameter exceeded 17 mm, 250 mg of rhCG (Ovitrelle, Serono) was administered to trigger final oocyte maturation. Oocyte retrieval was performed at 34 hours after the trigger.

IVF Outcomes

Oocyte denudation was performed four hours after retrieval, and intracytoplasmic sperm injection (ICSI) was applied to all mature oocytes. Embryos were cultured until day 3 or day 5. The decision on the embryo transfer day was based on the preferences of both the patient and the treating physician.

The protocol for frozen embryo transfer (FET) included the initiation of oral contraceptives on days 2-5 of the menstrual cycle after ovarian stimulation, followed by subcutaneous injection of 3.75 mg of leuprolide acetate depot (Lucrin; Abbott) in the midluteal phase. Endometrial priming was initiated with oral estradiol (Estrofem; Novo Nordisk) at a daily dose of 4 mg for 5 days, which was gradually increased to 8 mg per day. After 14 days of estradiol administration, if the endometrial thickness reached 8 mm or more and the progesterone level remained below 1.0 ng/mL, vaginal progesterone (Crinone gel 8% BID; Merck) was introduced twice daily, accompanied by 17-hydroxyprogesterone caproate (Proluton Depot; Bayer) administered twice weekly, and FET was scheduled. In cases in which the endometrial thickness was 8 mm, a 7.8 mg estradiol patch (Climara; Bayer) was added, and the patient was reassessed after 4 days. If the lining was still measured below 8 mm, the cycle was canceled. For fresh ET, luteal phase support consisted of daily vaginal progesterone (Crinone gel 8% BID; Merck) initiated the day following oocyte retrieval.

Pregnancy outcomes were assessed 12 days after ET using serum β-hCG measurement. Clinical pregnancy was defined by the presence of a gestational sac with detectable fetal heart activity, and live birth was defined as delivery after 28 gestational weeks.

Statistical Analysis

Data analysis was performed using SPSS software version 22 (SPSS-IBM 2.3, Inc., Chicago, IL, USA). The Shapiro-Wilk test was employed to assess the normality assumption of continuous variables, which was further evaluated visually through histograms and Q-Q plots. Comparisons between two groups were conducted using the Mann-Whitney U test. Continuous variables were expressed as medians with interquartile ranges (IQR), whereas categorical variables were described using frequencies and percentages (%). A p-value 0.05 was considered statistically significant.

Results

A total of 85 patients diagnosed with POR were enrolled in the study. The sociodemographic characteristics of the patients are summarized in Table 1. The median age of the patients was 41 (IQR: 37-44) years. The median duration of infertility among the couples was calculated as 48 (IQR:24-96) months. Ovarian reserve parameters including AFC, FSH, and AMH were also analyzed in the study (Median levels were 2.0 (IQR: 1-3), 23 (IQR: 11.6-37.7), 0.07 (IQR: 0.14-0.2), repectively.

The flowchart of the IVF outcomes is presented in Figure 2. After cessation of gonadotropin treatment, an increase in follicle diameter was detected in 34 of 85 (40%) patients. Among the patients who underwent egg retrieval, 29.4% (10/34) had no oocytes; 11.8% (4/34) experienced premature ovulation, and 17.6% (6/34) were unable to obtain an oocyte during the egg retrieval procedure. A total of 35 oocytes (minimum-maximum: 1-4) were obtained from the remaining 24 patients. Thirty of these 35 oocytes were mature (M2). One of the M2 oocytes was frozen. The remaining 29 M2 oocytes underwent ICSI,

Table 1. Patient characteristics and cycle parameters are presented as medians and IQR

	Median (IQR)
Age (year)	41 (37-44)
Partner's age (year)	40 (36-46)
BMI (kg/m^2)	25.7 (23-28.4)
Infertility duration (months)	48 (24-96)
Number of previous IVF attempts	2 (24-96)
Number of AFC	2.0 (1-3)
FSH (mIU/mL)	23 (11.6-37.7)
AMH (ng/mL)	0.07 (0.14-0.2)

AMH: Anti-Müllerian hormone, FSH: Follicle stimulating hormone, BMI: Body mass index, IVF: In vitro fertilization, AFC: Antral follicle count, IQR: Interquartile range

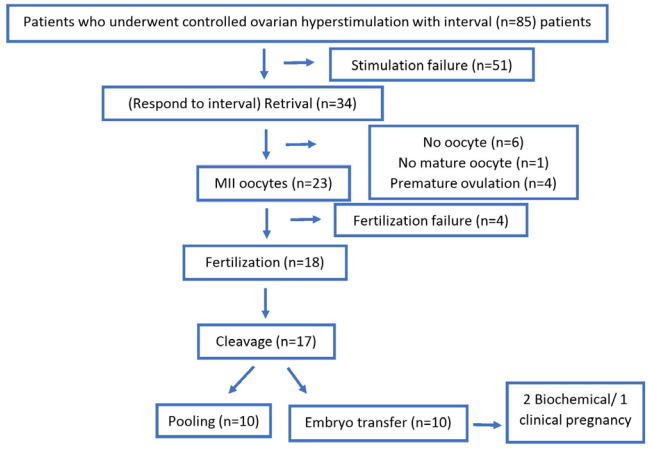

Figure 2. The flowchart of IVF outcomes

IVF: In vitro fertilization

leading to 23 2pn embryos in 18 patients (52.9%). Among these patients, 1 patient had 2 blastocyst stage embryos, 3 patients had 2 cleavage stage embryos and, 13 patients had 1 cleavage stage embryos. One patient did not have embryos that reached the cleavage stage. All the embryos of 7 patients were frozen based on the patient preference or embryo banking. Ten patients among 85 patients underwent embryo transfer (11.8%). Out of 10 patients who underwent embryo transfer, two was fresh and 8 was frozen embryo transfers. Two frozen embryo transfers were performed with cleavage embryos that resulted in a positive pregnancy test but did not result in clinical pregnancy. A blastocyst transfer was performed in another patient, resulting in a healthy live birth.

Parameters for patients with a rebound (group 1) and no rebound (group 2) to gonadotropin cessation were compared. Two groups were similar regarding age, AFC, and AMH levels. None of the patients in group 1 had significantly higher FSH levels compared to group 2 [13.1 (IQR: 9.1-27.0)] vs. 26.5 (IQR: 18.6-45); p=0.032). Also, the days of stimulation and total gonadotropin doses were similar between the two groups (Table 2).

Discussion

POR typically refers to patients with low ovarian reserve who cannot respond sufficiently to stimulation[6]. These patients account for approximately 9-24% of clinical practice[7]. ASRM/SART data show that 14.1% of IVF cycles are canceled and that 50% of canceled cycles consist of patients with a poor response[8]. As the number of obtained oocytes decreases, the declining pregnancy rates cause anxiety in both clinicians and couples. Optimal treatment for improving ovarian response in patients with POR is currently a controversial issue. The current data are insufficient to demonstrate the superiority of any treatment protocol or drug in terms of pregnancy rates among patients with a poor response[9,10]. In the present study, we showed that discontinuing the use of gonadotropins for 5-7 days in patients who did not respond to high-dose stimulation resulted in continued follicle development during the drug-free period in 40% of the patients. In total, 30 MII oocytes were obtained from 24 of 34 patients whose follicles continued to develop. Therefore, cessation of gonadotropins in this difficult-to-manage patient group may be important for the patient to have a chance of pregnancy with her own oocytes before being directed toward experimental treatments or donation.

In the present study, three patients got pregnant, two of which resulted in biochemical pregnancy and one resulted in healthy live birth. To the best of our knowledge, this study is the first to report a live birth after cessation of gonadotropin. Consistent with our results, previous studies have shown that pregnancy rates are quite low in poor responders[11]. Yin et al.[12] reported a per-patient cumulative live birth rate (CLBR) of 6.1% and a per-cycle CLBR of 2.7% in 244 patients aged 40-50 years classified as poor ovarian responders according to the Bologna criteria, and emphasized that these rates were significantly lower than those of normo-responder patients. When cumulative live birth rates were stratified by age, CLBR per cycle and per patient were found to be 4.3% and 8.8%, respectively, in the 40-43 age group. No significant differences were observed among the other age groups, and these rates were reported as 1.8% and 4.0% for patients aged 44-45, and 0.8% and 2.3% for patients aged over 45 years, respectively[12]. Polyzos et al.[13] evaluated the efficacy of ovarian stimulation in 485 patients fulfilling Bologna criteria and reported a pregnancy rate of 9.9% after fresh embryo transfer. Another study including 26,697 cycles of 19,781 patients stratified by the POSEIDON criteria in a Chinese population concluded that the cumulative live birth rates in POSEIDON groups 3 and 4 were 14.7% and 6.58%, respectively[14]. A recent study by Fanton et al.[15] suggested that the cumulative live birth rate is positively associated with the number of retrieved oocytes, and this relationship remained significant even after stratifying the patients by age, AMH, BMI, and infertility diagnosis.

One of the largest series in the literature that examined the relationship between the number of collected oocytes and live birth rates was reported by Sunkara et al.[16] this study included 400,135 cycles with fresh embryo transfer, and 49% of the patients were women aged 35 years or older. The authors reported that although live birth rates decrease with advanced maternal age, there is a strong correlation between the number of retrieved oocytes and live birth rates. Polyzos et al.[13] reported

Table 2. Hormone profiles, AFC, and IVF stimulation parameters were compared between the groups

Variables	Group 1 no response (n=51)	Group 2 response (n=34)	p
Age (year)	40 (34-44)	41 (39-44)	0.117
FSH (mIU/mL), median (IQR)	26.5 (18.6-45.0)	13.1 (9.1-27.0)	0.032
AMH (ng/mL)	0.03 (0.01-0.11)	0.08 (0.03-0.20)	0.112
Number of AFC	2 (1-3)	2 (1-3)	0.492
Day of stimulation	8 (7-9)	8 (7-11)	0.152
Total gonadotropin dose (IU)	4200 (3600-4800)	4800 (3300-5700)	0.430

All parameters are presented as median (IQR)
FSH: Follicle stimulating hormone, IQR: Interquartile range, AMH: Anti-Mullerian hormone, AFC: Antral follicle count, IVF, In vitro fertilization

that the number of retrieved oocytes was the only variable significantly associated with live births in poor responders. The authors also mentioned that there was no notable difference in live birth rates between patients aged 40 years and those aged >40 years of age. On the other hand, considering the increased risk of aneuploidy with advancing age, it is clear that the number of oocytes needed to increase the rates of live birth will vary. It has been reported that younger patients with expected POR require an average of 4-7 oocytes to obtain one euploid blastocyst, while older patients require an average of 12 oocytes[17]. Our approach may be a viable option for poor responders who struggle to achieve an adequate number of oocytes because each additional oocyte has been reported to increase the live birth rate by 5%[16,18].

The physiological basis of ovarian response in a group of patients after cessation of gonadotropin stimulation is controversial. During the menstrual cycle, the released FSH isoforms change. This change also occurs in different stages of the reproductive period as women age. This structural heterogeneity, which is called glycoform, arises from the hormone's varying degrees of glycosylation and glycan compositions[19,20]. The acidic isoforms of FSH are abundant in the early and mid-follicular phases, whereas they are less prevalent during the pre-ovulatory and ovulatory periods. In addition, the abundant form during the menopausal period is the acidic form. It has been shown that different types of FSH isoforms have diverse functions in granulosa cells, subsequently affecting oocyte maturation[21]. To the best of our knowledge, the only published report on a series of patients who exhibited follicular growth following cessation of gonadotropin was by Gleicher et al.[22]. They investigated 49 patients who did not respond to the maximum dose of exogenous gonadotropin. Gonadotropin stimulation was ceased for 4-6 days and follicular response was reported in 24 of the patients (49%). The authors suggested that follicular development may occur in patients who respond to stimulation during the stimulation-free period because of the increase in endogenous FSH, which is more compatible than exogenous FSH. After discontinuation of exogenous FSH in some patients, there may be an increase in the release of FSH isoforms that are more compatible for FSH receptors in granulosa cells in the late follicular-preovulatory phase due to negative feedback between gonadotropin withdrawal and hypothalamus.

The physiological variability in the biological structure of FSH with age and ovarian reserve raises questions about the suitability of standard FSH preparations for controlled ovarian stimulation in all patients. Although the detection of FSH isoforms in peripheral blood is not yet commonly practiced in clinical settings, identifying the gonadotropin structure that is necessary for effective ovarian stimulation based on a woman's age and formulating appropriate preparations could potentially offer higher success rates and cost[23].

Berker et al.[24] investigated the impact of early administration of human menopausal gonadotropin (hMG) on IVF outcomes in POSEIDON group 3 and 4 poor responders and found that initiating hMG in the early follicular phase significantly improved live birth rates and reduced cycle cancelations due to fertilization failure. Although this approach did not increase the total number of oocytes retrieved, it appeared to enhance embryo quality, which is a key factor in maximizing pregnancy potential in poor responders. These results highlight the importance of optimizing gonadotropin timing to improve clinical outcomes in patients with limited ovarian response. As an alternative approach, the STOP-START protocol has been suggested for those unresponsive to high-dose controlled ovarian stimulation, providing an option before cycle cancelation[25]. This method involves temporarily pausing rFSH stimulation, followed by weekly ultrasound to detect new follicular growth, which is consistent with the "second wave" theory, which posits multiple follicle recruitment waves within a menstrual cycle. In a case series of 11 women, 63.6% underwent successful oocyte retrieval, and two patients (18.2%) achieved live births. The protocol's proposed mechanism suggests that suspending rFSH may help prevent receptor down-regulation and internalization, allowing FSH receptors to become available again, and supporting follicle development upon reinitiating stimulation. These results are consistent with our approach, in which temporarily pausing gonadotropins facilitated continued follicular development. Together, these strategies demonstrate the potential of intermittent stimulation methods to foster follicular growth in poor responders, offering a practical, time-efficient alternative for patients unresponsive to high-dose stimulation.

Recent advancements in the management of POR have also highlighted promising strategies, such as intraovarian injections of autologous platelet-rich plasma (PRP). In our recent large cohort study, we demonstrated that PRP treatment significantly improved ovarian reserve markers, including an increase in AFC and AMH levels, along with a reduction in FSH levels in patients with POR[26]. These findings support the notion that enhancing ovarian response in patients with POR may require innovative approaches, and PRP could serve as an adjunctive therapy before considering oocyte donation.

In the present study, no difference was detected in terms of demographic characteristics, the number of antral follicles, and AMH levels between the groups that showed a rebound effect on cessation of gonadotropins or not. However, FSH levels were significantly higher in the group that did not rebound from the gonadotropin gap. Gleicher et al.[22] also found no significant difference between the two groups regarding the highest FSH levels, last FSH levels, and initial estradiol levels. Only patients in the responding group were older, although not significantly older. The current data indicate that it is not yet possible to determine a parameter for predicting which patients will achieve follicular development by discontinuing stimulation. Therefore, it may be appropriate to provide a chance for a rebound response by discontinuing stimulation in all patients who do not respond to high-dose stimulation.

Study Limitations

The limitations of our study include its retrospective nature and relatively small sample size, which may limit the generalizability of the findings. However, this study provides valuable data regarding a management modality in patients with POR to high-dose gonadotropin stimulation.

Conclusion

Although pregnancy rates are not high, cessation of gonadotropin administration during ovarian stimulation could potentially offer patients the opportunity to conceive with their own biological oocytes in cases where high-dose stimulation is not effective. In countries where donation is not legal or in cases where it is not an acceptable option for couples, this strategy might be an alternative.

Ethics

Ethics Committee Approval: The study was approved by the Acıbadem Mehmet Ali Aydınlar University Medical Research Ethics Committee (decision no: ATADEK-2021-04/31, date: 24.02.2021).
Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Z.E.U.K., A.Y., Ö.K., Ş.Y.K., Ç.Y., Y.Ç., B.T., Concept: Z.E.U.K., A.Y., Ö.K., Y.Ç., B.T., Design: Z.E.U.K., Y.Ç., B.T., Data Collection or Processing: Z.E.U.K., A.Y., Ö.K., Ş.Y.K., Ç.Y., Y.Ç., B.T., Analysis or Interpretation: Z.E.U.K., Y.Ç., B.T, Literature Search: Z.E.U.K., Writing: Z.E.U.K., Y.Ç., B.T.
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References

1. Conforti A, Esteves SC, Picarelli S, Iorio G, Rania E, Zullo F, et al. Novel approaches for diagnosis and management of low prognosis patients in assisted reproductive technology: the POSEIDON concept. Panminerva Med. 2019;61:24-9.
2. Abu-Musa A, Haahr T, Humaidan P. Novel physiology and definition of poor ovarian response; clinical recommendations. Int J Mol Sci. 2020;21:2110.
3. Poseidon Group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number); Alviggi C, Andersen CY, Buehler K, Conforti A, De Placido G, et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. Fertil Steril. 2016;105:1452-3.
4. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L; ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011;26:1616-24.
5. Roque M, Haahr T, Esteves SC, Humaidan P. The POSEIDON stratification-moving from poor ovarian response to low prognosis. JBRA Assist Reprod. 2021;25:282-92.
6. Pantou A, Giannelou P, Grigoriadis S, Maziotis E, Tzonis P, Koutsouni A, et al. Evaluating different strategies for poor ovarian response management: a retrospective cohort study and literature review. Ann N Y Acad Sci. 2021;1500:93-111.
7. Ubaldi F, Vaiarelli A, D'Anna R, Rienzi L. Management of poor responders in IVF: is there anything new? Biomed Res Int. 2014;2014:352098.
8. Badawy A, Wageah A, El Gharib M, Osman EE. Prediction and diagnosis of poor ovarian response: the dilemma. J Reprod Infertil. 2011;12:241-8.
9. Kyrou D, Kolibianakis EM, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Fertil Steril. 2009;91:749-66.
10. Shin J, Kwon H, Choi DH, Park C, Kim JH, Kim J, et al. Accumulated vitrified embryos could be a method for increasing pregnancy rates in patients with poor ovarian response. J Clin Med. 2022;11:4940.
11. Yang R, Zhang C, Chen L, Wang Y, Li R, Liu P, et al. Cumulative live birth rate of low prognosis patients with POSEIDON stratification: a single-centre data analysis. Reprod Biomed Online. 2020;41:834-44.
12. Yin H, Jiang H, He R, Wang C, Zhu J, Cao Z. Cumulative live birth rate of advanced-age women more than 40 with or without poor ovarian response. Taiwan J Obstet Gynecol. 2019;58:201-5.
13. Polyzos NP, Nwoye M, Corona R, Blockeel C, Stoop D, Haentjens P, et al. Live birth rates in Bologna poor responders treated with ovarian stimulation for IVF/ICSI. Reprod Biomed Online. 2014;28:469-74.
14. Li Y, Li X, Yang X, Cai S, Lu G, Lin G, et al. cumulative live birth rates in low prognosis patients according to the POSEIDON criteria: an analysis of 26,697 cycles of in vitro fertilization/intracytoplasmic sperm injection. Front Endocrinol (Lausanne). 2019;10:642.
15. Fanton M, Cho JH, Baker VL, Loewke K. A higher number of oocytes retrieved is associated with an increase in fertilized oocytes, blastocysts, and cumulative live birth rates. Fertil Steril. 2023;119:762-9.
16. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod. 2011;26:1768-74.
17. Haahr T, Esteves SC, Humaidan P. Individualized controlled ovarian stimulation in expected poor-responders: an update. Reprod Biol Endocrinol. 2018;16:20.
18. De Geyter C, Fehr P, Moffat R, Gruber IM, von Wolff M. Twenty years' experience with the Swiss data registry for assisted reproductive medicine: outcomes, key trends and recommendations for improved practice. Swiss Med Wkly. 2015;145:w14087.
19. Wide L, Eriksson K. Molecular size and charge as dimensions to identify and characterize circulating glycoforms of human FSH, LH and TSH. Ups J Med Sci. 2017;122:217-23.
20. Anobile CJ, Talbot JA, McCann SJ, Padmanabhan V, Robertson WR. Glycoform composition of serum gonadotrophins through the normal menstrual cycle and in the post-menopausal state. Mol Hum Reprod. 1998;4:631-9.
21. Yding Andersen C. Effect of FSH and its different isoforms on maturation of oocytes from pre-ovulatory follicles. Reprod Biomed Online. 2002;5:232-9.

22. Gleicher N, Weghofer A, Darmon SK, Barad DH. Rate of rebound in follicle growth after cessation of ovarian stimulation in initial non-responders: a prospective cohort study. J Ovarian Res. 2021;14:11.

23. Orvieto R, Seifer DB. Biosimilar FSH preparations- are they identical twins or just siblings? Reprod Biol Endocrinol. 2016;14:32. Erratum in: Reprod Biol Endocrinol. 2016;14:59.

24. Berker B, Şükür YE, Özdemir EÜ, Özmen B, Sönmezer M, Atabekoğlu CS, et al. Human menopausal gonadotropin commenced on early follicular period increases live birth rates in POSEIDON group 3 and 4 poor responders. Reprod Sci. 2021;28:488-94.

25. Atabekoğlu CS, Şükür YE, Özmen B, Sönmezer M, Berker B, Aytaç R. A feasible option before cycle cancellation for poor responders; STOP-START protocol. Int J Fertil Steril. 2021;15:300-2.

26. Cakiroglu Y, Yuceturk A, Karaosmanoglu O, Kopuk SY, Korun ZEU, Herlihy N, et al. Ovarian reserve parameters and IVF outcomes in 510 women with poor ovarian response (POR) treated with intraovarian injection of autologous platelet rich plasma (PRP). Aging (Albany NY). 2022;14:2513-23.

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Retrospective analysis of the indications, methods, and complications of pregnancy termination

Gebelik terminasyonlarının endikasyon, yöntem ve komplikasyonlarının retrospektif analizi

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Abstract

Objective: To evaluate the indications and methods of termination of pregnancy (TOP) and to identify maternal complications that occur during TOP.

Materials and Methods: This retrospective study was conducted at a single tertiary center with a total of 231 patients who underwent TOP from April 2019 to March 2023. The patients were divided into two groups based on gestational age at the time of TOP and the presence of complications. Group 1 consisted of patients with a gestational age of 11-22+6 weeks (n=196), while Group 2 comprised patients with a gestational age of 23-30 weeks (n=35). Additionally, the patients were categorized based on complications into those with complications (n=63) and those without complications (n=168). The TOP protocol involves misoprostol, a uterine balloon, a combination of misoprostol and balloon, or oxytocin. Procedure-related complications included the following: Rehospitalization, rest placenta, infection, uterine rupture, blood transfusion, and repeated manual vacuum curettage.

Results: The median gestational age at TOP was 18.0±3.3 weeks for women without complications and 19.5±5.1 weeks for those with complications, it was 19.5±5.1 weeks (p=0.037). In the group with complications, the combined misoprostol-balloon method was used significantly more frequently, and the rate of previous cesarean sections was higher (p<0.05). The induction time was longer in the oxytocin group (p<0.05). The misoprostol-balloon combination group had the highest rate of uterine rupture (p<0.05).

Conclusion: TOP during advanced gestation is associated with increased rates of maternal complications, such as increased transfusion, uterine rupture, and hysterotomy. Higher gestational age and previous uterine surgery are the main causes of TOP-related maternal complications.

Keywords: Fetal defect, maternal complications, prenatal screening, termination of pregnancy

Öz

Amaç: Gebelik terminasyonlarının (GT) endikasyonlarını ve yöntemlerini değerlendirmek ve GT sırasında ortaya çıkan maternal komplikasyonları belirlemek.

Gereç ve Yöntemler: Bu retrospektif çalışma, Nisan 2019 ile Mart 2023 arasında GT uygulanan toplam 231 hasta ile üçüncü basamak merkezde yürütülmüştür. Hastalar, GT sırasındaki gebelik haftası ve komplikasyonların varlığına göre iki gruba ayrılmıştır. Grup 1, gebelik haftası 11-22+6 hafta olan hastalardan (n=196) oluşurken, Grup 2, gebelik haftası 23-30 hafta olan hastalardan (n=35) oluşmuştur. Ek olarak, hastalar komplikasyonlara göre komplikasyon gelişenler (n=63) ve komplikasyon gelişmeyenler (n=168) olarak kategorize edilmiştir. GT protokolü misoprostol, uterin balon veya misoprostol-balon kombinasyonu veya oksitosin içermektedir. İşlemle ilişkili komplikasyonlar şunları içermekteydi: Tekrar hastaneye yatış, rest plasenta, enfeksiyon, uterin rüptür, kan transfüzyonu ve tekrar manuel vakum küretaj.

Bulgular: Komplikasyon gelişmeyen grupta medyan gebelik terminasyon haftası 18,0±3,3 iken, komplikasyon gelişen grupta 19,5±5,1 hafta idi (p=0,037). Komplikasyon gelişen grupta kombine misoprostol-balon yöntemi önemli ölçüde daha sık kullanılmıştı ve geçirilmiş sezaryen oranı daha yüksekti (p<0,05). Oksitosin grubunda indüksiyon süresi daha uzundu (p<0,05). Uterin rüptür oranı misoprostol-balon kombinasyon grubunda en yüksek saptandı (p<0,05).

Sonuç: İleri gebelik haftasında GT, artmış transfüzyon ihtiyacı, artmış uterin rüptür ve histerotomi gibi maternal komplikasyonlarla ilişkilidir. İleri gebelik haftası ve geçirilmiş uterin cerrahi, GT ile ilişkili maternal komplikasyonların ana nedenlerindendir.

Anahtar Kelimeler: Fetal defekt, maternal komplikasyonlar, prenatal tarama, gebelik terminasyonu

PRECIS: Termination of pregnancy in advanced gestation is associated with increased maternal complications such as increased transfusion, uterine rupture, and hysterotomy.

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Introduction

Congenital anomalies include fetal structural and functional disorders[1,2]. Major fetal anomalies are detected in 2-3% of all live births[1]. The incidence rate of fetal anomalies leading to intrauterine pregnancy loss and termination of pregnancy (TOP) can reach 4-5%[2]. Through the efforts to include every pregnant woman in modern obstetric care and pregnancy follow-up and the enhanced utilization of technology in obstetric examinations, the detection of fetal structural defects has increased and become possible in earlier weeks of pregnancy[3]. Even life-compatible forms of structural fetal defects cause parents to reassess their decisions concerning the continuation of pregnancy because of the difficulty in determining the prognosis of the newborn's mental state at later ages[4]. The advancement of technology and its subsequent implementation in obstetrics have led to the development of antenatal screening tests and the increased detection of fetal and chromosomal abnormalities in the antenatal period through the widespread utilization of comprehensive anatomical screening at 18-22 weeks of gestation[5]. Consequently, parents and their relatives are now confronted with more situations that may necessitate the consideration of TOP.

In our country, the consent of the mother and father is sufficient for TOP within the first 10 weeks of pregnancy (law no. 2827-5, 1983 Population Planning Law). For TOP at 10 weeks of gestation and above, signatures from at least two specialist physicians or a council decision are required. While pregnancy termination is performed upon the request of patients in 113 countries worldwide, it is prohibited in 86 countries[6,7]. TOP requires addressing medical problems and social, ethical, and belief-related issues. Medical problems are encountered when determining the reasons for pregnancy termination, both during and after the termination process[8]. Maternal diseases, previous surgery, and increased gestational age have been reported to increase the rate of complications at every stage of the termination process[9]. Nevertheless, only a limited number of studies exist, and these studies show considerable inconsistencies regarding the maternal outcomes of first- and second-trimester abortion in heterogeneous groups[10].

This study aimed to analyze TOP data from our clinic, which has substantial experience in TOP, and to identify and present the dependent factors that contribute to the incidence of complications during termination management.

Materials and Methods

This retrospective study was conducted at a single tertiary hospital. Patients between the ages of 18-44 years who underwent pregnancy termination in the high-risk pregnancies unit of the tertiary center from April 2019 to March 2023 were included in the study. The principles of the Declaration of Helsinki were followed. Approval for the study was received from the Ankara Bilkent City Hospital Clinical Research Ethics Committee (decision no: E2-23-3732, date: 27.03.2023). All patients who were included signed an informed consent form.

The clinicodemographic data of the patients, body mass index (BMI) values, gestational week at which fetal anomalies were diagnosed, indications for termination, gestational week at which pregnancy was terminated, termination methods, length of hospital stay, hemoglobin level before and after the procedure, delta hemoglobin value as an indicator of the change in the amount of hemoglobin, and complications that occurred during and after the procedure were retrospectively recorded by screening the hospital's database. Complications included uterine rupture, resting placenta, rehospitalization, infection treatment, and repeated manual vacuum curettage.

Patients with multiple pregnancies and those with intrauterine pregnancy loss detected before hospitalization for pregnancy termination were excluded from the study. The patients included in the study were divided into two groups according to the gestational age at the time of termination. Group 1 [from the earliest week included in the study (11 weeks) to the 22nd week of gestation corresponding to the viability limit in terms of estimated fetal weight] and Group 2 (from the 23rd gestational week to the 30th gestational week, which was the latest gestational week included in the study). Pregnancies presenting with more than one anomaly were classified by considering the primary indication leading to pregnancy termination. We grouped patients with neural tube defects separately because of their large number and broad clinicophysiological outcomes.

Our hospital is an important multidisciplinary, tertiary reference center serving approximately 8-9 million individuals, including those living in nearby provinces. Patients are referred to TOP due to fetal anomalies or maternal diseases detected either during routine examinations or during secondary anatomical screening performed at 18-22 weeks of gestation. Antenatal screening tests and detailed anatomical screening at 18-22 weeks of gestation are undertaken with an ultrasound examination in all pregnant women who are followed up. Detailed counseling is provided to patients whose pregnancy is to be terminated for any reason. Chromosomal analysis is performed on samples obtained via chorionic villus sampling, amniocentesis, or cordocentesis. Patients undergo a thorough evaluation by a multidisciplinary council of experts from branches relevant to the characteristics of fetal anomaly and maternal disease, with the routine participation of specialists in perinatology, genetics, and neonatology. The termination process begins with obtaining signatures from the mother and father on a detailed consent form. All pregnancy terminations are performed in the high-risk pregnancy unit of our hospital. There is no gestational age limit for TOP in our country.

The method used in our unit for pregnancy termination consists of the misoprostol protocol according to international guidelines and balloon application using a Foley catheter, depending on gestational age and uterine surgery history[11-13]. In patients with detected induction failure and a history of three

or more cesarean sections, direct hysterotomy is performed. In later weeks of gestation, TOP may be preferred by administering oxytocin. A signature is obtained on the informed consent form indicating the methods and medications to be administered before termination. The decision to feticulate is made according to the estimated fetal weight and gestational age when the fetus is above the viability limit (above 21-22nd weeks of gestation). Manual vacuum curettage is performed in cases where retained products of conception are suspected after termination.

Statistical Analysis

SPSS v. 22.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The Kolmogorov-Smirnov and Shapiro-Wilks tests were used to analyze the suitability of the data for a normal distribution. The chi-square test was used to compare categorical variables. Student's t-test was used to compare normally distributed variables. Descriptive statistics are presented as means, standard deviations, and percentages. A p-value 0.05 was considered statistically significant.

Results

A total of 231 patients who underwent pregnancy termination were included in the study. There were 196 patients in Group 1 (11-22$^{6/7}$ weeks of gestation) and 35 patients in Group 2 (23-30 weeks). Age, gravida, parity, and miscarriage rates were similar in both groups, and there was no statistically significant difference. However, there was a statistically significant difference in BMI (p=0.044) (Table 1).

The length of hospital stay of the patients in both groups was similar, and there was no statistically significant difference (p=0.430). No statistically significant difference was found between the pre-and post-termination hemoglobin levels of Groups 1 and 2 or the delta hemoglobin values showing the change in hemoglobin (Table 1).

No significant difference was observed throughout the groups for the gestational age at which the anomaly or maternal disease that caused the TOP was diagnosed (p=0.564); however, the gestational age at which pregnancy was terminated statistically significantly differed (p<0.001).

The most common indication for TOP in group 1 was neural tube defect (27%), followed by anhydramnios (22.9%), other anomalies originating from the central nervous system (CNS) (15.8%), trisomies (13.3%), and other anomalies originating from the skeletal-muscular system (7.7%). Similarly, in group 2, neural tube defect (45.7%) constituted the most common indication of TOP, and this was followed by fetal cardiac diseases (34.3%), trisomies (11.4%), anhydramnios (5.7%), and other anomalies originating from the CNS (2.9%) (Table 2).

The rates of rehospitalization and systemic infections were similar between the two groups. However, the rate of rest placenta after TOP was significantly higher in group 2 (10.2% vs. 37.1%, p<0.001). The repeat manual vacuum curettage rate was significantly higher in group 2 than in group 1 (8.7% vs. 22.9%, p<0.013). The rate of uterine rupture during TOP was significantly higher in group 2 than in group 1 (p=0.012). The requirement for blood transfusion

Table 1. Gestational age at diagnosis and termination, length of hospital stay, and clinicodemographic data of patients undergoing cesarean delivery

	Group 1 (11-22$^{6/7}$ weeks of gestation) (n=196)	Group 2 (23-30 weeks of gestation) (n=35)	p-value
Age in years (n)	**29.8±5.8**	**28.8±6.8**	0.702
Gravida (n)	2.3±1.3	2.8±1.7	0.171
Parity (n)	1.04±1.07	1.23±1.3	0.361
Miscarriage (n)	0.4±0.9	0.5±0.8	0.310
BMI (kg/m^2)	26.7±2.8	27.8±3.4	0.044
Termination week	17.3±3.1	24.8±1.9	0.000
Termination duration (hour)	12.3±10.1	11.1±6.2	0.373
Feticide (n, %)	34 (17.3%)	35 (100%)	0.000
History of previous cesarean section (n)	40 (20.4%)	8 (22.8%)	0.356
Length of hospital stay (day)	3.4±2.5	3.0±1.2	0.430
Week after diagnosis	14.2±2.3	21.6±1.4	0.564
Pre-termination hemoglobin (g/dL)	11.9±1.2	11.5±1.4	0.132
Post-termination hemoglobin (g/dL)	10.2±1.3	9.9±1.2	0.187
Delta hemoglobin	1.6±1.4	1.6±1.5	0.961

BMI: Body mass index

during pregnancy termination was significantly higher in group 2 than in group 1 (p=0.009) (Table 3).

The complication rate during TOP was 27.2% (n=63). In women without complications, the median gestational age at TOP was 18.0±3.3 weeks, whereas in those with complications, it was 19.5±5.1 weeks (p=0.037) (Table 4). In the group with complications, the combined misoprostol-balloon method was used significantly more frequently as a termination method, and the rate of previous cesarean section was higher (p<0.01, p<0.034).

The induction time was longer in the oxytocin group. The misoprostol-balloon combination group had the highest rate of uterine rupture (p=0.012) (Table 5). The hysterotomy rates did not differ between the groups. In the group that used the combination method (misoprostol and balloon), the hysterotomy rate was 8.3%; in the group that terminated the balloon method, the hysterotomy rate was 0%; in the group that terminated the balloon method, the hysterotomy rate was 10%. None of the patients developed complications requiring hysterectomy. There was no maternal deterioration that required follow-up in the intensive care unit or resulted in maternal death.

Table 2. Indications for pregnancy termination

Variables	Group 1 (11-22$^{6/7}$ weeks of gestation) (n=196)	Group 2 (23-30 weeks of gestation) (n=35)
Trisomies (n)	26 (13.3%)	4 (11.4%)
Fetal cardiac anomaly (n)	15 (7.7%)	12 (34.3%)
Neural tube defect (n)	53 (27.0%)	16 (45.7%)
Other CNS anomalies (n)	31 (15.8%)	1 (2.9%)
PPROM-anhydramnios (n)	45 (22.9%)	2 (5.7%)
Renal agenesis (n)	6 (3.0%)	0 (0.0%)
Musculoskeletal disorders (n)	7 (7.7%)	0 (0.0%)
Maternal diseases (n)	5 (2.6%)	0 (0.0%)

CNS: Central nervous system, PPROM: Preterm prelabour rupture of membranes

Table 3. Analysis of the termination-related complications in the study groups

Variables	Group 1 (11-22$^{6/7}$ weeks of gestation) (n=196)	Group 2 (23-30 weeks of gestation) (n=35)	p-value
Rehospitalization	18 (9.2%)	5 (14.3%)	0.353
Rest placenta	20 (10.2%)	13 (37.1%)	<0.001
Systemic infection	8 (4.1%)	2 (5.7%)	0.651
Uterine rupture	1 (0.5%)	2 (5.7%)	0.012
Blood transfusion	3 (1.5%)	4 (11.4%)	0.009
Repeat vacuum curettage	17 (8.7%)	8 (22.9%)	0.013

Table 4. Characteristics of the no-complication and complication groups

	No-complication group (n=168)	Complication group (n=63)	p-value
Maternal age (years)	29.6±5.8	28.0±6.3	0.070
Gestational age at TOP (week)	18.0±3.3	19.5±5.1	0.037
Gestational age at the TOP			0.027
11-22$^{6/7}$ week	76.5% (150/196)	23.5% (46/196)	
23-30 weeks	51.4% (18/35)	49.6% (17/35)	

Table 4. Continued

	No-complication group (n=168)	Complication group (n=63)	p-value
TOP protocol: 11-22$^{6/7}$ weeks			<0.001
Misoprostol	104/150 (69.3%)	24/46 (52.1%)	
Balloon	21/150 (14.0%)	7/46 (15.2%)	
Combination (misoprostol + balloon)	22/150 (14.6%)	9/46 (19.5%)	
Oxytocin	3/150 (2.0%)	6/46 (13.0%)	
TOP protocol 23-30 weeks			<0.001
Misoprostol	7/18 (38.8%)	6/17 (35.2%)	
Balloon	3/18 (16.6%)	4/17 (23.5%)	
Combination (misoprostol + balloon)	3/18 (16.6%)	3/17 (17.6%)	
Oxytocin	5/18 (27.7%)	4/17 (23.5%)	
Body mass index (kg/m^2)	26.8±2.8	26.7±3.1	0.959
Gravidity	2.4±1.4	2.5±1.4	0.977
Parity	1.0±1.1	1.2±1.1	0.754
Previous cesarean section	71/168 (42.3%)	36/63 (57.1%)	0.043

TOP: Termination of pregnancy

Table 5. Comparison of the complications of termination methods

Variables	Misoprostol (n=30)	Balloon (n=11)	Combination (misoprostol + balloon) (n=12)	Oxytocin (n=10)	p-value
Induction time (hours)	9.3 (7.2-16.5)	10.3 (8.9-17.3)	12.8 (9.4-21.5)	14.8 (7.6-19.1)	0.018
Rehospitalization	12 (40%)	3 (27.2%)	3 (25%)	4 (40%)	0.353
Rest placenta	8 (26.6%)	4 (36.3%)	4 (33.3%)	3 (30%)	0.822
Systemic infection	5 (16.6%)	1 (9.0%)	2 (16.6%)	1(10%)	0.651
Uterine rupture	0 (0%)	0 (0%)	3 (25%)	0 (0%)	0.012
Blood transfusion	3 (10%)	1 (9.0%)	2 (16.6%)	1 (10%)	0.349
Repeat vacuum curettage	6 (20%)	2 (18.1%)	3 (25%)	2 (20%)	0.980
Hysterotomy	5 (16.6%)	0 (0%)	1 (8.3%)	1 (10%)	0.507

Discussion

This study aimed to examine the indications and methods of TOP, complications during and after the procedure, and factors predicting these complications. The most common reason for termination was found to be CNS anomalies. Maternal complications, such as rest placenta, uterine rupture, and transfusion need, were more frequent in terminations at an advanced gestational age. Misoprostol-balloon method was associated with higher TOP-related uterine rupture.

TOP is a process in which difficult decisions are made for family and relatives[14]. This process's medical and social management, which is closely affected by social rules, traditions, beliefs, and laws, poses difficulties for families and healthcare providers[15-17]. Previous studies have identified CNS defects as the predominant cause of TOP during the second and third trimesters[18-20]. In the current study, neural tube defects and other CNS anomalies were the most common reasons for TOP in both groups, which is similar to the results of many related studies. Concerning hysterotomy performed due to pregnancy termination, Aslan et al.[21] reported non-significant rates. Similarly, we found no significant difference in the hysterotomy rates between the groups. Although no difference was observed between the groups in terms of the week of diagnosis of the anomaly or maternal disease that led to termination, differences were noted in the weeks of pregnancy termination. These results

could be attributed to the prolonged decision-making process of patients diagnosed at earlier stages of pregnancy and the misconception that the identified issue can be remedied with further advancement in gestational weeks.

Another finding of our study was that the rate of feticide applied for pregnancy termination increased as the gestational age approached the fetal survival limit, resulting in a significant difference between the groups. This finding is in agreement with previous studies[19,22]. Special conditions caused by the physiology of pregnancy and obstetric history affect the rates of complications that may occur during pregnancy termination[23]. Research on maternal complications that occur during pregnancy termination has shown that increasing gestational age, previous surgery, and the number of previous surgical procedures are associated with possible complications[24,25]. In this study, we observed a significantly higher rate of rest placenta and the subsequent requirement of repeat manual vacuum curettage among patients who underwent pregnancy termination in advanced weeks of gestation (especially later than the 22nd week). Although this rate was similar to that reported by Garofalo et al.[9], Spingler et al.[25] found no difference in the rate of blood transfusion requirement between the termination method groups. In a study conducted by Garofalo et al.[9], the rate of uterine rupture was found to be non-significant. In contrast, our groups significantly differed in terms of the uterine rupture rate. All patients with ruptures had previously undergone at least one cesarean section.

We also observed that blood transfusion requirement increased with gestational age and the number of previous cesarean sections. The rate of blood transfusion requirement we determined is different from the data presented by Spingler et al.[25]. Our study showed that CNS abnormalities are the main cause of top TOP and that maternal complications, such as repeated manual curettage and uterine rupture, increase in late TOP. This situation emphasizes the importance of making the decision for TOP as early as the first week of pregnancy and taking a multidisciplinary approach because many factors can influence the decision. The complication rates varied depending on the termination method. The misoprostol + balloon combination was found to be associated with serious complications, such as uterine rupture. The balloon plus misoprostol method is generally preferred in advanced-stage pregnancy and in patients undergoing previous uterine surgery. Therefore, the high complication rate may depend on the week of pregnancy when the method is used[25].

A strength of our study is that the indications for TOP, termination methods, and maternal complications were examined in detail in the same study.

Study Limitations

Our study had some limitations, including its retrospective design and reliance on data from a single center.

Conclusion

Each patient who will undergo pregnancy termination should be evaluated individually, including the gestational age at which the procedure will be performed and their obstetric history. TOP during advanced gestation is associated with increased maternal complications, such as increased transfusion, uterine rupture, and hysterotomy.

Ethics

Ethics Committee Approval: Approval for the study was received from the Ankara Bilkent City Hospital Clinical Research Ethics Committee (decision no: E2-23-3732, date: 27.03.2023).

Informed Consent: All patients who were included signed an informed consent form.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Z.A., M.H., H.C., G.İ., Concept: Z.A., A.T., M.H., H.C., G.İ., R.D., Ö.K., D.Ş., Design: Z.A., A.T., M.H., H.C., G.İ., R.D., Ö.K., D.Ş., Data Collection or Processing: Z.A., M.H., H.C., G.İ., Analysis or Interpretation: Z.A., A.T., M.H., H.C., Ö.K., D.Ş., Literature Search: Z.A., M.H., H.C., Writing: Z.A., A.T., M.H., G.İ., Ö.K., D.Ş.

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References

1. Edwards L, Hui L. First and second trimester screening for fetal structural anomalies. Semin Fetal Neonatal Med. 2018;23:102-11.
2. Whitworth M, Bricker L, Mullan C. Ultrasound for fetal assessment in early pregnancy. Cochrane Database Syst Rev. 2015;2015:Cd007058.
3. Aldridge N, Pandya P, Rankin J, Miller N, Broughan J, Permalloo N, et al. Detection rates of a national fetal anomaly screening program: a national cohort study. BJOG. 2023;130:51-8.
4. Heaney S, Tomlinson M, Aventin Á. Termination of pregnancy for fetal anomaly: a systematic review of the healthcare experiences and needs of parents. BMC Pregnancy Childbirth. 2022;22:441.
5. Carmen Prodan N, Hoopmann M, Jonaityte G, Oliver Kagan K. How to perform a second trimester anomaly scan. Arch Gynecol Obstet. 2023;307:1285-90.
6. WHO Guidelines Approved by the Guidelines Review Committee. Abortion care guidelines. Geneva: World Health Organization; 2013.
7. Lavelanet AF, Johnson BR, Ganatra B. Global Abortion Policies Database: A descriptive analysis of the regulatory and policy environment related to abortion. Best Pract Res Clin Obstet Gynaecol. 2020;62:25-35.
8. Boyle J, Eerden PV, McNamara M. Second trimester abortion for fetal anomalies or fetal death: labor induction compared with dilation and evacuation. Obstet Gynecol. 2011;118:362-3.

9. Garofalo G, Garofalo A, Sochirca O, Alemanno MG, Pilloni E, Biolcati M, et al. Maternal outcomes in first and second trimester termination of pregnancy: which are the risk factors? J Perinat Med. 2018;46:373-8.
10. Rørbye C, Nørgaard M, Vestermark V, Nilas L. Medical abortion. defining success and categorizing failures. Contraception. 2003;68:247-51.
11. Combination of mifepristone and misoprostol for the termination of pregnancy. Int J Gynaecol Obstet. 2011;115:1-4.
12. ACOG Practice Bulletin No. 200: Early pregnancy loss. Obstet Gynecol. 2018;132:e197-207.
13. Emery NJ. Cognitive ornithology: the evolution of avian intelligence. Philosophical Transactions of the Royal Society B. Biol Sci. 2006;361:23-43.
14. Lotto R, Smith LK, Armstrong N. Clinicians' perspectives of parental decision-making following diagnosis of a severe congenital anomaly: a qualitative study. BMJ Open. 2017;7:e014716.
15. Zaręba K, Makara-Studzińska M, Ciebiera M, Gierus J, Jakiel G. The role of social and informational support in determining pregnancy termination for medical reasons. Int J Environ Res Public Health. 2018;15.
16. Zaręba K, La Rosa VL, Ciebiera M, Makara-Studzińska M, Gierus J, Jakiel G. Psychosocial profile and reproductive decisions among women undergoing pregnancy termination for medical reasons: a cross-sectional study. Int J Environ Res Public Health. 2019;16.
17. Yapar EG, Senöz S, Urkütür M, Batioglu S, Gökmen O. Second trimester pregnancy termination including fetal death: comparison of five different methods. Eur J Obstet Gynecol Reprod Biol. 1996;69:97-102.
18. Ozyuncu O, Orgul G, Tanacan A, Aktoz F, Guleray N, Fadiloglu E, et al. Retrospective analysis of indications for termination of pregnancy. J Obstet Gynaecol. 2019;39:355-8.
19. Maurice P, Letourneau A, Benachi A, Jouannic JM. Feticide use in second- and third-trimester pregnancy termination for fetal anomalies: Results of a national survey. Prenat Diagn. 2019;39:1269-72.
20. Koşar Can Ö, Kaleli B. Retrospective clinical evaluation of indications for pregnancies termination due to fetal anomaly. J Turk Ger Gynecol Assoc. 2022;23:28-32.
21. Aslan H, Yildirim G, Ongut C, Ceylan Y. Termination of pregnancy for fetal anomaly. Int J Gynaecol Obstet. 2007;99:221-4.
22. De Costa CM. Feticide and late pregnancy termination: An essential component of reproductive health care. Med J Aust. 2022;217:400-1.
23. Kazma JM, van den Anker J, Allegaert K, Dallmann A, Ahmadzia HK. Anatomical and physiological alterations of pregnancy. J Pharmacokinet Pharmacodyn. 2020;47:271-85.
24. Elasy AN, Ibrahem MA, Elhawy LL, Hamed BM. Vaginal misoprostol versus combined intracervical foley's catheter and oxytocin infusion for second trimester pregnancy termination in women with previous cesarean sections: a randomised controlled trial. J Obstet Gynaecol. 2022;42:2962-9.
25. Spingler T, Sonek J, Hoopmann M, Prodan N, Abele H, Kagan KO. Complication rate after termination of pregnancy for fetal defects. Ultrasound Obstet Gynecol. 2023;62:88-93.
26. Aslam FN, Loveday TA, Junior P, Truty M, Smoot R, Bekaii-Saab T, et al. APRI score is not predictive of post-surgical outcomes in patients with cholangiocarcinoma. Ann Gastroenterol. 2024;37:95-103.

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Association between serum copeptin levels and non-obese normoglycemic polycystic ovary syndrome: A case control study

Serum kopeptin düzeyleri ile obez olmayan normoglisemik polikistik over sendromu arasındaki ilişki: Bir olgu kontrol çalışması

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Abstract

Objective: Copeptin is a glycopeptide that increases under stress and is present in polycystic ovary syndrome (PCOS) patients with metabolic system disorders. We examined the relationship between copeptin and reproductive function in patients with normoglycemic PCOS with anovulatory cycles and normal weight.

Materials and Methods: Women with unexplained infertility (n=52) and women with PCOS (n=57) were included in the study. PCOS was determined using the Rotterdam criteria. Biochemical tests including estradiol, follicle-stimulating hormone, luteinizing hormone, anti-Müllerian hormone (AMH), insulin, and copeptin were performed. Serum copeptin concentrations were measured using enzyme immunoassay.

Results: There were no significant differences in demographic data, insulin levels, and insulin resistance between the PCOS and healthy volunteers. Copeptin levels were lower in the PCOS group ($p<0.001$). A significant negative correlation was observed between AMH and copeptin in the control group ($r=-0.402$, $p= 0.013$). In the PCOS group, a negative correlation was observed between antral follicle count and copeptin, as well as between AMH and copeptin ($r=-0.544$, $p<0.01$). Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive value of copeptin levels. The estimated areas under the ROC curves for serum concentration were found to be statistically significant ($p<0.001$) with a cut-off value of 2.78 (95% confidence interval 0.701-0.896), sensitivity of 0.87, and specificity of 0.70.

Conclusion: This study showed that copeptin levels are lower in patients with PCOS in the absence of insulin resistance and obesity than in healthy volunteers, and there is a negative correlation between copeptin and reproductive markers.

Keywords: Copeptin, infertility, PCOS, obesity, insulin resistance, antral follicle

Öz

Amaç: Copeptin stres durumunda artan bir glikopeptid olup metabolik sistem bozukluklarında polikistik over sendromu (PKOS) hastalarında artmaktadır. Anovulatuar siklusları olan ve normal kilolu normoglisemik PKOS hastalarında copeptinin reproduktif fonksiyonlarla ilişkisini incelemeyi amaçladık.

Gereç ve Yöntemler: Çalışmaya açıklanamayan infertil kadınlar (n=52) ve PKOS kadınlar (n=57) alındı. PKOS roterdam kriterleri ile belirlendi. Estradiol, folikül uyarıcı hormon, luteinize edici hormon, anti-Müllerian hormon (AMH), insülin ve copeptin içeren biyokimyasal testler analiz edildi. Serum copeptin konsantrasyonlarının analizleri, enzim immünoassay vasıtasıyla ölçüldü.

PRECIS: Polycystic ovary syndrome is a multifactorial disease that is associated with anovulation and infertility and can include many findings of metabolic syndrome such as insulin resistance. Copeptin is a glycopeptide that increases under metabolic stress. In our study, we evaluated Copeptin levels in patients with Polycystic ovary syndrome who do not have metabolic syndrome findings. We found that Copeptin levels did not increase despite anovulation when there were no metabolic syndrome findings. However, we observed a negative correlation between copeptin and other markers indicating reproductive function.

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Bulgular: PKOS grubunda ve sağlıklı gönüllerde demografik veriler, insülin düzeyleri ve insülin rezistansları arasında fark yoktu. Copeptin düzeyleri PKOS grubunda daha düşük saptandı (p<0,001). Kontrol grubunda AMH ile copeptin arasında anlamlı negatif korelasyon gözlendi (r=-0,402, p= 0,013). PKOS grubunda AMH ve copeptinnin yanı sıra (r=-0,544, p<0,01) Antral folikül sayısı ve copeptin arasında negatif korelasyon gözlendi. PKOS prediktivitesinin belirlenmesi için copeptin düzeylerine ROC analizi yapıldı. Serum konsantrasyonu için alıcı işletim karakteristiği eğrileri altındaki tahmini alanların 2,78'lik (95% güven aralığı 0,701-0,896) bir kesme değeri, 0,87 duyarlılık ve 0,70 özgüllük ile istatistiksel olarak anlamlı (p<0,001) olduğu bulundu.

Sonuç: Bu çalışma PKOS hastalarında insülin rezistansı ve obezite olmadığında copeptin düzeylerinin sağlıklı gönüllülere kıyasla daha düşük olduğunu, copeptin ile reproduktif belirteçler arasında ise negatif yönlü korelasyon olduğunu göstermektedir.

Anahtar Kelimeler: Copeptin, infertilite, PKOS, obezite, insülin rezistansı, antral folikül

Introduction

Polycystic ovary syndrome (PCOS) is a clinical condition characterized by irregular menstruation, anovulation with increased androgen levels, and polycystic ovaries[1]. It is the most common multisystem endocrinological disease in women of reproductive age, affecting approximately 10-13% of the population from adolescence to menopause[2]. Insulin resistance may be observed in patients with PCOS, and this resistance is aggravated by obesity[3]. In patients with PCOS, oxidative stress, proinflammatory cytokine, and adipokine changes arrest follicular development, cessation of corpus luteum functions, anovulation, and hyperandrogenism. Impaired steroidogenesis and ovulation due to hyperandrogenism are the main causes of infertility in PCOS patients[4]. Infertility treatment for patients with PCOS should begin with preconception counseling and weight control, followed by ovulation induction and selection of assisted reproductive treatments[5].

Copeptin is a 39-amino acid glycopeptide that is the C-terminal product of preprovasopressin (pre-proAVP). It is secreted in response to stress and is correlated with plasma arginine vasopressin (AVP) levels[6]. Studies have shown that high serum copeptin levels are associated with obesity, insulin resistance, type 2 diabetes mellitus, hypertension, and hyperlipidemia[7]. The World Health Organization defines obesity as a body mass index (BMI) ≥ 30 kg/m^2. Obesity changes the secretion of various adipokine, such as adiponectin and leptin, and increased leptin levels have been found in women with ovulatory disorders[8]. When obese and non-obese women with PCOS were compared, copeptin levels were found to be higher in obese women[9].

The effects of copeptin on the reproductive system have been shown in patients with poor ovarian reserve, and it has been mentioned that it may have predictive value[10]. However, there are no clinical studies in which the relationship between obesity and copeptin has been eliminated and the reproductive functions of patients with non-obesity PCOS have been evaluated. We aimed to compare copeptin levels between non-obese patients with PCOS and healthy volunteers and to determine the relationship with reproductive markers.

Materials and Methods

Setting

Our research was conducted at Hitit University Faculty of Medicine with participants in the Gynecology and Obstetrics Clinic between 2021 and 2023. The research, which was designed as a case-control study, was carried out prospectively. Research ethics committee and approval were obtained from Hitit University Faculty of Medicine Clinical Research Ethics Committee (decision no: 383, date: 20.01.2021). Women participating in the study were provided with informative information, and written consent for the study was obtained.

Study Population

Gynecological and reproductive anamnesis of the participants were recorded, gynecological examinations were performed, and laboratory techniques were planned. Anamnesis was performed in women included in the study, and physical examination was performed. Patients who had undergone pelvic surgery, medication, or radiotherapy that affected their reproductive functions were excluded from the study. Patients with metabolic syndrome, obese patients (BMI over 30 kg/m^2), and patients with systemic diseases affecting reproduction were not included in the study. Infertile women aged between 20 and 40 years were included in the study, and antral follicle counts were performed by transvaginal sonogram (Toshiba Xario 100) in the early follicular phase of the women's menstrual cycle.

Unexplained infertility is defined as the inability to achieve pregnancy without an explanatory cause despite 1 year of unprotected intercourse. The 109 women included in the study were divided into two groups according to their ovarian reserves. The control group consisted of 52 women, and the women in this group were diagnosed with unexplained infertility. There were 57 women in the study group. The study participants were infertile women diagnosed with PCOS according to the Rotterdam criteria.

Biochemical Evaluations

Blood samples were collected from the antecubital vein. Blood samples collected during the early follicular period of the menstrual cycle were separated into 5 mL tubes (BD Vacutainer, Becton Dickinson, New Jersey, USA). The blood samples were centrifuged at 1000 g for 20 min. Laboratory workers who were unaware of the source of the samples placed the blood samples in an autoanalyzer (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany) for hormonal evaluation and measured follicle-stimulating hormone, estradiol, and luteinizing hormone (LH) levels using the electrochemiluminescence immunoassay (ECLIA) method.

After centrifugation, venous blood samples were stored at 80°C until the day of anti-Müllerian hormone (AMH) and copeptin

analyses. AMH analysis was performed using the ECLIA method (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany). Copeptin levels were analyzed using an ELISA method (Human Vasopressin-Neurophysin 2-copeptin ELISA kit: EIAab Wuhan EIAab Science Co. Ltd.; East Lake Hi-Tech Development Zone, Wuhan 430079, China).

Sample Size Estimation and Matching Analysis

Priori power analysis was performed during the study planning; the test was used for the Stundent's t-test. The literature was reviewed, and the number of participants was estimated with the help of Cohen effect size[10]. In the analysis, a minimum of 35 participants were required for both the study and control groups for the 95% power hypothesis, 0.005 error, and 0.8 effect size. The R software was used to remove the age effect from the data (Version 1.2.5042, R Core Team, Vienna, Austria). The MatchIt library version 3.0.1 was used for case-control matching and for confounding due to age.

Statistical Analysis

Statistical analysis of the research data was performed using the SPSS software (Statistical Packages for Social Sciences, Chicago, IL, USA) version 21. The normality of the data distribution was analyzed using the Shapiro-Wilk test. The normal and non-normal data of the groups formed by the participants were examined with the Student's t-test and Mann-Whitney U test. After the analysis according to the statistical distribution, the data were presented in the tables as mean (± standard deviation) and median (minimum-maximum). The correlation between copeptin and the other parameters was examined using the Pearson correlation test. To determine the distinguishing power of the obtained data (sensitivity and specifity), receiver operating characteristic (ROC) analysis was performed, and graphs were drawn. The area under the curve in this figure was estimated with a 95% confidence interval. The Youdan index was used to determine the best intersection point in the ROC curve of the sensitivity and specificity values for the recognition of patients with POR. For the data to be significant, the p-value was set at <0.05.

Results

In total, 109 volunteers were included in our study; 52 of these volunteers were in the control group with a diagnosis of unexplained infertility, while 57 of them were participants with PCOS. No statistically significant difference was found between the demographic characteristics of the participants (p>0.05). The reproductive data and laboratory results are presented in Table 1. The AMH levels of the patients in the PCOS group were higher than those in the control group (p<0.001). Copeptin levels were higher in the control group (p<0.001).

Table 1. Comparison of the clinical and biochemical characteristics of the study parameters

	Control group (n=52) Mean ± std median (min-max)	PCO group (n=57) Mean ± std median (min-max)	p
Age (years)	31.1±4.3 31 (22-38)	29.9±5.1 30 (21-40)	0.321
BMI (kg/m^2)	22.1±2.5 21.8 (18.2-26.6)	22.7±2.4 22.2 (17.1-27.3)	0.412
Glucose (mg/dL)	81.2±12.5 83.0 (62.0-103.0)	83.2±9.7 81.0 (66.0-108.0)	0,745
Insuline (IU/mL)	13.6±3.3 (8.8-21.6)	12.8±2.5 (10.4-17.9)	0,195
HOMA IR	2.1±0.2 (1.0-2.3)	1.8±0.7 (15-2.4)	0.098
FSH (IU/L)	5.6±0.8 5.5 (4.2-8.3)	5.4±1.5 5.3 (2.4-9.6)	0.336
LH (IU/L)	5.1±0.9 5.2 (3.7-6.9)	5.9±1.3 5.8 (3.5-9.8)	**0.022***
AMH (ng/dL)	3.6±1.1 3.8 (1.7-5.8)	6.5±1.5 6.7 (3.3-9.7)	**<0.001***
AFC	13.0±4.1 13 (6-21)	14.8±4.8 15 (4-24)	0.111
Copeptin (ng/mL)	3.4±0.8 3.3 (2.7-5.3)	2.7±0.9 2.2 (1.9-6.6)	**<0.001***

BMI: Body mass index, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, AFC: Antral follice counts, Min: Minimum, Max: Maximum, Std: Standard

Correlation analysis was performed between copeptin and other reproductive parameters because the levels of copeptin were high in the control group and low in the PCOS group. A negative correlation was observed between Copeptin and AMH levels and LH levels (r=-227, p=0.047; r=-236, p=0.039, respectively), and no statistically significant correlation was found between other parameters and copeptin (p>0.05). Then, a new correlation analysis was performed between the reproductive parameters of the patients within the groups and their chronic periodontitis (CP) levels. In the correlation analysis performed in the control group, there was a negative correlation between copeptin and AMH (r=-0.402, p=0.013), while no statistically significant correlation was found between other reproductive parameters (p>0.05). In the correlation analysis performed within the PCOS group, a stronger negative correlation was found between AMH and copeptin levels (r=-0.544, p<0.01) also antral follice counts (AFC) and copeptin levels (r=-0.392, p=0.029). Correlation data within the PCO group are presented in Table 2.

ROC analysis of the CP levels was performed to determine PCOS predictivity. The estimated areas under ROC curves for serum concentration were found to be statistically significant (p<0.001) with a cut-off value of 2.78 (95% confidence interval 0.701-0.896), sensitivity of 0.87, and specificity of 0.70 (Table 3).

Discussion

This study aimed to determine the relationship between copeptin and reproductive function in patients with infertile PCOS. Copeptin levels were decreased in patients with PCOS without obesity and insulin resistance compared with healthy volunteers. The reproductive outcomes of patients with PCOS were negatively correlated with copeptin levels, and low copeptin levels had predictive value.

Copeptin levels are precursors of AVP and can be used as a stress marker in patients with PCOS. Because it is more stable in peripheral blood, biochemical measurements are considered more reliable. Copeptin is also used for diabetes insipidus and other diseases exhibiting vasopressin secretion changes. There is evidence that copeptin, like vasopressin, can be used in the follow-up of diabetes mellitus and respiratory and cardiovascular diseases[11,12]. Cardiovascular system diseases, diabetes mellitus, and obesity are frequently observed in PCOS patients[13].

Table 2. Correlation analysis of serum copeptin concentration and fertility parameters

	Copeptin control group (n=52)		Copeptin PCO (n=57)	
	r	p	r	p
Age (years)	0.252	0.327	-0.301	0.074
BMI (kg/m^2)	0.532	0.105	0.212	0.193
E2 (pg/mL)	0.224	0.466	0.351	0.091
FSH (IU/L)	0.481	0.179	0.377	0.038*
LH (IU/L)	0.195	0.281	0.401	0.052
AMH (ng/dL)	-0.480	0.033*	-0.544	<0.001*
AFC	-0.299	0.074	-0.392	0.029*

BMI: Body mass index, E2: Estradiol, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, AMH: anti-Müllerian hormone, AFC: Antral follice counts

Table 3. Serum low copeptin concentrations for predicting PCO development

Copeptin (ng/mL)	
AUC (95 % CI)	0.799 (0.701-0.896)
Cut-off	2.78
Sensitivity	0.87 (0.75-0.94)
Specificity	0.70 (0.66-0.88)
PPV	0.78 (0.65-0. 0.88)
NPV	0.88 (0.75- 0.95)
LR+	4.26 (2.55-7.12)
p	**<0.001**

AUC: Area under the curve, PPV: Positive predictive value NPV: Negative predictive value, LR: Learning rate

Stress markers and copeptin levels were elevated in patients with PCOS. Copeptin is believed to play an important role in the metabolic response and development of atherosclerosis in patients with insulin-resistant, hyperandrogenemic PCOS. It has been shown that copeptin levels are higher in patients with PCOS and accompanying increased carotid intima-media thickness, increased low-density lipoprotein levels, and insulin resistance compared with healthy volunteers[14]. Studies have shown that metabolic syndrome (MetS) and high Copeptin levels coexist, and PCOS patients have also been observed to have high copeptin levels in cases where MetS symptoms are present, and a correlation has been observed with insulin resistance, which is a component of MetS[15-17]. In our participants, fasting blood sugar levels were normal, insulin resistance was not present, and copeptin levels were lower than in the control group.

In our study, we found high AFC and AMH levels in the PCOS group, consistent with previous studies. We examined the relationship between copeptin levels and these two markers, which are markers of reproductive function, in both groups. An inverse correlation was observed between AMH and copeptin levels in both groups. It is known that inflammation is present in patients with PCOS and MetS, and interleukin (IL)-1 has a role in inflammation. Although copeptin levels are high in these patients, it has been shown that they do not change when IL-1 receptors are blocked in healthy volunteers and patients with PCOS who have MetS findings. In this case, the relationship between AMH and copeptin may develop through mechanisms other than hormonal changes[18].

Animal studies have also shown vasopressin neurons in the lamina terminalis (the area associated with sexual development) in the hypothalamic region. Because copeptin is produced from the c-terminus of pre-proAVP, the negative correlation with AMH in both groups may indicate changes in the hypothalamic region[19].

In our study, the relationship between AFC and copeptin was examined in both groups. An inverse correlation was found in the PCOS group, whereas no significant correlation was found in the control group. Because copeptin is known to be an osmodependent stress and inflammatory biomarker, the AFC count is expected to decrease during inflammatory and stress processes[20]. Although there were no MetS and proinflammatory stress findings in the PCOS group in our study, the negative correlation between AFC and copeptin may be attributed to changes in the follicular microenvironment.

Study Limitations

Although our study is specific because it evaluated patients with PCOS without MetS findings, it did not include follicle fluid analyses that can show hormonal and tissue-level changes that evaluate the hypothalamic-pituitary axis, such as corticotropin releasing hormone, AVP, and gonadotrophin-releasing hormone. Eliminating these limitations in future studies will increase the reliability of the data.

Conclusion

This study showed that low copeptin levels were lower in non-obese and insulin-resistant patients (without MetS symptoms) than in healthy volunteers. We showed that this finding was negatively correlated with AFC and AMH levels and that patients with PCOS with high copeptin levels (cut-off 2.7 ng/mL) may experience negative reproductive outcomes. Further studies are needed to investigate the effects of copeptin on tissue and follicular fluid levels in patients with PCOS who do not have MetS findings.

Ethics

Ethics Committee Approval: The research, which was designed as a case-control study, was carried out prospectively. Research ethics committee and approval were obtained from Hitit University Faculty of Medicine Clinical Research Ethics Committee (decision no: 383, date: 20.01.2021).

Informed Consent: Informed consent was obtained from all participants included in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ü.G., Concept: E.Y., Ü.G., Design: E.Y., Data Collection or Processing: E.Y., Analysis or Interpretation: E.Y., Literature Search: Ü.G., Writing: E.Y., Ü.G.

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References

1. Teede HJ, Tay CT, Laven JJE, Dokras A, Moran LJ, Piltonen TT, et al. Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. J Clin Endocrinol Metab. 2023;108:2447-69.
2. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, et al. Polycystic ovary syndrome. Nat Rev Dis Primers. 2016;2:16057.
3. Lim SS, Norman RJ, Davies MJ, Moran LJ. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Rev. 2013;14:95-109.
4. Haddad-Filho H, Tosatti JAG, Vale FM, Gomes KB, Reis FM. Updates in diagnosing polycystic ovary syndrome-related infertility. Expert Rev Mol Diagn. 2023;23:123-132.
5. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008;23:462-77.
6. Saleem U, Khaleghi M, Morgenthaler NG, Bergmann A, Struck J, Mosley TH Jr, et al. Plasma carboxy-terminal provasopressin (copeptin): a novel marker of insulin resistance and metabolic syndrome. J Clin Endocrinol Metab. 2009;94:2558-64.
7. Polak K, Czyzyk A, Simoncini T, Meczekalski B. New markers of insulin resistance in polycystic ovary syndrome. J Endocrinol Invest. 2017;40:1-8.
8. Estienne A, Bongrani A, Reverchon M, Ramé C, Ducluzeau PH, Froment P, et al. Involvement of novel adipokines, chemerin, visfatin, resistin and

apelin in reproductive functions in normal and pathological conditions in humans and animal models. Int J Mol Sci. 2019;20:4431.

9. Taskin MI, Bulbul E, Adali E, Hismiogulları AA, Inceboz U. Circulating levels of obestatin and copeptin in obese and nonobese women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2015189:19-23.
10. Görkem Ü, Yıldırım E. Copeptin: A potential marker for the prediction of poor ovarian reserve in the infertile women. Turk J Obstet Gynecol. 2022;19:281-6.
11. Schill F, Timpka S, Nilsson PM, Melander O, Enhörning S. Copeptin as a predictive marker of incident heart failure. ESC Heart Fail. 2021;8:3180-8.
12. Jalleh R, Torpy DJ. The emerging role of copeptin. Clin Biochem Rev. 2021;42:17-25.
13. Orio F, Muscogiuri G, Nese C, Palomba S, Savastano S, Tafuri D, et al. Obesity, type 2 diabetes mellitus and cardiovascular disease risk: an uptodate in the management of polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2016;207:214-9.
14. Karbek B, Ozbek M, Karakose M, Topaloglu O, Bozkurt NC, Cakır E, et al. Copeptin, a surrogate marker for arginine vasopressin, is associated with cardiovascular risk in patients with polycystic ovary syndrome. J Ovarian Res. 2014;7:31.
15. Rojas-Humpire R, Soriano-Moreno DR, Galindo-Yllu B, Zafra-Tanaka JH. Association between copeptin and metabolic syndrome: a systematic review. J Nutr Metab. 2022;2022:5237903.
16. Alı AI, Hassan WN, Alrawı S. A copeptin as a predictor marker for insulin resistance among women with polycystic ovary syndrome. Curr Womens Health Rev. 2022;18:67-72.
17. Coelho JM, D'cunha P, Shıvashankara AR. Serum copeptin as a biomarker of polycystic ovarian syndrome and its correlation with metabolic syndrome components: a cross-sectional analytical study. Journal of Clinical and Diagnostic Research. 2024;18:7.
18. Popovic M. The role of interleukin-1 in the pathophysiology of polycystic ovary syndrome and regulation of copeptin [dissertation]. Basel (Switzerland): University of Basel; 2021.
19. Tata B, Mimouni NEH, Barbotin AL, Malone SA, Loyens A, Pigny P, et al. Elevated prenatal anti-Müllerian hormone reprograms the fetus and induces polycystic ovary syndrome in adulthood. Nat Med. 2018;24:834-46.
20. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol. 2005;3:28.

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Comparison of obstetric, neonatal, and surgical outcomes of emergency and planned deliveries in pregnancies complicated by placenta previa and in subgroups with and without placenta accreta spectrum

Plasenta previa ile komplike olan gebeliklerde ve plasenta akreta spektrumu olan ve olmayan alt gruplarda acil ve elektif doğumların obstetrik, neonatal ve cerrahi sonuçlarının karşılaştırılması

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Abstract

Objective: This study aimed to compare emergency and planned cesarean section cases in pregnancies complicated with placenta previa (PP) and subgroups with and without placenta accreta spectrum (PAS) in terms of obstetric, neonatal, and surgical outcomes.

Materials and Methods: This retrospective cohort study included 128 patients diagnosed with PP who underwent cesarean section. Obstetric, neonatal, and surgical outcomes of all cases with PP and subgroups with and without PAS were compared according to whether they were emergency or planned cesarean section.

Results: Of the 128 women with PP, 60 planned and 68 underwent emergency cesarean section. In all patients with PP and in the PAS and non-PAS subgroups, the neonatal outcomes of patients who underwent emergency cesarean section were more negative than those of patients who underwent planned cesarean section. It was observed that more hysterectomy were performed in the emergency group than in the elective group in all patients with PP and PAS patients (p=0.027 and p=0.012 respectively). It was observed that patients with PP and non-PAS were hospitalized after cesarean section for a longer period of time in the emergency group than in the planned group (p=0.044 and p=0.002 respectively).

Conclusion: Planned cesarean section leads to better obstetric, neonatal, and surgical outcomes compared with emergency cesarean section in pregnancies complicated by PP, especially in those with PAS. Our findings suggest that planned delivery strategies should be considered for patients with PP. Decisions regarding the timing of delivery should balance maternal risks and benefits with fetal and fetal risks and benefits.

Keywords: Placenta previa, placenta accreta spectrum, emergency delivery, hysterectomy, surgical outcomes

PRECIS: The current study investigated the obstetric, neonatal and surgical outcomes of emergency and planned delivery in patients with placenta previa.

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Öz

Amaç: Bu çalışmanın amacı, plasenta previa (PP) ile komplike gebeliklerde ve plasenta akreta spektrumu (PAS) olan ve olmayan alt gruplarda acil ve planlı sezaryen olgularını obstetrik, neonatal ve cerrahi sonuçlar açısından karşılaştırmaktır.

Gereç ve Yöntemler: Bu retrospektif kohort çalışması, sezaryenle doğum yapan PP tanısı almış 128 hasta ile yürütülmüştür. PP'li tüm olguların ve PAS'lı ve olmayan alt grupların obstetrik, neonatal ve cerrahi sonuçları, acil veya planlı sezaryen olmalarına göre karşılaştırılmıştır.

Bulgular: PP'li 128 kadından 60'ı planlı, 68'i ise acil sezaryenle doğum yapmıştı. PP'li tüm hastalarda ve PAS ve PAS olmayan alt gruplarda, acil sezaryenle doğum yapan hastaların neonatal sonuçlarının planlı sezaryenle doğum yapan hastalara kıyasla daha olumsuz olduğu gözlenmiştir. Acil grupta, elektif gruba kıyasla tüm PP hastalarında ve PAS hastalarında daha fazla histerektomi yapıldığı gözlemlendi (sırasıyla p=0,027 ve p=0,012). PP'li ve PAS olmayan hastaların sezaryen sonrası acil grupta, planlı gruba kıyasla daha uzun süre hastanede yattığı gözlemlendi (sırasıyla p=0,044 ve p=0,002).

Sonuç: PP ile komplike gebeliklerde, özellikle PAS'lı olanlarda, planlı sezaryenler acil sezaryenlere kıyasla daha iyi obstetrik, neonatal ve cerrahi sonuçlara yol açmaktadır. Bulgularımız, PP'li hastalarda planlı doğum stratejilerinin desteklenmesi gerektiğini göstermektedir. Doğum zamanlaması ile ilgili kararlar, anne riskleri ve faydaları ile fetüs veya yenidoğan için riskler ve faydaları dengelemelidir.

Anahtar Kelimeler: Plasenta previa, plasenta akreata spektrumu, acil doğum, histerektomi, cerrahi sonuçlar

Introduction

Placenta previa (PP) refers to the abnormal implantation of the placenta into the lower segment of the uterus, causing complete or partial closure of the cervix[1]. Increased maternal, fetal, and neonatal morbidity and mortality in pregnancies complicated by PP. These pregnancies often present with painless, recurrent vaginal bleeding in the third trimester and are at increased risk of morbidity, including blood transfusion, peripartum hysterectomy, postpartum hemorrhage, infection, and longer hospital stays[2]. Placenta accreta spectrum (PAS; placenta accreta, increta, or percreta), that is, invasion of placental villi beyond the decidua basalis, may be observed in PP. This can lead to catastrophic bleeding, multiple complications, and even death[2,3]. Additionally, newborns born to patients with PP face problems such as prematurity, low birth weight, low Apgar scores, neonatal intensive care unit (NICU) requirements, and increased fetal mortality[1,2].

Optimal timing of birth is important because of the potential morbidity risks of PP and PAS. However, there is no consensus in the literature regarding the optimal timing of childbirth for patients with PP[1]. In general, women with uncomplicated PP are recommended to give birth at 36-37 weeks of pregnancy[2,4,5]. In cases of PAS, it has been stated in some studies that catastrophic bleeding is common after the 36th week and that planned birth at 34-35 weeks of pregnancy after antenatal steroids leads to a decrease in blood loss and blood transfusions[5,6].

Identifying the differences between the obstetric, neonatal, and surgical outcomes of emergency and elective cesarean sections in PP cases and evaluating these differences between subgroups of patients with and without PAS will contribute to the literature in terms of determining the optimal timing of birth in PP patients with and without PAS.

The aim of this study was to compare emergency and planned cesarean section cases in pregnancies complicated with PP and subgroups with and without PAS in terms of obstetric, neonatal, and surgical outcomes.

Materials and Methods

This study retrospectively examined PP cases at Health Sciences University Turkey, Derince Training and Research Hospital between October 2012 and October 2022. Ethical approval for the study was given by the Health Sciences University Turkey, Kocaeli Derince Training and Research Hospital Clinical Research Ethics Committee (protocol number: 2022-145, date: 12/01/2023).

Women with singleton pregnancies who underwent cesarean delivery after 24 weeks of pregnancy with a diagnosis of PP were included in the study. Women who had a cesarean section elsewhere, whose PP diagnosis could not be confirmed during cesarean section, who had multiple pregnancies, and who had a miscarriage were excluded from the study.

One hundred and twenty-eight women with PP who met the inclusion criteria were included in the study. We recorded whether these women had PAS and whether they had an emergency or elective cesarean section. Whether the patients had PAS or not was decided according to the pathology results in patients who underwent hysterectomy or lower uterine resection, while in other patients, it was decided based on preoperative placental ultrasonography findings and intraoperative placental bleeding and observation. Of the 128 patients with PP, 54 were PAS and 74 were non-PAS.

The demographic characteristics and obstetric, neonatal, and surgical outcomes of all cases with PP and the subgroup of cases with and without PAS were compared according to whether they were emergency or planned cesarean section. Women who were followed up in the clinic and had a cesarean section at the planned time were considered "planned", while those who presented with symptoms requiring urgent cesarean section, such as bleeding, labor pain, fetal distress, and premature rupture of membranes, were considered "emergency". Additionally, demographic characteristics, obstetric outcomes, neonatal outcomes, and surgical outcomes of patients who underwent emergency cesarean section were compared according to PAS status.

The demographic and obstetric characteristics analyzed were age, gravidity, parity, number of abortions, number of vaginal

births and cesarean sections, time of birth, type of PP (total, partial, marginal and low-lying), and dominant localization of the placenta (anterior, posterior). Birth weight, first and fifth minute Apgar scores, NICU stay, and invasive mechanical ventilation were defined as neonatal outcome parameters. The amount of blood products transfused, the mother's treatment in the intensive care unit, the duration of hospital stay after birth, and interventions other than routine cesarean section (hysterectomy, internal iliac artery ligation, intrauterine sutures, Bakri balloon, compression sutures) were defined as surgical outcome parameters.

Statistical Analysis

Statistical analysis was performed using IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA). Numerical variables were presented as mean ± standard deviation and median (interquartile range), whereas categorical variables were presented as frequency (percentage). Normality tests (Kolmogorov-Smirnov test, Shapiro-Wilk test) were applied to numerical variables. Numerical variables with normal distribution were analyzed with the Student-t test, and numerical variables with non-normal distribution were analyzed using the Mann-Whitney U test. Relationships between categorical variables were evaluated using a ch-square test and Fisher's exact test. To test two-sided hypotheses, $p<0.05$ was considered sufficient for statistical significance.

Results

One hundred and twenty-eight women diagnosed with PP during the research period were included in the study. Of these, 68 were in the emergency group and 60 were in the planned surgery group. Of the 128 patients with PP, 54 were PAS and 74 were non-PAS. Of the 74 non-PAS cases, 41 were in the emergency group and 33 were in the planned group. Of the 54 patients with PAS, 27 were in the emergency and 27 in the planned group. There were no significant differences between the emergency and planned groups in terms of the presence or absence of PAS (Table 1).

Table 1 shows a comparison of the demographic and obstetric characteristics between the emergency and planned groups. No significant difference was observed between the emergency and planned cesarean groups in terms of age, gravity, parity, dominant localization of the placenta, and type of PP (total or others). Of all PP patients included in the study, those who had a planned cesarean section gave birth on the 259.83rd day (approximately 37 weeks and 1 day) on average, and those who had an emergency cesarean section gave birth on the 235.25th day (approximately 33 weeks and 4 days) on average. In all patients with PP and in the subgroup of patients with and without PAS, the gestational period was shorter in patients who underwent emergency cesarean section than in those who underwent planned cesarean section, and birth rates before the 37th and 34th weeks were higher in these patients. It was observed that both in all patients and in the PAS and non-PAS subgroups, those who had an emergency cesarean had a higher number of previous cesareans, whereas those who had a planned cesarean had a higher number of previous vaginal births. When 128 patients with PP were examined in the study, a weak but significant negative correlation was found between the number of previous cesarean sections and the duration of pregnancy. ($r=-0.184$ $p=0.037$ in Spearman analysis). Accordingly, the higher the number of previous cesarean sections, the earlier was the gestational age at birth.

Table 2 presents the comparison of neonatal outcomes between emergent and planned patients. In all patients with PP and in the PAS and non-PAS subgroups, the neonatal outcomes of patients who underwent emergency cesarean section were more negative than those of patients who underwent planned cesarean section. In all patient groups, newborns from patients who underwent emergency cesarean section had lower birth weights, 1st and 5th minute Apgar scores were lower, rates of admission to the NICU were higher, and rates of receiving invasive mechanical ventilation were higher compared with newborns from patients who underwent planned cesarean section.

The surgical outcomes between the emergency and planned groups are presented in Table 3. No significant difference was observed in terms of transfused blood products (total blood products, erythrocyte suspension, platelet suspension) between patients who underwent emergency and planned cesarean section, both in all patients with PP and in the PAS and non-PAS subgroups. Of the 128 patients with PP included in the study, 23 (17.9%) underwent hysterectomy along with cesarean section. Of the 23 hysterectomized patients, 2 were in the non-PAS group, and both patients were emergency cases. Twenty-one of the 23 patients who underwent hysterectomy, 21 had PAS. Among the 15 patients who underwent emergency cesarean section, 6 were included in the planned cesarean group. In all patients with PP and PAS, more hysterectomy were performed in the emergency group than in the elective group, which was statistically significant ($p=0.027$ and $p=0.012$ respectively). Of the 128 patients with PP included in the study, 30 had intrauterine sutures after cesarean section. Table 3 shows that in all patients with PP and PAS, intrauterine suturing was performed in statistically more patients in the planned group than in the emergency group ($p=0.013$ and $p<0.001$ respectively). No significant difference was observed between the emergency and planned groups in terms of intrauterine suture application in non-PAS patients. No significant differences were observed in all patients with PP, nor in the PAS and non-PAS subgroups, in terms of internal iliac artery ligation rates, bari balloon insertion rates, and compression suture placement rates between patients who underwent emergency and planned cesarean sections.

Table 1. Comparison of demographic and obstetric characteristics between the emergency and planned group

Total number of cases (n=128)	**Planned (n=60)**	**Emergency (n=68)**	**p**
Age	31.62±6.07 / 32 (11)	31.78±5.13 / 33 (7)	0.787*
Gravidity	3.22±1.62 / 3 (2)	3.04±1.04 / 3 (2)	0.618*
Parity	1.90±1.46 / 2 (2)	1.72±1.19 / 2 (1)	0.439*
Number of previous abortions	0.30±0.67 / 0 (0)	0.32±0.60 / 0 (1)	0.569*
Number of previous vaginal births	0.8±1.41 / 0 (1)	0.18±0.71 / 0 (0)	***<0.001****
Number of Previous cesarean sections	1.05±1.06 / 1 (2)	1.5±1.08 / 1 (1)	***0.024****
Delivery time			
Delivery day	259.83±9.64 / 260 (13.8)	235.25±21.25 / 240 (14.8)	***<0.001*****
Preterm birth (<37 weeks)	24 (40 %)	59 (86.8 %)	***<0.001****
Preterm birth (<34 weeks)	0 (0.0 %)	24 (35.3 %)	***<0.001****
Placenta previa			
Total placental previa	36 (60.0%)	48 (70.6 %)	0.208**
Others (partial, marginal, low lying)	24 (40.0 %)	20 (29.4 %)	
Predominant placental			
Anterior	28 (46.7 %)	35 (51.5 %)	0.587**
Posterior	32 (53.3 %)	33 (48.5 %)	
PAS status			
PAS cases	27 (45%)	27 (39.7 %)	0.545**
Non-PAS cases	33 (55.0)	41 (60.3 %)	
Non-PAS cases (n=74)	**Planned (n=33)**	**Emergency (n=41)**	**p**
Age	30.79±6.06 / 30 (26-36.5)	31.17±5.7 / 32 (28.5-35.5)	0.781****
Gravidity	3.12±2.02 / 3 (3)	2.61±1.3 / 2 (1)	0.317*
Parity	1.76±1.87 / 2 (2)	1.32±1.05 / 1 (1)	0.476*
Number of previous abortions	0.30±0.64 / 0 (1)	0.32±0.61 / 0 (1)	0.819*
Number of previous vaginal births	1.24±1.75 / 0 (2)	0.24±0.74 / 0 (0)	***<0.001****
Number of Previous cesarean sections	0.58±0.94 / 0 (1)	1±0.86 / 1 (2)	***0.015****
Delivery time			
Delivery day	262.67±7.90 / 263 (11.5)	237.58±25.38 / 244 (27)	***<0.001*****
Preterm birth (<37 weeks)	10 (30.3 %)	33 (80.5 %)	***<0.001****
Preterm birth (<34 weeks)	0 (0.0 %)	17 (41.5 %)	***<0.001****
Placenta previa			
Total placental previa	17 (51.5 %)	26 (63.4 %)	
Others (partial, marginal, low lying)	16 (48.5 %)	15 (36.6 %)	
Predominant placental			
Anterior	12 (36.4 %)	17 (41.5%)	0.655**
Posterior	21 (63.6 %)	24 (58.5%)	
PAS cases (n=54)	**Planned (n=27)**	**Emergency (n=27)**	**p**
Age	32.63±6.05 / 33 (11)	32.88±4.03 / 33.5 (6)	0.958****
Gravidity	3.33±0.96 / 3 (1)	3.69 ±1.16 / 3.5 (3-4.25)	0.227*
Parity	2.07±0.73 / 2 (1)	2.31±1.15 / 2 (1.75-3)	0.543*
Number of previous abortions	0.30±0.72 / 0 (0)	0.35±0.63 / 0 (0-1)	0.567*
Number of previous vaginal births	0.44±0.64 / 0 (1)	0.08±0.39 / 0 (0-0)	***0.004****
Number of previous cesarean sections	1.63±0.93 / 2 (0)	2.23±0.95 / 2 (1.75-3)	***0.035****
Delivery time			
Delivery day	256.37±10.56 / 258 (16)	239.31±13.32 / 238 (7.5)	***<0.001*****
Preterm birth (<37 weeks)	14 (51.9 %)	26 (96.3 %)	***<0.001****
Preterm birth (<34 weeks)	0 (0.0 %)	7 (25.9%)	***0.010*****
Placenta previa			
Total placental previa	19 (70.4 %)	22 (81.5 %)	0.340**
Others (partial, marginal, low lying)	8 (29.6 %)	5 (18.5 %)	
Predominant placental			
Anterior	16 (59.3 %)	18 (66.7 %)	0.573**
Posterior	11 (40.7 %)	9 (33.3 %)	

Variables are given as mean ± standard deviation / median (interquartile range) or frequency (percentage), *: Mann Whitney U test, **: Chi-square test, ***: Fisher exact test, ****: Student-t test, Bold / italics value signifies statistical significance, PAS: Placenta accreta spectrum

Table 2. Comparison of neonatal outcomes between the emergency and planned groups

Total number of cases (n=128)	Planned (n=60)	Emergency (n=68)	p
Neonatal birth weight (g)	2949.38±448.17	2368.73±693.55	***<0.001*****
Apgar score			
1st minute	7.88±0.64 / 8 (8-8)	6.40±1.67 / 7 (3)	***<0.001****
5th minute	9.40±0.74 / 9.5 (1)	8.10±1.47 / 8 (2)	***<0.001****
1st minute <7	2 (3.3 %)	31 (45.6 %)	***<0.001****
5th minute <7	1 (1.7 %)	16 (23.5 %)	***<0.001****
Neonatal intensive care unit	13 (21.7 %)	44 (64.7 %)	***<0.001****
Neonatal invasive mechanical ventilation	6 (10 %)	26 (38.2 %)	***<0.001****
Non-PAS cases (n=74)	**Planned (n=33)**	**Emergency (n=41)**	**p**
Neonatal birth weight (g)	2939.94±513 / 2810 (795)	2386.71±771 / 2440 (1015)	***<0.001*****
Apgar score			
1st minute	7.82±0.72 / 8 (8)	6.44±1.75 / 7 (3)	***<0.001****
5th minute	9.39±0.86 / 10 (1)	8.17±1.44 / 9 (2)	***<0.001****
1st minute <7	1 (3 %)	18 (43.9 %)	***<0.001****
5th minute <7	1 (3 %)	9 (22.0 %)	***0.036****
Neonatal intensive care unit	6 (18.2 %)	25 (61 %)	***<0.001****
Neonatal invasive mechanical ventilation	3.9% (9.1 %)	14 (34.1 %)	***0.011****
PAS cases (n=54)	**Planned (n=27)**	**Emergency (n=27)**	**p**
Neonatal birth weight (g)	2960.93±362/2950 (550)	2340.38±563/2272.5 (830)	***<0.001*****
Apgar score			
1st minute	7.96±0.52/8 (0)	6.27±1.56/6 (3)	***<0.001****
5th minute	9.41±0.57/9 (1)	7.92±1.49/8 (3)	***<0.001****
1st minute <7	1 (3.7 %)	13 (48.1 %)	***<0.001****
5th minute <7	0 (0.0 %)	7 (25.9 %)	***0.010*****
Neonatal intensive care unit	7 (25.9 %)	19 (70.4 %)	***0.001****
Neonatal invasive mechanical ventilation	3 (11.1 %)	12 (44.4 %)	***0.006****

Variables are given as mean ± standard deviation / median (interquartile range) or frequency (percentage), *: Mann Whitney U test, **: Chi-square test, ***: Fisher exact test, ****: Student-t test, Bold / italics value signifies statistical significance, PAS: Placenta accreta spectrum

There were no significant differences in the rates of admission to the maternal intensive care unit between patients who underwent emergency and planned cesarean section, both in all patients with PP and in those with PAS and non-PAS. It was observed that patients with PP and non-PAS were hospitalized after cesarean section for a longer period of time in the emergency group than in the planned group (p=0.044 and p=0.002 respectively). In patients with PAS, no significant difference was observed between the emergency and planned group in terms of hospitalization time after cesarean section.

Table 4 presents the demographic characteristics and obstetric, neonatal, and surgical outcomes of patients who underwent emergency cesarean section according to PAS status. Among patients with PP who underwent emergency cesarean section, those with PAS had a higher number of previous cesarean sections, gravida, and parity than those without (p<0.05 for all). There were no significant differences between the two groups in the number of previous vaginal births. The pregnancy duration was similar between the two groups (average 239 days in PAS patients, average 237 days in non-PAS patients). There were no significant difference in PP type (total and other). The predominant placental location was more anterior in PAS-positive emergency patients, whereas it was more posterior in non-PAS emergency cases (p=0.042). No significant difference was observed between the groups with and without PAS in emergency cases in terms of the neonatal outcomes examined (birth weight, Apgar score, need for neonatal intensive care stay, and need for invasive mechanical ventilation of the newborn). In emergency cases, the amount of blood products transfused, hysterectomy rate, internal iliac artery ligation rate, postoperative hospital stay, and maternal intensive care unit admission rate were higher in the PAS group than in the non-PAS group (p<0.05 for all).

Table 3. Comparison of surgical outcomes between the emergency and planned groups

Total number of cases (n=128)	Planned (n=60)	Emergency (n=68)	p
Total blood products (units)	0.68±1.56 / 0 (0)	0.80±1.50 / 0 (1)	0.466*
Erythrocyte suspension (units)	0.47±0.99 / 0 (0)	0.54±0.97 / 0 (1)	0.477*
Platelet suspension (units)	0.22±0.61 / 0 (0)	0.26±0.59 / 0 (0)	0.324*
Intervention other than routine cesarean section			
Hysterectomy	6 (10 %)	17 (25 %)	***0.027*****
Internal iliac artery ligation	26 (43.3 %)	32 (47.1 %)	0.673**
Intrauterine sutures	20 (33.3 %)	10 (14.7 %)	***0.013*****
Bakri balloon	13 (21.7%)	13 (19.1 %)	0.721**
Compression sutures (B-Lynch, etc.)	4 (6.7 %)	6 (8.8 %)	0.749***
Postoperative hospital stay (days)*	3.12±1.63 / 2 (2)	3.72±2.19 / 3 (2)	***0.044****
Maternal intensive care unit	7 (11.7 %)	17 (25.0 %)	0.054**
Non-PAS cases (n=74)	**Planned (n=33)**	**Emergency (n=41)**	**p**
Total blood products (units)	0.15±0.71 / 0 (0)	0.39±1.06 / 0 (0)	0.242*
Erythrocyte suspension (units)	0.09±0.38 / 0 (0)	0.24±0.62 / 0 (0)	0.234*
Platelet suspension (units)	0.06±0.35 / 0 (0)	0.15±0.48 / 0 (0)	0.266*
Total number of cases (n=128)	**Planned (n=60)**	**Emergency (n=68)**	**p**
Intervention other than routine cesarean section			
Hysterectomy	0 (0.0 %)	2 (4.9 %)	0.304***
Internal iliac artery ligation	6 (18.2 %)	10 (24.4 %)	0.519**
Intrauterine sutures	6 (18.2 %)	7 (17.1 %)	0.901**
Bakri balloon	5 (15.2 %)	7 (17.1 %)	0.824**
Compression sutures (B-Lynch, etc.)	0 (0.0 %)	3 (7.3 %)	0.249***
Postoperative hospital stay (days)*	2.42±1.00 / 2 (0)	3.37±2.22 / 3 (2)	***0.002****
Maternal intensive care unit	0 (0.0 %)	4 (9.8 %)	0.124***
PAS cases (n=54)	**Planned (n=27)**	**Emergency (n=27)**	**p**
Total blood products (units)	1.33±2.04 / 0 (2)	1.50±1.83 / 0.5 (3)	0.696*
Erythrocyte suspension (units)	0.93±1.30 / 0 (2)	1.04±1.22 / 0.5 (2)	0.731*
Platelet suspension (units)	0.41±0.80 / 0 (0)	0.46±0.71 / 0 (1)	0.552*
Intervention other than routine cesarean section			
Hysterectomy	6 (22.6 %)	15 (55.6 %)	***0.012*****
Internal iliac artery ligation	20 (74.1 %)	22 (84.5 %)	0.745
Intrauterine sutures	14 (51.9 %)	3 (11.1 %)	***0.001*****
Bakri balloon	8 (29.6 %)	6 (22.2 %)	0.535**
Compression sutures (B-Lynch, etc.)	4 (14.8 %)	3 (11.1 %)	0.500***
Postoperative hospital stay (days)*	3.96±1.85 / 3 (2)	4.26±2.07 / 3 (3)	0.677*
Maternal intensive care unit	7 (25.9 %)	13 (48.1 %)	0.091**

Variables are given as mean ± standard deviation / median (interquartile range) or frequency (percentage), *: Mann Whitney U test, **: Chi-square test, ***: Fisher exact test, ****: Student-t test, Bold / italics value signifies statistical significance, PAS: Placenta accreta spectrum

Table 4. Examination of demographic characteristics and obstetric, neonatal, and surgical outcomes of patients who underwent emergency cesarean section according to PAS status

Emergency cases (n=68)	PAS (n=27)	Non-PAS (n=41)	p
Age	32.88±4.03 / 33.5 (6)	31.17±5.7 / 32 (28.5-35.5)	0.232****
Gravidity	3.69±1.16 / 3.5 (3-4.25)	2.61±1.3 / 2 (1)	***0.006****
Parity	2.31±1.15 / 2 (1.75-3)	1.32±1.05 / 1 (1)	***0.006****
Number of previous abortions	0.35±0.63 / 0 (0-1)	0.32±0.61 / 0 (1)	0.843*
Number of previous vaginal births	0.08±0.39 / 0 (0-0)	0.24±0.74 / 0 (0)	0.441*
Number of previous cesarean sections	2.23±0.95 / 2 (1.75-3)	1±0.86 / 1 (2)	***<0.001****
Delivery time			
Delivery day	239.31±13.32 / 238 (7.5)	237.58±25.38 / 244 (27)	0.321*
Preterm birth (<37 weeks)	26 (96.3 %)	33 (80.5 %)	0.060**
Preterm birth (<34 weeks)	7 (25.9%)	17 (41.5 %)	0.190**
Placenta previa			
Total placental previa	22 (81.5 %)	26 (63.4 %)	0.110**
Others (partial, marginal, low lying)	5 (18.5 %)	15 (36.6 %)	
Predominant placental			
Anterior	18 (66.7 %)	17 (41.5%)	***0.042****
Posterior	9 (33.3 %)	24 (58.5%)	
Neonatal birth weight (g)	2340.38±563 / 2272.5 (830)	2386.71±771 / 2440 (1015)	0.723****
Apgar score			
1st minute	6.27±1.56 / 6 (3)	6.44±1.75 / 7 (3)	0.826*
5th minute	7.92±1.49 / 8 (3)	8.17±1.44 / 9 (2)	0.549*
1st minute <7	13 (48.1 %)	18 (43.9 %)	0.731**
5th minute <7	7 (25.9 %)	9 (22.0 %)	0.705**
Neonatal intensive care unit	19 (70.4 %)	25 (61 %)	0.428**
Neonatal invasive mechanical ventilation	12 (44.4 %)	14 (34.1 %)	0.393**
Total blood products (units)	1.50±1.83 / 0.5 (3)	0.39±1.06 / 0 (0)	***0.006****
Erythrocyte suspension (units)	1.04±1.22 / 0.5 (2)	0.24±0.62 / 0 (0)	***0.006****
Platelet suspension (units)	0.46±0.71 / 0 (1)	0.15±0.48 / 0 (0)	***0.035****
Intervention other than routine cesarean section			
Hysterectomy	15 (55.6 %)	2 (4.9 %)	***<0.001****
Internal iliac artery ligation	22 (84.5 %)	10 (24.4 %)	***<0.001****
Intrauterine sutures	3 (11.1 %)	7 (17.1 %)	0.729***
Bakri balloon	6 (22.2 %)	7 (17.1 %)	0.597**
Compression sutures (B-Lynch, etc.)	3 (11.1 %)	3 (7.3 %)	0.675***
Postoperative hospital stay (days)*	4.26±2.07 / 3 (3)	3.37±2.22 / 3 (2)	***0.029****
Maternal intensive care unit	13 (48.1 %)	4 (9.8 %)	***<0.001****

Variables are given as mean ± standard deviation / median (interquartile range) or frequency (percentage), *: Mann Whitney U test, **: Chi-square test, ***: Fisher exact test, ****: Student-t test, Bold / italics value signifies statistical significance, PAS: Placenta accreta spectrum

Discussion

This study aimed to elucidate differences in obstetric, neonatal, and surgical outcomes between emergency and elective cesarean sections in pregnancies complicated by PP, including subgroups with and without PAS. Our results showed that planned cesarean section was associated with a reduced incidence of maternal complications, such as the need for emergency hysterectomies, particularly in PAS cases. These outcomes highlight the importance of careful prenatal management and timely intervention for pregnancies complicated by PP and PAS. In our study, we observed that the number of previous cesarean sections was higher in those who had emergency cesarean sections, both in all patients and in the PAS and non-PAS subgroups. In addition, we observed that as the number of previous cesarean sections increased, the gestational age at delivery also became earlier. Our findings are consistent with those of Ruiter et al.[7], who found a history of cesarean section as a predictor of emergency delivery in patients with PP. Additionally, studies have shown that the presence of antepartum bleeding in patients with PP increases the risk of emergency cesarean delivery[4,7,8]. In the study by Pivano et al.[8], 3 or more episodes of antepartum bleeding and the first antepartum bleeding occurring before the 29th week of gestation were associated with emergency cesarean section. Similarly, Oğlak et al.[4] found that a first episode of antepartum bleeding occurring at or before the 28th week of pregnancy increases the risk of emergency cesarean section. We did not evaluate the relationship between emergency cesarean section and antepartum hemorrhage in our retrospective study because there was not enough information in the files regarding the number of antepartum bleeding episodes and when the bleeding episodes first started.

The optimal timing of delivery of PP is unclear. In a consensus study published in 2018, delivery was recommended between 34 and 35$^{6/7}$ weeks of gestation in stable PAS-positive patients. In this study, waiting beyond the 36th week in PAS-positive patients is not recommended because of the increased risk of bleeding[9]. In contrast, Wang et al.[10] recommended planned surgery around 36-37 weeks of gestation for PAS-positive patients because their data showed that waiting until 36 weeks did not significantly increase the rate of emergency delivery. In another study describing the management of PP and accreta, elective cesarean delivery is recommended at 38 weeks of gestation when there is no antepartum hemorrhage in PAS cases, whereas elective cesarean delivery was recommended at 36 weeks of gestation to reduce the risk of emergency delivery in cases with a history of antepartum vaginal hemorrhage[11]. Erfani et al.[12] recommend planned surgery at 36-37 weeks in patients with PP without placental adhesion, provided that there is no significant bleeding.

In our study, the average gestational period for urgent cesarean sections was approximately 235 days (33 weeks and 4 days) in all patients. We observed that the frequency of hysterectomy was increased, the postoperative hospital stay was prolonged, and maternal comorbidity was higher in patients with PP who underwent emergency delivery than in those who underwent planned delivery. Therefore, we believe that maternal morbidity can be reduced by planning earlier births in patients with PP compared to those with uncomplicated pregnancies. In our study, no significant difference was observed in terms of gestational age at delivery between emergency cases with and without PAS. However, among patients who had emergency deliveries, PAS cases had more frequent hysterectomies, required more blood transfusions, prolonged postoperative hospital stay, and higher rates of maternal intensive care unit admission compared with non-PAS cases. In other words, maternal morbidity was more frequent in patients with PAS who underwent emergency cesarean delivery. Therefore, we recommend that PAS cases be delivered earlier than non-PAS cases to reduce the number of emergency cesareans. Neonates born between 34 and 36 weeks of gestation are considered late preterm category[13,14]. Although they are at greater risk than term neonates, those born during this period are known as late preterm neonates because they have the closest physiological development to term. In cases of elective PAS, we recommend elective cesarean delivery at 34-35 weeks of gestation to avoid increasing the frequency of emergency cesarean sections and to wait at least until late preterm. Considering the neonatal problems caused by premature birth, we believe that this timing may vary depending on the patient's and fetus's condition (such as the presence of additional symptoms like bleeding or fetal development). We suggest choosing the most appropriate timing for delivery in which maternal risks, such as bleeding from delayed delivery, and fetal risks from early birth are balanced. Although we observed less morbidity in emergency cases without PAS spectrum, we recommend planning cesarean section before 38 weeks of gestation in cases of PP without PAS to ensure the comfort of the procedure in elective conditions. The concept of term in newborns begins at 37 weeks[14]. We believe that elective cesarean section between 37 and 37$^{6/7}$ weeks of gestation is appropriate to reduce the frequency of emergency cesarean section in cases with PP without PAS.

Our data showed that neonatal outcomes were significantly better in the planned cesarean group. Neonates born by emergency cesarean delivery had lower birth weights, worse Apgar scores, and higher rates of NICU admission and mechanical ventilation. This finding was likely due to the lower gestational age of the emergency cases. Adverse neonatal outcomes may also be attributed to the fetus being affected by the mother's adverse hemodynamics in the emergency setting. Similar to our study, in the study conducted by Durukan et al.[1] with 313 patients, newborns born by emergency cesarean section had lower birth weights, worse Apgar scores, and increased rates of NICU admission and mechanical ventilation. However, when the analysis was restricted to term neonates,

no significant difference in NICU requirement was observed between the emergency and planned groups. Balayla et al.[13] examined neonatal outcomes in PP and reported that early term ($37^{0/7}$ and $38^{6/7}$ weeks of gestation) delivery in PP was associated with fewer complications and did not carry more risk than late preterm ($34^{0/7}$ and $36^{6/7}$ weeks of gestation) delivery.

The surgical results of our study support the planning of cesarean section. The shorter hospital stay for planned procedures and lower incidence of emergency hysterectomies, especially in patients with PAS, suggest that elective surgeries allow for better preparation and management of possible complications. In their study comparing the maternal and neonatal outcomes of emergency and elective cesarean sections in patients with PP without PAS, Gedik Özköse et al.[2] observed better results in the planned group in terms of preoperative and discharge hemoglobin levels, maternal intensive care unit admission rate, and hospital stay duration. A retrospective study of Asıcıoglu et al.[15] reported that the rates of intraoperative estimated blood loss, bladder damage, uterine vessel injury, and hysterectomy were higher in cases of emergency delivery with PP and that more blood transfusion was required in emergency cases. A retrospective study conducted by Durukan et al.[1] observed that the amount of blood transfused in the emergency group of patients with PP was higher and the number of days the mother was admitted to the intensive care unit was higher. In a retrospective study by Taşgöz et al.[16], complications, hysterectomy, and re-laparotomy rates in patients with previa did not differ between emergency and planned deliveries, whereas admission to the adult intensive care unit and longer hospital stay were more common in emergency patients. Studies conducted to date, including our study, have shown that emergency delivery in patients with PP is associated with more adverse surgical and maternal outcomes than planned delivery. However, there is no clear consensus on whether serious adverse outcomes, such as hysterectomy, are observed more frequently in emergency cases. The retrospective nature of the studies conducted so far on this subject and the inadequacy of the sample size make it difficult to reach a clear conclusion in this regard.

In our study, we evaluated emergency cases separately according to the presence and absence of PAS. In emergency cases, we observed that patients with PAS required more transfusions, were hospitalized for longer periods, and required more maternal intensive care compared to patients without PAS. Therefore, we recommend a more careful approach, such as preparing more blood products and providing intensive care conditions before performing a cesarean section, in patients with PAS compared to patients without PAS, even in emergency situations.

Study Limitations

The retrospective nature and single-center design of this study may limit the generalizability of the findings. Another limitation of our study was that prepartum bleeding episodes and estimated blood loss were not evaluated. On the other hand, the evaluation of both PAS and non-PAS patients and the evaluation and treatment of all patients by an experienced surgical team in a tertiary center are strengths of the study.

Conclusion

In conclusion, our study suggests that planned cesarean sections in pregnancies complicated by PP, especially with PAS, lead to better obstetric, neonatal, and surgical outcomes compared with emergency cesarean sections. These findings advocate proactive prenatal management and planned delivery strategies to improve maternal and neonatal health in patients with PP and PAS. Decisions regarding the timing of delivery should balance maternal risks and benefits with fetal and fetal risks and benefits.

Ethics

Ethics Committee Approval: Ethical approval for the study was given by the Health Sciences University Turkey, Kocaeli Derince Training and Research Hospital Clinical Research Ethics Committee (protocol number: 2022-145, date: 12/01/2023).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

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

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References

1. Durukan H, Durukan ÖB, Yazıcı FG. Planned versus urgent deliveries in placenta previa: maternal, surgical and neonatal results. Arch Gynecol Obstet. 2019;300:1541-9.
2. Gedik Özköse Z, Oğlak SC, Ölmez F. The comparison of maternal and neonatal outcomes between planned and emergency cesarean deliveries in placenta previa patients without placenta accreata spectrum. Ginekol Pol. 2022;93:217-23.
3. Fan D, Xia Q, Liu L, Wu S, Tian G, Wang W, Wu S, Guo X, Liu Z. the incidence of postpartum hemorrhage in pregnant women with placenta previa: a systematic review and meta-analysis. PLoS One. 2017;12:e0170194.
4. Oğlak SC, Ölmez F, Tunç Ş. Evaluation of antepartum factors for predicting the risk of emergency cesarean delivery in pregnancies complicated with placenta previa. Ochsner J. 2022;22:146-53.
5. Spong CY, Mercer BM, D'Alton M, Kilpatrick S, Blackwell S, Saade G. Timing of indicated late-preterm and early-term birth. Obstet Gynecol. 201;118:323-33.
6. Warshak CR, Ramos GA, Eskander R, Benirschke K, Saenz CC, Kelly TF, et al. Effect of predelivery diagnosis in 99 consecutive cases of placenta accreta. Obstet Gynecol. 2010;115:65-9.

7. Ruiter L, Eschbach SJ, Burgers M, Rengerink KO, van Pampus MG, Goes BY, et al. Predictors for emergency cesarean delivery in women with placenta previa. Am J Perinatol. 2016;33:1407-14.
8. Pivano A, Alessandrini M, Desbriere R, Agostini A, Opinel P, d'Ercole C, et al. A score to predict the risk of emergency caesarean delivery in women with antepartum bleeding and placenta praevia. Eur J Obstet Gynecol Reprod Biol. 2015;195:173-6.
9. American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine. Obstetric Care Consensus No. 7: Placenta accreta spectrum. Obstet Gynecol. 2018;132:e259-75.
10. Wang Y, Zeng L, Niu Z, Chong Y, Zhang A, Mol B, et al. An observation study of the emergency intervention in placenta accreta spectrum. Arch Gynecol Obstet. 2019;299:1579-86.
11. Allahdin S, Voigt S, Htwe TT. Management of placenta praevia and accreta. J Obstet Gynaecol. 2011;31:1-6.
12. Erfani H, Kassir E, Fox KA, Clark SL, Karbasian N, Salmanian B, et al. Placenta previa without morbidly adherent placenta: comparison of characteristics and outcomes between planned and emergent deliveries in a tertiary center. J Matern Fetal Neonatal Med. 2019;32:906-9.
13. Balayla J, Wo BL, Bédard MJ. A late-preterm, early-term stratified analysis of neonatal outcomes by gestational age in placenta previa: defining the optimal timing for delivery. J Matern Fetal Neonatal Med. 2015;28:1756-61.
14. Karnati S, Kollikonda S, Abu-Shaweesh J. Late preterm infants-changing trends and continuing challenges. Int J Pediatr Adolesc Med. 2020;7:36-44.
15. Asıcıoglu O, Şahbaz A, Güngördük K, Yildirim G, Asıcıoglu BB, Ülker V. Maternal and perinatal outcomes in women with placenta praevia and accreta in teaching hospitals in Western Turkey. J Obstet Gynaecol. 2014;34:462-6.
16. Taşgöz FN, Yenigül NN, Kender Erturk N, Kırşan İleri E, Yaşa FN. Comparison of maternal and neonatal outcomes between emergency and planned cesarean delivery in women with placenta previa. Eur Res J. 2022;8:359-67.

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Efficacy of conservative laparoscopic surgical treatment for acute ovarian torsion in pediatrics and adolescent populations: A single-armed meta-analysis

Pediatrik ve adolesan popülasyonda akut over torsiyonunun konservatif laparoskopik cerrahi tedavisinin etkinliği: Tek kollu bir meta-analiz

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Abstract

Conservative laparoscopic torsioning with or without cystectomy or oophoropexy has emerged as a promising approach for the management of ovarian torsion, particularly in pediatric populations. We sought to understand the efficacy of this approach. We comprehensively reviewed the relevant literature. We formulated a search strategy by combining keywords related to laparoscopic surgery, conservative management, and ovarian torsion in pediatric and adolescent populations. Data were retrieved from Web of Science, PubMed, Medline, Cochrane Library, and ClinicalTrials. The Gov and Scopus databases. Eligible articles met the following criteria: Involved pediatric or adolescent populations with ovarian or adnexal torsion and evaluating the use of any method of laparoscopic torsioning surgery. We included the following outcomes; recurrence, conversion to laparotomy, need for oophorectomy, mean time to the onset of symptoms, abdominal pain at the time of presentation, fever, and evidence of ovarian function on postoperative ultrasound. In our meta-analysis, conducted using OpenMeta[Analyst], we analyzed both continuous and dichotomous data with mean difference and risk ratio, respectively, along with 95% confidence intervals (CI). First, the incidence of recurrence was reported in five studies, where 17 cases experienced recurrence out of 391 cases experienced recurrence. Additionally, instances requiring open surgery were documented in five studies, with 22 out of 360 cases requiring this conversion to open surgery, resulting in a combined ratio of 0.051 [95% CI (0.018, 0.083), p=0.02]. Moreover, six studies provided data on cases necessitating oophorectomy, with 27 out of 437 cases requiring the procedure. Furthermore, the mean time from symptom onset to surgery was 51.9 h. Abdominal pain was prevalent at presentation, affecting 264 out of 324 patients. Fever was less frequently reported, with 19 out of 324 patients experiencing it. Finally, a high percentage of patients showed evidence of ovarian function on postoperative ultrasound, with a pooled proportion of 0.69. Our analysis performed the conservative management of ovarian torsion in young women. Recurrence occurred in 17 out of 391, and 22 out of 360 required conversion to open surgery. Furthermore, oophorectomy was necessary in 27 of 437 patients, and the mean time from symptom onset to surgery was 51.9 h. Abdominal pain was the most common symptom, affecting 264 out of 324 cases, whereas fever was less common.

Keywords: Torsion, pediatrics, conservative management, laparoscopy

Öz

Sistektomi veya ooforopeksi ile birlikte veya bunlar olmaksızın konservatif laparoskopik detorsiyon, özellikle pediatrik popülasyonlarda over torsiyonunun tedavisinde umut verici bir yaklaşım olarak ortaya çıkmıştır. Veriler Web of Science, PubMed, Medline, Cochrane Library, ClinicalTrials.Gov ve SCOPUS veri tabanlarından elde edildi. İlk olarak, toplam 391 olgudan 17'sinde nüks görüldüğü beş çalışmada nüks insidansı bildirilmiştir. Ek olarak, açık cerrahi gerektiren olgular 5 çalışmada belgelenmiştir. Üç yüz altmış olgudan 22'sinde açık cerrahiye geçilmesi gerekmiştir ve bu da 0,051'lik bir birleşik oranla sonuçlanmıştır [%95 güven aralığı (0,018-0,083), p=0,02]. Ayrıca, 6 çalışma ooferektomi gerektiren vakalar hakkında veri sağlamıştır ve 437 olgudan 27'sinde bu prosedür gerekmiştir. Ayrıca, semptomların başlangıcından ameliyata kadar geçen ortalama süre 51,9 saat olarak bildirilmiştir. Başvuru sırasında

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karın ağrısı sık olarak bildirilmiştir ve 324 olgunun 264'ünü etkilemiştir. Ateş daha az sıklıkla rapor edilmiş olup 324 olgunun 19'unda görülmüştür. Son olarak, genel olarak, ameliyat sonrası ultrasonda yumurtalık fonksiyonuna dair kanıt bulunan hastaların oranı yüksek olup, havuzlanmış oran 0,69'dur. Analizimiz genç kadınlarda over torsiyonunun konservatif tedavisini değerlendirdi. Rekürrens 391 olgunun 17'sinde görüldü ve 360 olgunun 22'sinde açık cerrahiye geçilmesi gerekti. Ayrıca, 437 olgunun 27'sinde ooferektomi gerekti ve semptom başlangıcından ameliyata kadar geçen ortalama süre 51,9 saatti. Karın ağrısı 324 olgunun 264'ünü etkileyen baskın bir semptomken, ateş daha az görüldü.

Anahtar Kelimeler: Torsiyon, pediatri, konservatif tedavi, laparoskopi

Introduction

Ovarian and adnexal torsion are rare but critical gynecologic emergencies. The prevalence among women aged 18 years was approximately 4.9 per 100,000 individual[1]. Approximately 3% of acute abdominal pain cases in children where urgent surgical intervention is required[2]. This syndrome describes torsion of the ovary and vascular pedicle, thereby interrupting blood flow. Consequently, Ischemia and necrosis may occur if not promptly treated. In children, this condition can also present with non-specific symptoms such as severe abdominal pain, nausea, and vomiting[3,4]. Several factors might be implicated in the ovarian torsion of children, ranging from anatomical abnormalities to unusual presentation of ovarian masses, or even hormonal variations[5]. Timely diagnosis is vital to avoid complications and to preserve the function of the ovaries. Unfortunately, many times the diagnosis remains undetermined due to the condition's inconsistent presentation and the potential overlap with other intra-abdominal pathologies in children. Imaging techniques, including ultrasound (US) and magnetic resonance imaging, have an undoubtedly significant role in making a diagnosis; however, clinical awareness and suspicion are also critically important components to accurate diagnosis[6,7]. Despite advancements in diagnostic methods and treatment approaches, challenges persist in the diagnosis of ovarian torsion in children. Thus, a collaborative, multidisciplinary approach involving pediatricians, gynecologists, and radiologists is crucial to improve outcomes and mitigate complications effectively[8]. The conventional treatment for ovarian torsion has typically favored oophorectomy over detorsion because of concerns about post-detorsion thromboembolic events, as well as the risks of peritonitis or infection from the presence of a necrotic ovary in the abdomen. However, there is a growing trend toward conservative, ovary-sparing management, which recognizes its advantages over oophorectomy. In the USA, the rates of conservative management have increased from approximately 20% of all surgically managed ovarian torsion cases in 2001 to just over 25% in 2015[8]. Conservative procedures typically involve accessing the abdominal cavity via laparoscopy and performing detorsion to restore normal vascular supply to the ovaries, and may include oophoropexy or cystectomy[9]. In our analysis, we aimed to investigate the efficacy and perioperative complications of laparoscopic procedures to conserve the ovaries in pediatric or adolescent patients and to analyze recent population-based patterns in the surgical treatment of ovarian torsion.

Methodology

Our study adhered to the PRISMA guidelines and recommendations[10]. We formulated a search strategy by combining keywords related to laparoscopic surgery, conservative management, and ovarian torsion in pediatric and adolescent populations. We included all studies that considered themselves to be inclusive of a population that was described to be "child/(ren)," "young," "pediatric" or "adolescent" and excluded any studies that included at least one participant that was referenced as being an "adult" or included participants that were shown by the data to be greater than 18 years of age at time of study inclusion. Data were retrieved from Web of Science, PubMed, Medline, Cochrane Library, and ClinicalTrials. The Gov and Scopus databases.

Study Selection

The titles and abstracts were initially screened, followed by full-text screening. Eligible articles met the following criteria. First, we only included studies involving pediatric and adolescent females with ovarian or adnexal torsion in which the investigators evaluated management with laparoscopic torsioning or oophorectomy. We only included studies that reported on at least one of our preselected outcomes, which included recurrence rates, conversion to laparotomy rates, the need for oophorectomy, mean time to the onset of symptoms, abdominal pain at the time of presentation, fever, and follicular changes in postmenarche females. We chose to include randomized control trials (RCTs) and observational studies, excluding reviews, surveys, abstracts, and meta-analyses. Ultimately, no RCTs have been found on this topic. We did not restrict study selection by language or country of origin.

Quality Assessment

The NHLB quality assessment tools were used to assess the quality of the observational studies. Each study's risk of bias was categorized as low, high, or unclear[11].

Data Extraction

Data were extracted into three categories: patient demographics and baseline data, outcomes including recurrence, conversion to laparotomy, need for oophorectomy, mean time to the onset of symptoms, abdominal pain at the time of presentation, fever, evidence of ovarian function on a postoperative US, and data related to the quality assessment. Microsoft Excel was used to facilitate data collection.

Statistical Analysis

OpenMeta[Analyst][12] was used for the meta-analysis. Continuous data were analyzed using the mean difference and risk ratio for dichotomous outcomes, with a 95% confidence interval (CI). The fixed-effects and random-effects models were applied to the homogeneous and heterogeneous data, respectively. Heterogeneity was determined using the I^2 and chi-square tests. Heterogeneity was indicated by $p<0.1$ or $I^2>50\%$.

Results

Results of our literature search

Our review of the literature is depicted in Figure 1 using a PRISMA diagram. We identified seven articles[13-19] that satisfied our inclusion criteria and were from diverse databases. These studies focused on young females who experienced ovarian torsion and were managed through conservative laparoscopic detorsion. In total, 456 cases were included, including 157 on the right side, 137 on the left side, and 25 bilateral cases. Among these cases, 233 women were postmenarche. The average age of the participants was 12.3 years, with a standard deviation of 3.5. The complete demographics of the study are presented in Table 1.

Results of risk of bias assessment

The mean score of the observational studies was 10.7 out of 14. Table 2 provides a detailed illustration of the quality assessment of the observational studies.

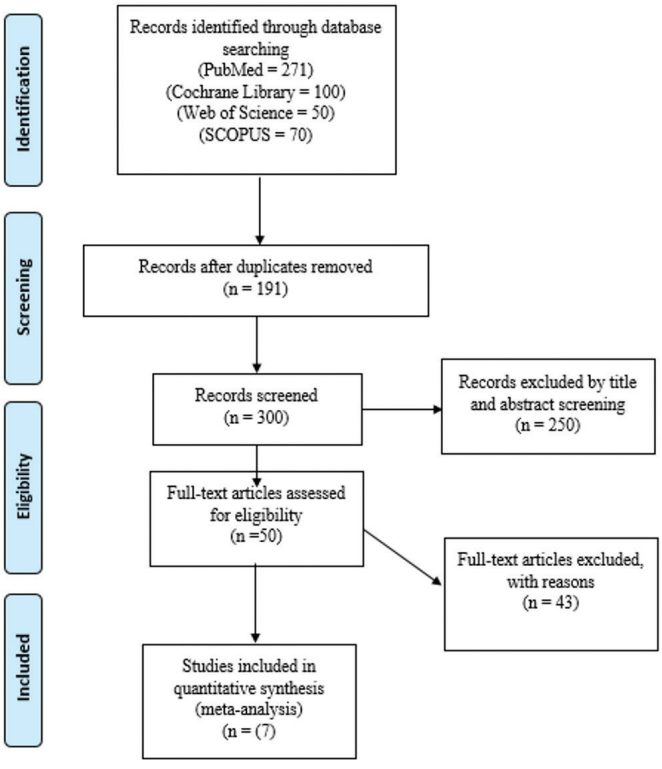

Figure 1. PRISMA flow diagram describing our literature search

Outcomes

1. Recurrence

Recurrence was difficult to define among the studies, as there was an inconsistent definition regarding whether recurrence referred to the recurrence of acute ovarian torsion, the recurrence of the benign or malignant process that led to the acute torsion, or if both were required to meet the definition. Ultimately, we accepted each included study's definition of "recurrence" of each included study for data synthesis. Accordingly, the incidence of recurrence was reported in five studies. Seventeen cases experienced recurrence from a total number of 391. The pooled ratio was 0.038 with a [95% CI (0.019, 0.057), p=0.01]. The pooled analysis was homogeneous (I^2=0%, p=0.54), as shown in Figure 2.

2. Required laparotomy

In five of the studies, we analyzed documented instances where laparoscopic surgery needed to be converted to laparotomy to complete the procedure. Among the 360 cases across these studies, 22 required this conversion. The combined ratio of cases requiring conversion to open surgery was calculated to be 0.051, with a [95% CI (0.018, 0.083), p=0.02]. The p-value associated with this analysis was 0.02. The pooled analysis was homogeneous (I^2=29%, p=0.22), as shown in Figure 3.

3. Required oophorectomy

The six studies included in our analysis provided data on cases in which conversion to oophorectomy (removal of the ovary) was considered necessary by the attending surgeon. Among the 437 patients who underwent laparoscopic detorsion, 27 required conversion to oophorectomy. The pooled ratio was 0.053 with a [95% CI (0.026, 0.080), p=0.01]. The pooled analysis was homogeneous (I^2=25%, p=0.24), as shown in Figure 4.

4. Mean time from diagnosis to surgical intervention (in hours)

Three studies reported the time elapsed from the diagnosis of ovarian torsion to surgical intervention. The pooled estimate was 51.9 hours with a [95% CI (37.2, 66.6), p=0.01]. The pooled analysis was heterogeneous (I^2=83.017%, p=0.03), as shown in Figure 5A. We performed a leave-one-out meta-analysis to display the effect size after excluding each study. Excluding Sriram et al.[19] seemed to have the largest influence on reducing the heterogeneity, producing an estimate of 60.25 with a [95% CI (55.4, 65.6), p=0.01], as seen in Figure 5B.

5. Abdominal pain at presentation

Abdominal pain was among the most commonly experienced symptoms at the time of the presentation. A total of 264 out of 324 patients suffered from abdominal pain. The pooled ratio was 0.842 with a [95% CI (0.734, 0.950), p=0.01]. The pooled analysis was heterogeneous (I^2=79.4%, p=0.02), as shown in Figure 6.

Table 1. Demographic and clinical characteristics of the participants

Country	Study design	Sample size	Age > years, (mean, SD/IQR)	Menarche (%)	The type of mass causing ovarian torsion					Side affected			Follow-up period: 1 month
					Immature teratoma	Follicular cyst	Dermoid cyst	Adenoma	Adeno-carcinoma	Right	Left	Bilateral	NR
India	Retrospective study	10	11 [6-17]	5 (50)	NR	1	NR	NR	NR	7	3	0	8.4
USA	Retrospective study	245	12.4 3.29	170 (60)	2 (0.8)	12 (4.8%)	35 (14%)	10 (4)	1 (0.4)	134	109	1	8.8
India	Retrospective study	46	14.3 [1-18]	-	NR	36 (80)	7 (15.2)	NR	NR	NR	NR	18	4-5 years
Australia	Retrospective study	54	9.80±3.95	14 (25.9)	NR	12 (21)	10 (18.51)	2 (3.70)	NR	16	21	2	NR
USA	Retrospective study	50	13.9±2.6	44 (88)	NR	16		NR	NR	NR	NR	NR	NR
Canada	Retrospective review of medical records	8	NR	NR	NR	4	1	NR	NR	NR	4	4	32.2
USA	Retrospective series	43	8.3 [4.1, 13.6]	NR	NR	10	5	3	NR	NR	NR	NR	NR

SD: Standard devation, IQR: Interquartile range

Table 2. Quality assessment of the included studies

Study ID	Sriram et al.[19]	Adeyemi-Fowode et al.[18]	Agarwal et al.[17]	Julania et al. [13]	Fang et al.[15]	Pansky et al.[14]	Walker et al.[16]
1. Was the research question or objective clearly stated in this paper?	**1**	**1**	**1**	**1**	**1**	**1**	**1**
2. Was the study population clearly defined?	**1**	**1**	**1**	**1**	**1**	**1**	**1**
3. Was the participation rate of eligible persons at least 50%?	**1**	**1**	**1**	**1**	**0**	**1**	**1**
4. Were all subjects selected or recruited from the same or similar populations (including the same time period)? Were the inclusion and exclusion criteria prespecified and applied uniformly to all participants?	**0**	**1**	**1**	**1**	**1**	**1**	**1**
5. A sample size justification, power description, or variance and effect estimates	**0**	**0**	**0**	**0**	**0**	**0**	**0**
6. For the analyses in this paper, were the exposure(s) of interest measured before the outcome(s) was measured?	**1**	**1**	**1**	**1**	**1**	**1**	**1**
7. Was the timeframe sufficient to reasonably expect an association between exposure and outcome if it existed?	**1**	**1**	**1**	**1**	**1**	**1**	**1**
8. For exposures that can vary in amount or level, did the study examine different levels of exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	**1**	**1**	**1**	**1**	**1**	**1**	**1**
9. Are the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all participants?	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table 2. continued

Study ID	Sriram et al.[19]	Adeyemi-Fowode et al.[18]	Agarwal et al.[17]	Julania et al.[13]	Fang et al.[15]	Pansky et al.[14]	Walker et al.[16]
10. Was the exposure(s) assessed more than once?	0	0	1	0	0	0	0
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all participants?	1	1	1	1	1	1	1
12. Were the outcome assessors blinded to the exposure status of the participants?	*	*	*	*	*	*	*
13. Did loss to follow-up after a baseline of 20% or less?	1	1	1	1	1	1	1
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	1	0	1	1	1	1	1
Total score (out of 14)	10/14	10/14	12/14	11/14	10/14	11/14	11/14

Key: 1= Yes, 0= No, *: Not reported, N/A: Not applicable

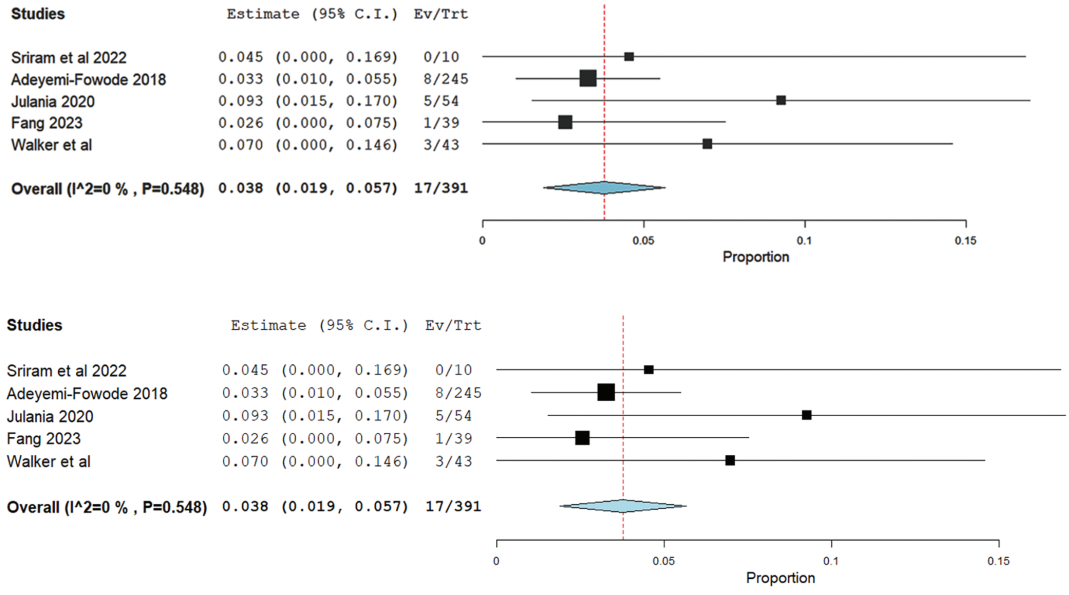

Figure 2. Meta-analysis of the incidence of recurrence

CI: Confidence interval

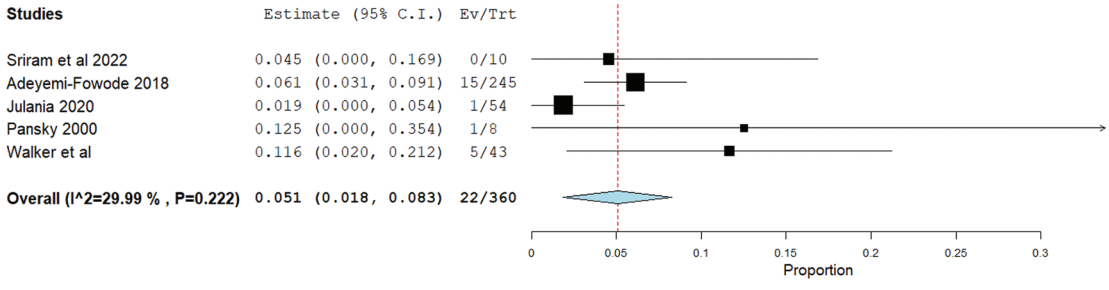

Figure 3. Meta-analysis of the incidence of conversion to laparotomy

CI: Confidence interval

6. Fever as a symptom at presentation

Fever at presentation was reported in 19 out of 324 patients. The pooled proportion was 0.055, with a [95% CI (0.030, 0.079), p=0.01]. The pooled analysis was homogeneous (I^2=0%, p=0.6), as shown in Figure 7.

7. Evidence of ovarian function in postoperative US

Almost all studies included assessments of ovarian function in the postoperative period. Studies varied on what was acceptable criteria for evidence of function, and some included the presence of follicles, normal results on Doppler flow studies, or simply a radiologist's interpretation of a normal appearing ovary for the patient's age. Studies also varied in the postoperative time frame, but all were within 8 weeks of surgery. The pooled proportion was 0.69 with a [95% CI (0.511, 0.87), p=0.01]. The pooled analysis was heterogeneous (I^2=85.7%, p=0.01), as shown in Figure 8.

Discussion

In our analysis, several key outcomes related to ovarian torsion were evaluated. First, the incidence of recurrence was reported in five studies, where 17 cases experienced recurrence out of 391 cases experienced recurrence. Additionally, instances requiring open surgery were documented in five studies, with 22 out of 360 cases requiring this conversion to open surgery, resulting in a combined ratio of 0.051 [95% CI (0.018, 0.083), p=0.02]. Moreover, six studies provided data on cases requiring oophorectomy, with 27 out of 437 cases requiring

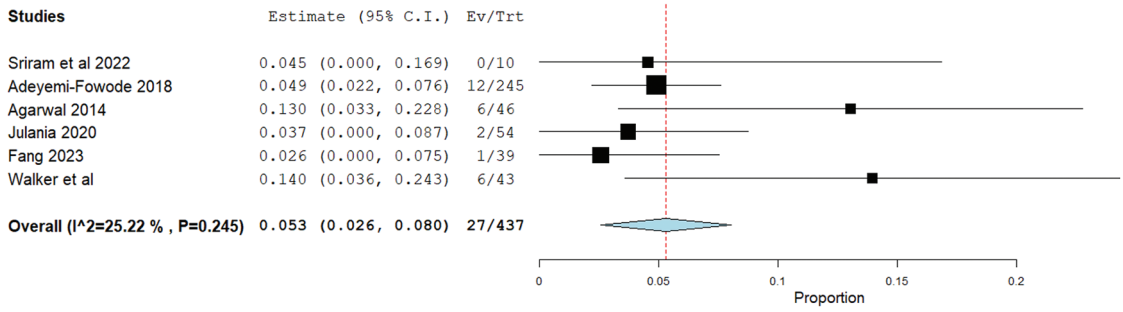

Figure 4. Meta-analysis of the incidence of required oophorectomy

CI: Confidence interval

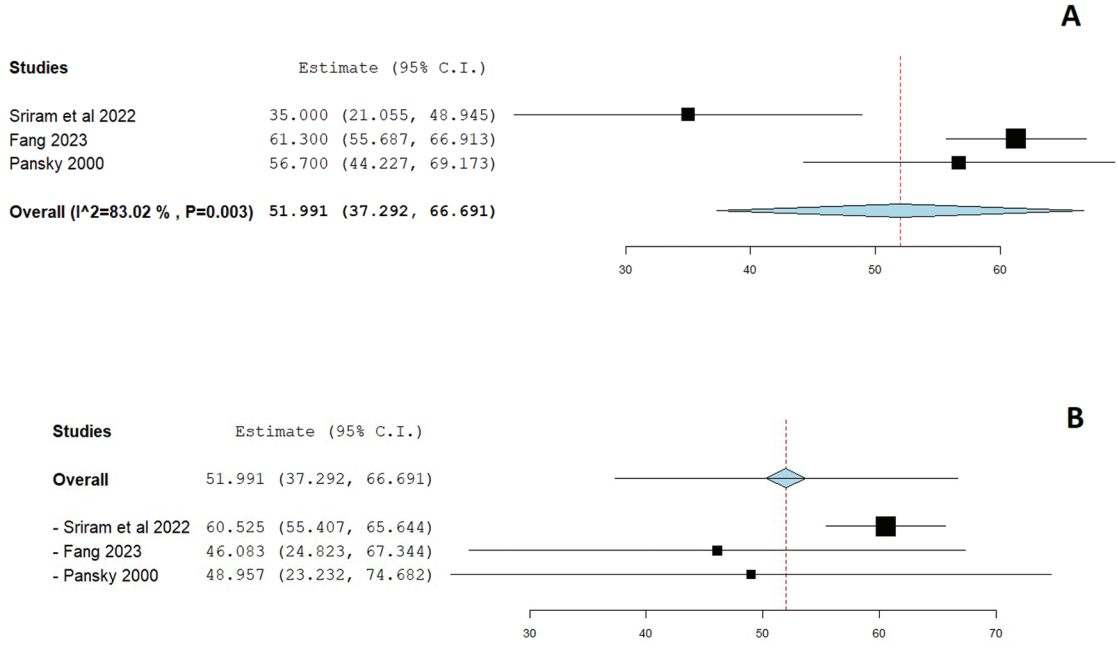

Figure 5. A) Meta-analysis of the mean time from diagnosis to surgical intervention, B) Meta-analysis of the mean time from diagnosis to surgical intervention after excluding Sriram et al.[19] in an attempt to solve heterogeneity

CI: Confidence interval

this conversion. The mean time from diagnosis to surgery was 51.9 hours. Abdominal pain was very prevalent at presentation, affecting 264 out of 324 patients. Fever was less frequently reported, with only 19 out of 324 cases experiencing it. Finally, most conservative surgeries seemed to be successful, with postoperative evidence of ovarian function showing a pooled proportion of 0.69. These findings shed light on the various aspects of ovarian torsion presentation, management, and

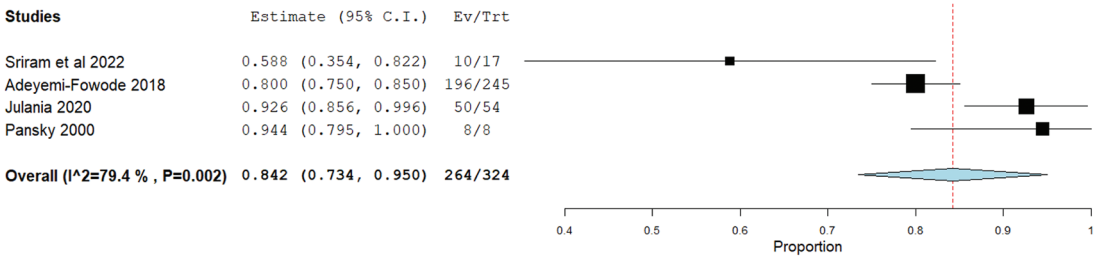

Figure 6. Meta-analysis of the presence of abdominal pain on initial presentation
CI: Confidence interval

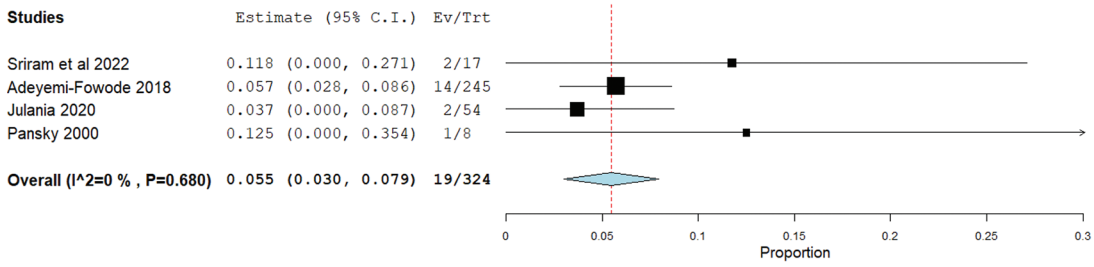

Figure 7. Meta-analysis of the presence of fever on initial presentation
CI: Confidence interval

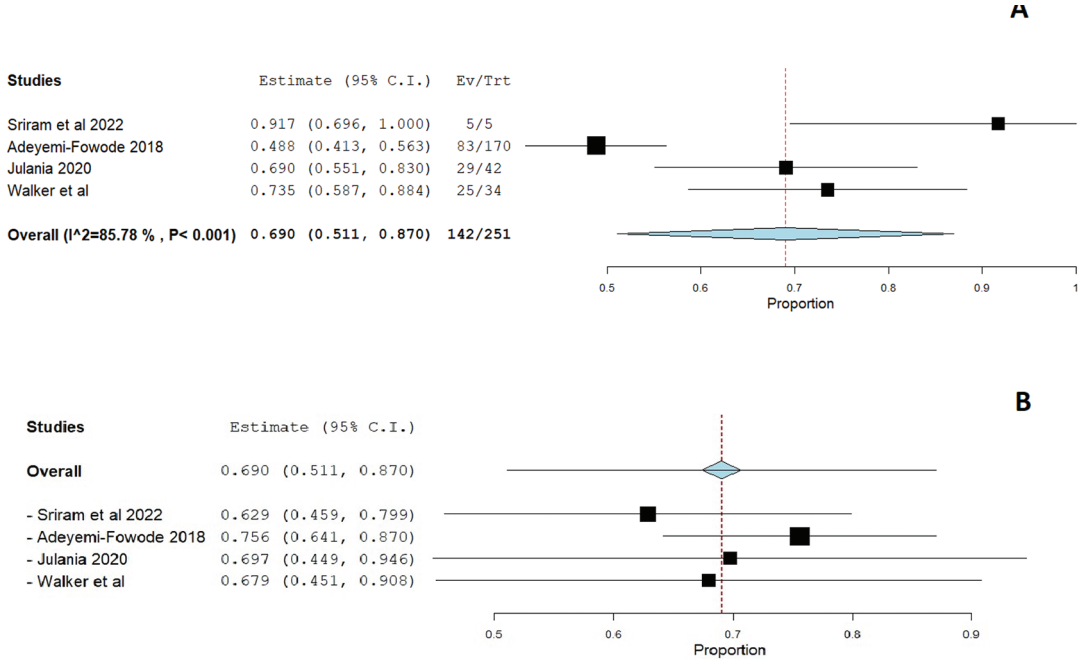

Figure 8. Meta-analysis of the presence of evidence of ovarian function on post-operative ultrasound
CI: Confidence interval

associated outcomes, thereby providing a deeper understanding of this condition.

In contrast to ovarian torsion in adults, pediatric cases rarely involve malignant masses, with a prevalence ranging from 0.4% to 1.4%[20]. Therefore, a higher rate of ovarian salvage may be possible in this population than in oophorectomy. The majority of ovarian masses in this age group are benign, with only a small percentage being malignant. Studies by Prieto et al.[21] and Ashwal et al.[22] indicated that benign masses or cysts are present in a significant proportion of pediatric ovarian torsion cases and that these were almost universally benign and usually related to hormonal influences. Additionally, ovarian neoplasms >5 cm in diameter are associated with a higher risk of torsion, albeit with lower rates of malignancy in cases involving both mass and torsion. The biggest negative of oophorectomy is the decrease in fertility through its side effects. It simply means that women who have had oophorectomy twice in their lives face difficulties conceiving naturally and also speed up the appearance of menopause. Before puberty, when both ovaries are removed there is abnormal sexual development, primary amenorrhea, and development of infertility[23]. Consequently, the interference with fertility should be considered before performing the first oophorectomy and should be avoided as long as it is possible too. Other than that, women with only one ovary also experience different levels of fertility impairment because of diminished ovarian reserve, as shown by the higher basal serum follicle stimulating hormone levels, lower anti-müllerian hormone levels, and poorer response to stimulation. Additionally, they are prone to teenage menopause and a shortened reproductive age span[24]. These features led to the variation in conservative care of ovarian twists within different age groups and children who are under 15 years were 40% conservatively treated, compared to women who are under 35 years at 20% and those who are over 50 years at 5%[9].

The growing adoption of conservative management for ovarian torsion is largely attributable to the increasing recognition of its safety and the notably high rates of ovarian viability following detorsion. Numerous studies have explored the outcomes of universally applied conservative treatment, yielding promising findings. For instance, in a medical center where all pediatric cases of ovarian torsion were conservatively managed, 92% of ovaries demonstrated good vascularity and follicular development on follow-up ultrasonography, despite initial presentation with darkened or necrotic appearance[25]. Another study by Moiety[26] detailed conservative management in 48 cases of ovarian torsion, all of which resulted in preserved ovarian tissue, as confirmed by various follow-up assessments, including Doppler ultrasonography and laparoscopy. Moreover, analysis of a large database comprising over 150,000 women treated for ovarian torsion, a quarter of whom underwent conservative management, revealed no increased risk of complications such as venous thromboembolism or sepsis[9]. These findings collectively suggest that a strategy of universally opting for conservative management of ovarian torsion could lead to high rates of ovarian function preservation with minimal complications. As supported by numerous literature studies, the ovaries that have been salvaged from torsion are reported to show a rate of follicle development that is comparable to normal ovaries. An experiment was conducted that showed within 12 weeks following detorsion of 42 torsed ovaries without follicles all specimens maintained full normal anatomy and the resumption of folliculogenesis. Different research surveyed showed that 74% of pediatric patients had visible follicles 18.7 weeks post detorsion, with a low oophorectomy rate of 2%[16,27]. The other important finding is that the anatomical appearance (sonographic morphology) of the involved ovary does not appear to be altered with or without the cystectomy at the time of detorsion[28].

Rousseau et al.[29] examined 40 cases of ovarian and adnexal torsion in children aged 16 years, excluding neonates, to analyze their clinical and therapeutic characteristics. Abdominal or pelvic pain was the primary symptom, with some children experiencing pain for several months before surgery and most presenting with associated vomiting. US diagnosed various ovarian lesions, including mature teratomas, cystadenoma, functional cysts, and malignant neoplasms. Conservative management was successful in 19 cases, but detorsion with incomplete tumor removal resulted in tumor enlargement and re-torsion in some cases. Bilateral ovarian pathology necessitated unilateral ovariectomy in some cases, whereas others underwent conservative treatment. The findings of the study advocate conservative treatment to preserve ovarian function whenever possible. Oskaylı et al.[30] investigated the diagnosis and treatment of ovarian torsion in children, focusing on conservative approaches and their long-term outcomes. The study involved 41 patients with 42 affected ovaries, most of whom presented with abdominal pain. Conservative detorsion was employed in 62% of the cases. Over time, there has been a shift toward conservative management. Follow-up for conservatively treated patients revealed mostly normal US results although some ovaries were smaller than their counterparts, yet with present ovarian follicles.

Study Limitations

This single-arm meta-analysis of retrospective studies has several limitations that warrant consideration. First, inherent biases are prevalent in retrospective studies due to the reliance on previously collected data, leading to potential selection bias and confounding variables that may not be adequately controlled for. Second, the nature of a single-arm analysis precludes a control or comparison group, which, by its nature, can invite selection bias and limit the interpretation of results. Although single-arm meta-analyses of retrospective studies can provide valuable insights into associations and trends, cautious interpretation is essential because of these inherent limitations. Our study was also limited by heterogeneity among the included studies, which most likely stemmed from the variability in

surgical practices across studies. However, in most cases, we were able to solve the heterogeneity problem using accepted techniques. Lastly, we were disappointed that we were unable to find any RCTs on this topic and were forced to utilize only observational studies to complete our analysis. The existence of RCTs on this topic would provide higher quality data and allow more insight into the details of the surgical treatment of ovarian torsion.

Conclusion

We found that recurrence occurred in 17 out of 391, with 22 out of 360 cases requiring conversion to open surgery. Furthermore, oophorectomy was necessary in 27 of 437 patients, and the mean time from symptom onset to surgery was 51.9 h. Abdominal pain was the predominant symptom, affecting 264 out of 324 cases, whereas fever was less prevalent, reported in only 19 cases. Additionally, evidence of retained ovarian function was frequently observed in postmenarche females, with a pooled proportion of 69%. These findings offer valuable insights into the presentation, management, and outcomes of ovarian torsion and contribute significantly to our understanding of this condition.

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Footnotes

Authorship Contributions

Concept: G.J.M., A.M., M.D., H.U., Design: G.J.M., B.H., H.U., Data Collection or Processing: A.M., A.A., D.H.G., B.H., K.R., M.R., M.D., H.U., Analysis or Interpretation: A.A., K.R., H.U., Literature Search: A.M., A.A., D.H.G., B.H., K.R., M.R., M.D., H.U., Writing: G.J.M., A.M., M.D, H.U.

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

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References

1. Guthrie BD, Adler MD, Powell EC. Incidence and trends of pediatric ovarian torsion hospitalizations in the United States, 2000–2006. Pediatrics. 2010;125:532-8.
2. Breech LL, Hillard PJA. Adnexal torsion in pediatric and adolescent girls. Curr Opin Obstet Gynecol. 2005;17:483-9.
3. Cohen A, Solomon N, Almog B, Cohen Y, Tsafrir Z, Rimon E, et al. Adnexal torsion in postmenopausal women: Clinical presentation and risk of ovarian malignancy. J Minim Invasive Gynecol. 2017;24:94-7.
4. Bridwell RE, Koyfman A, Long B. High-risk and low-prevalence diseases: Ovarian torsion. Am J Emerg Med. 2022;56:145-50.
5. Varras M, Tsikini A, Polyzos D, Samara C, Hadjopoulos G, Akrivis C. Uterine adnexal torsion: pathological and gray-scale ultrasonographic findings. Exp Obstet Gynecol. 2004;31:34-8.
6. Mashiach R, Melamed N, Gilad N, Ben-Shitrit G, Meizner I. Sonographic diagnosis of ovarian torsion. J Ultrasound Med. 2011;30:1205-10.
7. Singh T, Prabhakar N, Singla V, Bagga R, Khandelwal N. Magnetic resonance imaging findings in ovarian torsion. Polish J Radiol. 2018;83:564-75.
8. Scheier E. Diagnosis and management of pediatric ovarian torsion in emergency departments: Current insights. Open Access Emerg Med. 2022;283-91.
9. Mandelbaum R, Smith M, Violette C, Matsuzaki S, Matsushima K, Klar M, et al. Conservative surgery for ovarian torsion in young women: perioperative complications and national trends. BJOG An Int J Obstet Gynaecol. 2020;127:957-65.
10. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6.
11. Robbins IM, Moore TM, Blaisdell CJ, Abman SH. National heart, lung, and blood institute workshop. Circulation. 2012.
12. Wallace BC, Dahabreh IJ, Trikalinos TA, Lau J, Trow P, Schmid CH. Closing the gap between methodologists and end users: R as a computational back-end. J Stat Softw. 2012;49.
13. Julania S, Chown I, Gera S, Hunter T. Management of adnexal torsion in the pediatric and adolescent population of Western Australia's single tertiary children's hospital over the last 10 years: Retrospective study. J Minim Invasive Gynecol. 2021;28:1183-9.
14. Pansky M, Abargil A, Dreazen E, Golan A, Bukovsky I, Herman A. Conservative management of adnexal torsion in premenarchal girls. J Am Assoc Gynecol Laparosc. 2000;7:121-4.
15. Fang ME, Crain C, Baquet E, Dietrich JE. Laparoscopic salvage procedures for adnexal torsion in pediatric and adolescent patients during the COVID-19 pandemic: a retrospective cohort study. Patient Saf Surg. 2023;17:1-7.
16. Walker SK, Lal DR, Boyd KP, Sato TT. Pediatric ovarian torsion: evidence of follicular development after ovarian preservation. Surg (United States). 2018;163:547-52.
17. Agarwal P, Agarwal P, Bagdi R, Balagopal S, Ramasundaram M, Paramaswamy B. Ovarian preservation for adnexal pathology in children, current trends in laparoscopic management and our experience. J Indian Assoc Pediatr Surg. 2014;19:65-9.
18. Adeyemi-Fowode O, Lin EG, Syed F, Sangi-Haghpeykar H, Zhu H, Dietrich JE. Adnexal torsion in children and adolescents: A retrospective review of 245 cases at a single institution. J Pediatr Adolesc Gynecol. 2019;32:64-9.
19. Sriram R, Zameer MM, Vinay C, Giridhar BS. Black Ovary: Experience with oophoropexy in pediatric ovarian torsion cases and review of relevant literature. J Indian Assoc Pediatr Surg. 2020;27:558.
20. Oltmann SC, Fischer A, Barber R, Huang R, Hicks B, Garcia N. Cannot exclude torsion—a 15-year review. J Pediatr Surg. 2009;44:1212-7.
21. Prieto JM, Kling KM, Ignacio RC, Bickler SW, Fairbanks TJ, Saenz NC, et al. Premenarchal patients present differently: A twist in a typical patient presenting with ovarian torsion. J Pediatr Surg. 2019;54:2614-6.

22. Ashwal E, Hiersch L, Krissi H, Eitan R, Less S, Wiznitzer A, et al. Characteristics and management of ovarian torsion in premenarchal compared With postmenarchal patients. Obstet Gynecol. 2015;126:514-20.
23. Gold MA, Schmidt RR, Parks N, Traum RE. Bilateral ovaries and distal fallopian tubes. A case report. J Reprod Med. 1997;42:375-7.
24. Lass A. Fertility potential of women with a single ovary. Hum Reprod Update. 1999;5:546-50.
25. Parelkar SV, Mundada D, Sanghvi BV, Joshi PB, Oak SN, Kapadnis SP, al. Should the ovary be preserved during torsion? A tertiary care institute experience. J Pediatr Surg. 2014;49:465-8.
26. Moiety FMS. Adnexal torsion: Management controversy: A case series. Middle East Fertil Soc J. 2017;22:156-9.
27. Geimanaite L, Trainavicius K. Pediatric ovarian torsion: Follow-up after preservation of ovarian tissue. J Pediatr Surg. 2019;54:1453-6.
28. Murphy NC, Elborno D, Kives S, Allen LM. Postoperative ovarian morphology on ultrasound after ovarian torsion—effect of immediate surgery: A retrospective cohort study. J Pediatr Adolesc Gynecol. 2022;35:353-8.
29. Rousseau V, Massicot R, Darwish AA, Sauvat F, Emond S, Thibaud E, et al. Emergency management and conservative surgery for ovarian torsion in children: A report of 40 cases. J Pediatr Adolesc Gynecol. 2008;21:201-6.
30. Oskaylı MÇ, Durakbaşa ÇU, Maşrabacı K, Mutuş HM, Zemheri IE, Okur H. Surgical approach to ovarian torsion in children. J Pediatr Adolesc Gynecol. 2015;28:343-7.

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