Assessment of Platelet-Rich Plasma (PRP) Effects on the Proliferation and Apoptosis of Cyclophosphamide-Induced Damage in Human Granulosa Cells

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Abstract
Background: Chemotherapy-induced premature ovarian failure is a prevalent issue for cancer patients, and current treatment options are associated with limitations. Platelet-rich plasma (PRP) has emerged as a safe and straightforward treatment option for various medical conditions, including infertility. The study aims to assess the impact of PRP on the follicular function of granulosa cells damaged with Cyclophosphamide (CTX).

Methods: The study evaluated the biological characteristics of Human Granulosa Cells (HGrC1) that were damaged with 4 hydroxy-cyclophosphamide (4-HC) as the active form of CTX. Then, cells were treated with different concentrations of platelet-rich plasma (PRP), and the expression of PI3K, K-RAS, BCL-2, P27, and Caspase 3 genes were analyzed. Cell viability and apoptosis were also assessed.

Results: In the MTT assay, it was observed that cells treated with CTX had a faster growth rate when exposed to high concentrations of PRP. The results of the apoptosis assay using Annexin-V and propidium iodide (PI) revealed that PRP effectively inhibited apoptosis and enhanced cell proliferation across all incubation periods (24, 48, and 72 hours). Compared to untreated cells, those treated with different concentrations of PRP showed significantly lower levels of apoptosis, indicating higher cell viability. Real-time PCR results demonstrated that PRP treatment decreased the expression of apoptotic factors P27 and caspase 3 while increasing the expression of genes that promote cell survival and proliferation, such as BCL-2, PI3K, and K-RAS. These findings are consistent with previous research, which suggests that the growth factors present in platelets have anti-apoptotic effects and promote high rates of cell proliferation.

Conclusion: The study suggests that PRP therapy may have potential benefits in promoting follicular growth and repairing ovarian tissue. However, further research is necessary to establish novel therapeutic strategies based on PRP therapy for infertility treatment, especially in elevated temperature and humidity.

Introduction
Reproductive problems are common among cancer patients, particularly women under the age of 40 who undergo chemotherapy or radiation therapy. These injuries are known as premature ovarian failure/insufficiency (POF/POI) and are caused by the toxicity of ovarian germ cells.1,2 Cyclophosphamide (CTX), an alkylating agent, is widely used to treat malignant and nonmalignant diseases that carry the highest risk of POF/POI.3,4 Studies conducted in vitro and in vivo have shown that these agents induce premature apoptosis of oocytes and granulosa cells, leading to the loss of follicles and reduced likelihood of successful ovulation.1 Women with POF/POI experience symptoms similar to menopause, such as hot flashes, vaginal dryness,
and increased risk of osteoporosis, which can significantly impact their quality of life.\(^5\)

It is unfortunate that there is no effective cure for POF/POI, and reversing the condition is a difficult task. Various methods have been used in recent years to preserve and restore ovarian function in women who receive chemotherapy. These methods include cryopreservation techniques, hormone replacement therapy, Gonadotropin-releasingagonist ( Gn-Rha) treatments, assisted reproductive technology (ART), and stem cell transplantation.\(^6-8\)

However, each method has its pros and cons, and the best method depends on several factors, such as the type and stage of cancer, the age and fertility status of the patient, and the availability and cost of the technique. Hormone replacement therapy, for instance, can increase the risk of breast cancer and other diseases if used for a long time.\(^9\)

ART may be unaffordable and pose ethical issues with egg donation and embryo transfer. One of the main issues is how the age of the egg donor affects the rights and interests of the child born from ART.\(^10,11\) Cryopreservation techniques require invasive procedures and have low success rates.

Gn-Rha treatments have controversial efficacy and may cause side effects, and have not been effective in treating severe premature ovarian failure.\(^11\) Also, stem cells from adult tissues (bone marrow, liver, fat, and skin) have limitations in supply and ability to multiply/differentiate with age\(^12\). Moreover, the transplantation of stem cells can lead to the development of infections and tumors, as well as secondary damage to the patient's tissues.\(^13-15\)

Platelet-rich plasma (PRP) is a simple treatment method that is widely used to treat various medical disorders, particularly in orthopedic and sports medicine. This treatment not only improves musculoskeletal conditions like arthritis, tendinitis, ligament sprains, and tears but has also been highly effective in treating athletic injuries by providing fast pain relief and enabling a speedy return to normal activities. Furthermore, it has shown promise in the areas of aging and rejuvenation.\(^16\)

PRP therapy is being studied for use in barren women and women with diminished ovarian reserve, with PRP injected into the ovary under ultrasound guidance in a procedure similar to ovum retrieval in IVF.\(^17\)

PRP has a high concentration of growth factors, including PDGF, TGF-\(\beta\), EGF, VEGF, IGF-I, b-FGF, and pro-inflammatory cytokines.\(^18,19\) These growth factors have been found to regulate the proliferation and differentiation of endometrial cells and have been demonstrated to increase endometrial thickness in patients undergoing IVF treatment.\(^20-23\)

Based on extensive clinical experience, PRP is considered a safe alternative and is more readily accessible compared to stem cells.\(^24\)

In reproductive medicine, PRP has been used to repair damaged endometrium. This study used CTX to damage human granulosa cells (HGrC1) as an in vitro model of human ovarian failure. This study aims to evaluate the effect of PRP on the inhibition and proliferation of granulosa cells damaged with CTX on follicular function through measures of cell viability, cellular proliferation, and gene expression.

**Methods**

**Cell line and cell culture**

HGrC1 is a human non-luteinized granulosa cell line that was a gift from Dr. Yashar Esmaeilian, Ph.D. Research Center for Translational Drugs, KoÇ University, Istanbul, Turkey. HGrC1 cells are derived from GCs of antral follicles. HGrC1 cells grew as an adherent monolayer until confluence and multi-layered after reaching confluence. The estimated doubling time of the cells was approximately 40 h in vitro.\(^25\)

HGrC1 cell lines were routinely cultured in DMEM-F12 + 2 mM glutamine + 10% FBS in a 37°C incubator with a humidified mixture of 5% CO\(_2\)+95% air. Cells from passage 19 were used for the experiments reported herein (Figure 1).

**Cytotoxicity determination of 4-hydroxy cyclophosphamide (4-HC)**

CTX is an inactive pro-drug that requires hepatic cytochrome P450 enzymes (CYP2A6, -2B6, -2C8, -2C9, and -3A4) to metabolize it into its active form, 4-hydroxy cyclophosphamide (4-HC).\(^26\) In this in vitro experiments, 4-HC (provided by Dr. Yashar Esmaeilian, Ph.D. Research Center for Translational Drugs, KoÇ University, Istanbul, Turkey) used instead of CTX. To determine the IC\(_{50}\) of 4-hydroxy cyclophosphamide, 5×10\(^4\) HGrC1 cells seeded into 96-well plates (sorf scp-11-096) with DMEM-F12 (Gibco 32500-035) + 10% FBS (Gibco). After incubating the plates at 37°C, 5% CO\(_2\), and 95% humidity for 24 hours, the drug was added to the wells at concentrations of 1, 5, 10, 25, 50, 75, and 100 μg/mL in DMEM-F12 + 10% FBS. The control groups devoid of pharmaceutical agents comprised solely of cells and cultur media. All controls and tests were performed in triplicate. The plates were then incubated for 12, 24, and 48 hours at 37°C, 5% CO\(_2\), and 95% humidity. Each well contained a final volume of 200 μl. After incubation, the media was removed from the wells and added 180 μL of fresh media without FBS and 20 μl MTT (Sigma M5655) (5 mg/mL) to each well. The plate was incubated at 37°C with 5% CO\(_2\), and 95% humidity for four hours. Following incubation, the media containing MTT was removed from wells and added 200 μl DMSO (Merck) to each well. The plate covered with foil to avoid direct light and shook it in the orbital shaker for 15 minutes at 37°C.

**Figure 1.** HGrC1 cell line that cultured in DMEM-F12 medium with 10% FBS (The second day of culture).
Finally, the absorbance of the plate at 590 nm measured using a microtitre plate reader (Awareness Technology Inc). After calculating cell viability, the IC₅₀ dosage of 4-HC estimated using GraphPad Prism 8 software.

**Cell viability test**

Cell viability was assessed using the MTT assay, a widely used method to measure cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. This assay utilizes a yellow tetrazolium salt (MTT) that turns into purple formazan crystals by metabolically active cells through a colorimetric reaction. The viable cells contain specific enzymes that facilitate this reduction process. The insoluble formazan crystals are then dissolved using dimethyl-sulfoxide (DMSO), forming a colored solution that can be quantified by measuring absorbance at 500-600 nanometers using a multi-well spectrophotometer.²⁷ In this experiment, cells were seeded in 96-well plates at a density of 5×10³ cells/well and incubated at 37°C with 5% CO₂ and 95% humidity in DMEM-F12 medium supplemented with 2 mM glutamine and 10% FBS for 24 hours. Subsequently, the culture medium was replaced with DMEM-F12 medium containing 10% FBS and 4-HC at the IC₅₀ dosage (22 μg/mL) in the test wells for 12 hours. The untreated negative controls consisted of cells incubated in DMEM-F12 medium with 10% FBS, while the untreated plasma controls included cells incubated in DMEM-F12 medium with 10% PPP (platelet-poor plasma). After 12 hours, the drug and medium were removed from the test wells, and PRP was added at concentrations of 10%, 30%, and 50%. The positive controls included cells incubated in DMEM-F12 medium with 10% FBS and 4-HC without any added PRP. The cells were then incubated for 24, 48, and 72 hours at 37°C with 5% CO₂ and 95% humidity. At the end of each incubation period, the culture medium was removed, and a mixture of 20 μL MTT solution (5 mg/mL) and 180 μL serum-free culture medium was added to each well. The cells were further incubated at 37°C with 5% CO₂ and 95% humidity for 4 hours. After the incubation, the MTT-containing medium was removed, and 200 μL DMSO was added to each well. The plate was covered with foil to protect it from direct light exposure and shaken in an orbital shaker for 15 minutes at 37°C. Finally, the absorbance of the plate was measured at 590 nm using a microtitre plate reader.

**PRP preparation**

The creation of platelet-rich plasma (PRP) is a secure procedure that involves the collection of blood, PRP preparation, and injection into the affected region. Various techniques exist for preparing PRP, with differences in centrifugation velocity and timing. A blood sample was collected from a volunteer donor with no blood disorders. The following steps illustrate a typical method for creating PRP:

1. A sterile tube containing 3.2% sodium citrate anticoagulant is used to obtain venous blood (15-50 mL) from the volunteer's antecubital vein in a 1:9 ratio.
2. To avoid platelet activation during centrifugation, it is recommended to maintain a temperature of 21-24°C during the processing.
3. The blood is centrifuged at 300 g for 10 minutes.
4. The blood is separated into three layers: an upper layer containing platelets and a small number of white blood cells, a thin intermediate layer (the buffy coat) that is abundant in white blood cells, and a bottom layer containing red blood cells.
5. The upper layer is transferred to an empty sterile tube. The plasma is centrifuged again at 800 g for 10 minutes to create soft pellets of platelets at the bottom of the tube.
6. The top two-thirds of the plasma is discarded because it is poor in platelets.
7. Pellets are homogenized in the lower third (5 mL) of the plasma to create PRP.
8. The PRP is now ready for activation. Approximately 30 mL of venous blood yields 3-5 mL of PRP.
9. For every 10 mL of platelet concentrate, 1 mL of thrombin and 1 mL of calcium gluconate 10% are added to the 15 mL falcon tube.
10. The tubes are incubated in a 37°C incubator for 2 hours.
11. After platelet gel formation, the tubes are centrifuged at 1400 g for 10 minutes.
12. The supernatant contains all growth factors released from platelet granules.
13. The growth factors are immediately frozen at -80°C in a mechanical refrigerator and are kept at this temperature until they are used.

**Apoptosis assay**

Cell apoptosis was assessed using Annexin V-FITC and PI staining with the Exbio apoptosis kit. HGrC1 cells were seeded in 24-well plates (Sorfa SCP-11-024) at a density of 5×10³ cells/well and incubated at 37°C with 5% CO₂ and 95% humidity in DMEM-F12 medium supplemented with 2 mM glutamine and 10% FBS for 24 hours. The culture medium was then replaced with DMEM-F12 medium containing 10% FBS and 4-HC at the IC50 dosage (22 μg/mL) for 12 hours. The negative controls consisted of cells incubated in DMEM-F12 medium with 10% FBS, without any added drug. After 12 hours, the drug and medium were removed from the test wells, and PRP was added at concentrations of 10%, 30%, and 50%. The positive controls included cells incubated in DMEM-F12 medium with 10% FBS and 4-HC without any added PRP. The cells were then incubated for 24, 48, and 72 hours at 37°C with 5% CO₂ and 95% humidity. At the end of each incubation period, the culture medium was removed, and a mixture of 20 μL MTT solution (5 mg/mL) and 180 μL serum-free culture medium was added to each well. The cells were further incubated at 37°C with 5% CO₂ and 95% humidity for 4 hours. After the incubation, the MTT-containing medium was removed, and 200 μL DMSO was added to each well. The plate was covered with foil to protect it from direct light exposure and shaken in an orbital shaker for 15 minutes at 37°C. Finally, the absorbance of the plate was measured at 590 nm using a microtitre plate reader.
binding buffer (100 μL) and evaluated using a FACS Calibur flow cytometer. The untreated unstained cells were used as the auto-fluorescence control group.

**RNA extraction and real-time PCR analysis**

To extract RNA and perform real-time PCR analysis, HGrC1 cells were seeded at a density of 3×10^5 cells/well in a 6-well plate following the protocol of the previous section. After 24, 48, and 72 hours of incubation, the cells were harvested and washed twice with PBS. RNA was then extracted using the High-Quality Nucleic Acid Extraction Kit (Payesh Gene, IR), and samples were treated with DNase to eliminate DNA contamination. The concentration and purity of the extracted RNA were measured using NanoDrop Spectrophotometry (NanoDrop™ One/One C microvolume-Thermo Scientific), and the quality and integrity of the total RNA were verified by electrophoresis on a 1.5% (w/v) agarose gel.

For real-time PCR amplification, 200 ng of RNA was reverse transcribed using the Easy cDNA Synthesis Kit (Applied Biosciences). Oligonucleotide primers were designed using Primer3, an open-source online tool available on NCBI, and they were designed across an exon-exon junction to eliminate genomic DNA amplification. The primer sequences can be found in Table 1. Real-time PCR amplification was carried out using SYBR Green Master Mix (Applied Biosciences) on the LightCycler 96 (Roche) Real-Time PCR System. All analyses were performed in triplicate, and the relative amount of the target was normalized using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

The reaction conditions included 10 µl of 2X PCR Master Mix, primer pair mix 5 pmol/µL (1 µL of each primer), 50 ng of template cDNA (2 µL), and nuclease-free water (6 µL). The total reaction volume was 20 µL. The cycling conditions were as follows: initial denaturation of samples was done for 10 min, and the run proceeded at 95°C for 10 s and 40 cycles. The annealing/extension stage was completed for 30 s at 58°C. Melting curve analysis was conducted to analyze the purity of products after real-time PCR.

The efficiency of each primer amplification was monitored via serial dilution. Additional dissociation curve analysis was performed, and in all cases, showed a single peak. To standardize the results and eliminate variation in cDNA quantity, the expression of each gene was normalized to the internal control GAPDH reference gene. Data were analyzed by using the comparative Ct method, where Ct is the cycle number at which fluorescence first exceeds the threshold. The Δ cycle threshold (ΔCt) values from each sample were obtained by subtracting the reference gene values from the Ct. For each experimental sample, relative expression levels were determined by the 2^−ΔΔCt method.

**Statistical analysis**

The data were analyzed using GraphPad Prism V8.2.1 (GraphPad Software Inc., San Diego, CA). The statistical differences between the groups were tested using one-way ANOVA followed by a least-significant-difference test. The data are presented as mean ± standard deviation, and a p-value <0.05 is considered significant. * P<0.05; ** P<0.01; n.s., non-significant.

**Results**

**Cytotoxicity determination of CTX (4-HC)**

The viability of HGrC1 cells in the presence of different concentrations of 4-HC was assessed using the MTT assay, and the results are presented in Figure 2. Based on the obtained data, the half-maximal inhibitory concentration (IC_{50}) of 4-HC was determined to be 22 μg/mL after a 12-hour incubation period. No significant difference was observed in the 24 and 48-hour incubation periods. The IC_{50} dosages of 4-HC were calculated using GraphPad Prism software (V. 8.2.1).

**Evaluation of cell viability and cell proliferation**

The MTT assay results indicated that cyclophosphamide-treated cells exhibited a faster growth rate when exposed to high concentrations of PRP. However, cells that did not receive PRP were unable to grow in the presence of 10% FBS in the culture medium. After 72 hours of incubation, cells treated with PRP at concentrations of 30% and 50% exhibited the highest growth rates, which were similar to the negative control group. To eliminate the effect of platelet-poor plasma on cell growth, untreated control cells + 10% PPP were used, and no significant growth was observed (Figure 3).

**Evaluation of apoptosis inhibition of PRP**

Annexin-V, a fluorescent agent, was used to stain apoptotic

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
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<tbody>
<tr>
<td>PIK3CA</td>
<td>AGAGATGAAGTAGCCCAGATGT</td>
<td>GAAACGCAAACCTCGAACC</td>
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<tr>
<td>BCL2</td>
<td>TGTGTTGAGAGCGTCAAC</td>
<td>GGGCGTACAGTTCCACAA</td>
</tr>
<tr>
<td>CASP3</td>
<td>GTGGAAATTGTAGGGTGTTT</td>
<td>ACTTCTCAAGCAGTCCTCT</td>
</tr>
<tr>
<td>K-RAS</td>
<td>AGCAAGAGAAAGAGAACAGAGTG</td>
<td>ATTTTCACACAGCCAGGAGT</td>
</tr>
<tr>
<td>CDKN1B(P27)</td>
<td>TCTGAGGACACGCATTGG</td>
<td>CTCTGTTGTCTGGCTTTTG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>TCGGAGTCACAGCGATTGG</td>
<td>TGGGTTGGAATCATATTGGAACA</td>
</tr>
</tbody>
</table>

**Table 1. Primers used for real-time PCR analysis.**
Figure 2. CTX IC\textsubscript{50} determination, *P<0.05; **P<0.01.

Figure 3. The growth rate of cells treated with cyclophosphamide in the vicinity of different PRP concentrations and various incubation times. The results showed no significant difference between the positive control group (CTX+FBS 10%) and the 10% PRP group, but significant differences were observed between the 30% and 50% PRP groups, which increased over time. The greatest amount of change was observed in the 50% PRP group at 72 hours of incubation. * P<0.05; ** P<0.01; n.s., non-significant.
cells, while propidium iodide (PI) was used to stain the nucleus of late apoptotic and necrotic cells. The impact of PRP on the inhibition of apoptosis and increased proliferation was observed. In all incubation periods (24, 48, and 72 hours), cells treated with various concentrations of PRP showed significantly lower levels of apoptosis (higher cell viability) compared to untreated cells. The most significant reduction in apoptosis was observed in cells treated with 30% and 50% PRP after 72 hours of incubation (Figure 4).

Investigation of proliferation and apoptosis inhibitory effects of PRP by real-time PCR

A real-time PCR test was conducted to investigate the apoptosis inhibition and proliferation pathway thoroughly. Treatment of granulosa cells with 4-HC in the control group (CTX+FBS10%) resulted in the upregulation of the expression of the cell cycle regulatory factor P27 and the pro-apoptotic gene caspase-3. The expression of genes involved in cell survival and proliferation, including BCL-2, PI3K, and K-RAS, was also downregulated. After being

![Figure 4. Flow cytometry results of cells (HGrC1) damaged by cyclophosphamide (CTX) and treated with different concentrations of PRP for 24, 48 and 72 h. Cell apoptosis were investigated using annexin V-FITC and PI.](image-url)
Figure 5. Real-time PCR: investigation of different genes expression. The results showed that PRP significantly upregulated the expression of BCL2, PI3K, and K-RAS, which are genes associated with proliferation. Conversely, PRP reduced the expression of caspase 3 and P27 genes, which are involved in apoptosis. Statistical analysis indicated that the results were significant (* P<0.05; ** P<0.01), while non-significant.
treated with PRP at various concentrations (10%, 30%, and 50%) and durations (24, 48, and 72 hours), there was a significant downregulation in the expression of \( P27 \) and \( \text{caspase-3} \). In contrast, the expression of \( \text{Bcl-2} \), \( \text{PI3K} \), and \( \text{K-RAS} \) was significantly upregulated and very close to the untreated control group (Figure 5).

**Discussion**

Cyclophosphamide (CTX) and other alkylating agents are frequently used to increase the chances of long-term survival in cancer patients. High dosages of chemotherapeutic agents can cause injuries such as nephrotoxicity, ototoxicity, and reproductive toxicity. Previous studies have reported that these agents can cause POI in clinical and nonclinical settings. Exposure of granulosa cells to chemotherapeutic agents can result in growth arrest, DNA damage, and increased apoptosis. CTX treatment can lead to temporary amenorrhea or permanent ovarian failure in women; it can also damage primordial and primary ovarian follicles, as well as preantral and antral follicles. Cyclophosphamide targets oocytes for apoptotic destruction in primordial and small primary follicles while inducing granulosa cell apoptosis followed by oocyte death in larger follicles. Granulosa cells, the largest group in the ovary, synthesize estrogen and progesterone and play a crucial role in follicle development, ovulation, and atresia.

Therefore, preserving fertility and gonadal function is a significant concern for chemotherapy patients of childbearing age. To mitigate these side effects, numerous studies have been conducted, leading to the development of protective adjuvants. PRP has gained significant attention, particularly for its therapeutic potential. The PRP is a blood-derived product that contains a high concentration of growth factors. Autologous cellular therapies that utilize PRP are emerging as potential adjunctive treatments in various regenerative medicine plans. Currently, PRP therapies are suitable treatment options with clinical benefits and encouraging patient outcomes have been reported.

In this study, we aimed to assess the impact of PRP on the function of granulosa cells damaged with CTX. Our findings indicate that PRP treatment effectively decreased the expression of apoptosis-related factors, \( P27 \) and \( \text{caspase-3} \), while increasing the expression of genes that promote cell survival and proliferation, such as \( \text{BCL-2} \), \( \text{PI3K} \), and \( \text{K-RAS} \). Based on the findings, it can be inferred that PRP therapy has the potential to promote follicular growth and repair ovarian tissue. These results are consistent with previous studies that have demonstrated the protective effects of PRP on ovarian function and fertility in various animal models and clinical settings. While the mechanisms by which PRP exerts its beneficial effects are not yet completely understood, its growth factors likely play a key role in modulating the cellular processes of granulosa cells. For instance, Dehghani et al. reported that cyclophosphamide therapy decreased cortical volume, the number of pre-antral follicles, and the diameters of follicular cells and oocytes in the antral follicle. However, treatment with PRP was found to prevent these negative effects. Indeed, PRP improves ovarian cortex volume, pre-antral follicle number, and antral follicle diameter in cyclophosphamide-induced ovarian failure in female rats. Likewise, in 4-vinyl-cyclohexene dioxide (VCD)-induced POI Wistar albino rats’ model, intra-ovarian injection of PRP promoted rejuvenation and restored ovulation rate and GCs frequency via angiogenesis modulation finally. In addition, in patients with poor ovarian response, autologous PRP local administration resulted in a significant increase in the number of oocytes and embryos, as well as estradiol levels. Several recent reports have clarified that the healing regenerative capabilities of PRP depend on the various growth factors, including TGF-β, insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and also hepatocyte growth factor (HGF). Furthermore, PRP comprises a protein known as Growth Differentiation Factor 9 (GDF-9) which originates from oocytes. GDF-9 plays a vital role in the oocytes’ maturation process and enables an increase in the number of primary and preantral follicles. Importantly, premature ovarian failure may result from GDF-9 gene mutation. PRP therapy has been shown to induce postnatal oogenesis, which facilitates the establishment of new primordial follicles in the ovaries of menopausal women through the stimulation of GnRH receptors. Meanwhile, local administration of PRP efficiently enhanced markers of the low ovarian reserve before ART in the 83 participants suffering from low ovarian reserve. It is interesting to note that treatment with PRP was found to be associated with a significant improvement in pregnancy rates, both during the biochemical and clinical stages. Several studies suggest that PRP treatment can enhance neoangiogenesis in the menopausal ovary and promote the development of ovarian stem cells into mature follicles. PRP may also have an inhibitory effect on the expression of \( P27 \), a protein that is known to act as a negative regulator of cell cycle progression and is considered a marker of ovarian aging. Conversely, PRP has been shown to enhance the expression of \( \text{K-RAS} \), a proto-oncogene that mediates the signaling of various growth factors and cytokines in ovarian cells. These findings are consistent with a previous research conducted by Huang et al. to investigate the effects of mobilized PBMCs combined with PRP on the apoptosis of granulosa cells in POI rats. The results indicate that this combination therapy was successful in reducing the apoptosis of granulosa cells. The plausible mechanism underlying this effect is the ability of PRP to enhance the expression of \( \text{BCL-2} \), which is a potent anti-apoptotic protein that can prevent mitochondrial dysfunction and oxidative stress in granulosa cells. In addition, PRP may stimulate the \( \text{PI3K/AKT} \) pathway, which is known to regulate cell survival, proliferation, and differentiation.
in ovarian follicles. The outcomes of our research have important implications for the development of novel therapeutic strategies based on PRP therapy for treating infertility caused by chemotherapy-induced ovarian failure. PRP therapy presents a safe, simple, and effective alternative or adjunctive treatment option for patients who wish to preserve or restore their fertility and ovarian function. However, our study also has some limitations and challenges that need to be addressed in future research. First, our study was performed on an in vitro model using a human granulosa cell line, which may not fully reflect the complex interactions and dynamics of ovarian follicles in vivo. Therefore, further studies are needed to validate our findings in animal models and clinical trials. Second, our study used a single dose and time point of CTX exposure to induce granulosa cell damage, which may not represent the realistic scenario of chemotherapy-induced ovarian failure. Therefore, further studies are needed to investigate the effects of PRP therapy on different doses and time points of CTX exposure. Third, our study did not measure the levels or activities of the growth factors present in PRP or their receptors on granulosa cells, which may limit our understanding of the mechanisms involved in PRP therapy. Therefore, further studies are needed to quantify and characterize the growth factors in PRP and their interactions with granulosa cells.

Conclusion

Although this study was performed on an in vitro model, our data suggest that PRP strongly affects granulosa cells determining a high proliferation rate and upregulation of genes that play an essential role in reproduction. The mechanisms by which PRP upregulates these genes remains unclear. However, it is conceivable that its growth factors are involved in these mechanisms. The results of the current study reveal the effectiveness of PRP in decreasing gene expression of the apoptotic factors such as P27 and caspase 3, while increasing the expression of genes like BCL-2, PI3K, and K- RAS for cell survival and proliferation. The study indicates that growth factors released from platelets have apoptosis-inhibiting effects and high cell proliferation, consistent with the findings of previous research. This superiority is consistent with the results of previous studies on this effect. It can be concluded that this positive effect may also be effective in follicular growth and in improving ovarian tissue repair.

Ethical Issues

This study was approved by the Ethical Committee of Tabriz University of Medical Sciences, Tabriz. IRCT code: (IR.TBZMED.REC.1398.079).

Acknowledgements

The authors of this work would like to express their gratitude to their colleagues, particularly Dr. Yashar Esmaeilian from the Research Center for Translational Drugs at KOÇ University in Istanbul, Turkey, for providing the gifted cell line (HGrC1) and 4-HC. We would also like to acknowledge the laboratory personnel of the Faculty of Advanced Medical Sciences for their assistance. This study was funded by the Faculty of Advanced Medical Sciences of Tabriz University of Medical Sciences (grant no. 62657) Tabriz University of Medical Sciences, Tabriz, Iran.

Author Contributions


Conflict of Interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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