Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2024.72002

Turk J Obstet Gynecol 2024;21:220-6

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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Received/Geliş Tarihi: 02.08.2024 **Accepted/Kabul Tarihi:** 29.09.2024

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

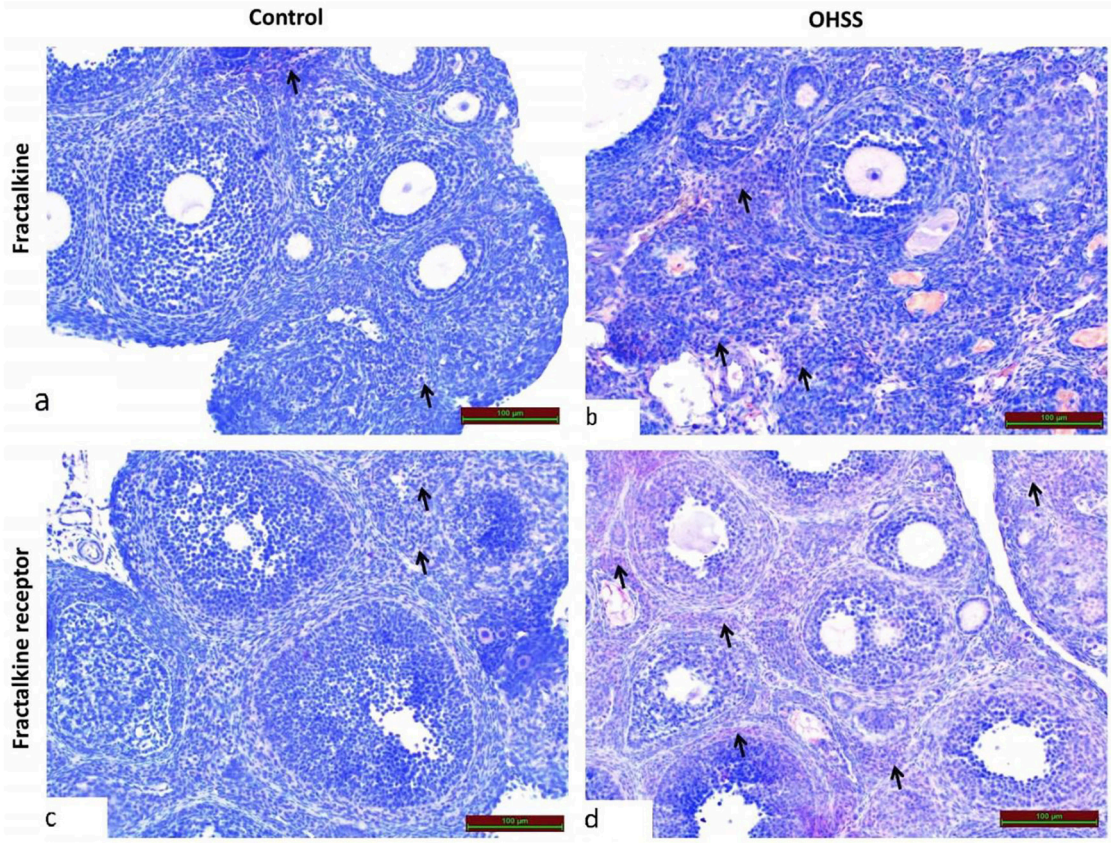

Figure 1. Immunohistochemical staining for fractalkine and fractalkine receptor in rat ovarian tissue belonging to the control and OHSS groups (a and b). Fractalkine and fractalkine receptor immunopositive cells are indicated by black arrows. A significant increase in fractalkine and fractalkine receptor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

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

Financial Disclosure: Fırat University Scientific Research Project Center (TF.23.21)

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