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The role of clinical and immunological factors in the outcomes of in vitro fertilization procedure in women

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The objective: **to determine the features of clinical and immunological factors in women undergoing in vitro fertilization (IVF) procedure and to determine a relationship between these parameters and the procedure outcomes.**
Materials and methods**. In the period from 2020 to 2022, 131 patients were examined at the Reproductive Department of the Caspian International Hospital. Of these, 103 (78.6%) are aged 19-35 years, 28 (21.4%) are aged 36-50. Patients were grouped and analyzed based on: IVF success (presence or absence of fertilization), pregnancy (yes, no), and infertility factors.**
Markers of peripheral blood mononuclear cells (CD16/56 and HLA-DR) were determined in 50 women. The cytokines – interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and interferon-ɣ (IFN-ɣ) were studied in blood serum of 84 patients. All samples were taken at the day of oocyte pick-up.
Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, and minimum and maximum), Student-t test and Mann–Whitney U test (for comparisons between two groups) were used while evaluating the study data. Statistical significance was accepted as p<0.05.
Results**. Female infertility occurred in 49 (37.4%) patients, 82 (62.6%) patients were fertile. There was no significant difference in CD16/56 and HLA-DR levels depending on pregnancy status, IVF outcomes and infertility factors (p>0.05). The levels of IL-1β and IFN-ɣ differed in the blood serum of patients with and without pregnancy (p=0.041 and p=0.037, respectively). Analysis of groups based on infertility factors showed that there were not significant differences (p>0.05) in the levels of CD16/56, HLA-DR, IL-1β, TNF-α and IFN-ɣ between groups.**
Conclusions**. Our study showed that there is an association between serum levels of IL–1β and IFN-y and successful pregnancy in the IVF procedure. We did not reveal the other relationships between clinical and immunological parameters in women with IVF procedure.**
Keywords*: in vitro fertilization, pregnancy, infertility, markers of peripheral blood mononuclear cells, cytokines.*

Роль клініко-імунологічних факторів у результатах процедури екстракорпорального запліднення у жінок

А. Ф. Сафарова

Мета дослідження: вивчення клініко-імунологічних параметрів у жінок, яким проведено екстракорпоральне запліднення (ЕКЗ), та встановлення зв'язку між цими параметрами та наслідками процедури.
Матеріали та методи. У період з 2020 до 2022 року у Репродуктивному відділенні Caspian International Hospital загалом було обстежено 131 пацієнтку. З них 103 (78,6%) жінки були у віці 19–35 років, 28 (21,4%) – 36–50 років. Дані пацієнток були згруповані та проаналізовані на підставі: результату ЕКЗ (наявність та відсутність запліднення), настання вагітності (наявність або відсутність) і факторів безплідності.
Маркери мононуклеарних клітин периферійної крові (CD16/56 та HLA-DR) були кількісно визначені у 50 жінок. У сироватці крові 84 пацієнток досліджували концентрацію цитокінів – інтерлейкіну-1β (ІЛ-1β), фактора некрозу пухлин-α (ФНП-α) та інтерферону-ɣ (ІФН-ɣ). Усі зразки було взято у день забору ооцитів.
Використовували описові статистичні методи (середнє значення, стандартне відхилення, медіана, частота, відсоток, мінімум і максимум), критерій Стьюдента та U-критерій Манна–Уітні (для порівняння між двома групами). Ці показники вважали статистично значущими за p<0,05.
Результати. Жіночу безплідність діагностували у 49 (37,4%) пацієнток, 82 (62,6%) жінки були фертильними. Достовірної різниці у рівнях CD16/56 та HLA-DR залежно від статусу вагітності, успіху ЕКЗ та факторів безплідності не відзначено (p>0,05). Показники ІЛ-1β та ІФН-ɣ відрізнялися у сироватці крові пацієнток з вагітністю та без вагітності (p=0,041 та p=0,037 відповідно). Достовірних відмінностей (p>0,05) у рівнях CD16/56, HLA-DR, ІЛ-1β, ФНП-α та ІФН-ɣ між групами за факторами безплідності також не виявлено.
Висновки. Установлено, що існує зв'язок між сироватковими рівнями ІЛ-1β та ІФН-ɣ та успішним перебігом вагітності за використання процедури ЕКЗ. Інших взаємозв'язків між клінічними та імунологічними показниками у жінок, які перенесли процедуру ЕКЗ, не виявлено.
Ключові слова: *екстракорпоральне запліднення, вагітність, безпліддя, маркери мононуклеарних клітин периферійної крові, цитокіни.*

Assisted reproductive technology (ART), as defined by the American Center for Disease Control, are any fertility-related treatments that manipulate eggs or embryos. Recently, the use of ART and the number of fertility clinics providing ART services have increased steadily [1–3]. At least 5 million of infants have been born as a result of ART and in some countries the proportion of infants born after ART now exceeds 5% [4].

Among the various ART methods, in vitro fertilization (IVF) technology is the most widely used in the treatment of infertility worldwide [1, 5].

ARTs are most frequently performed secondary to infertility. In patients with tubal factor infertility, IVF directly bypasses the fallopian tubes. Other infertility etiologies in which IVF is employed include male factor infertility, diminished ovarian reserve, ovarian failure (with donor eggs), ovulatory dysfunction, and unexplained infertility [6].

However, IVF is characterized by lower live birth rate (39-49% according to various sources) [1, 7].

The success of IVF procedure and outcomes depend on various factors: condition of women's general health, the features and diseases of reproductive system, hormonal status, etc. In addition, IVF involves various steps, including controlled ovarian stimulation, oocyte retrieval, fertilization, embryo culture, and embryo transfer [8]. Some authors noted, that pregnancy and delivery after ARTs were characterized by higher rates of preterm birth, caesarean section, assisted vaginal delivery, and massive obstetric bleeding [9]. Successful and consistent outcomes in human in vitro fertilization (IVF) can be readily achieved by optimization of each procedure associated with the collection and processing of gametes and transfer of healthy embryos [10].

One of the parameters influencing the success of the IVF procedure is the state of the immune system, which is actively involved in the regulation of ovarian functions, controlling steroidogenesis, folliculogenesis, ovulation, formation and atresia of the corpus luteum. [11, 12]

So, some cytokines support the embryonic development includes promotion of implantation and protection of blastomeres from cell stress and apoptosis. The correlation between embryo quality and the concentration of specific cytokines is considered to be an important predictor of successful treatment. On the other hand, deviations of some peripheral blood mononuclear cells (PBMCs) parameters beyond the normal range (increased expression of CD56, CD158a in T-lymphocytes, a decrease in the level of CD4 in T-lymphocytes, increased expression of HLA-DR) in a number of studies are considered as immune deviations that potentially predict IVF failure [13, 14].

Some authors noted that dysregulation of immune cells and cytokine profiles may play an important role in the competence of the oocyte and the development of the embryo [15]. Abnormal female immune response, which relates to CD4 + T-cell-related cytokines expression, especially IL-10 and IL-23, is one of the potential causes of unexplained infertility [16].

Thus, the study of changes in clinical and immunological factors is of interest in terms of assessing their role in success and predicting the outcomes of the IVF procedure.

The objective: to determine the features of clinical and immunological factors in women undergoing IVF procedure.

MATERIALS AND METHODS

A total of 131 patients, undergoing IVF procedure, with diagnosis such as Infertility, N.97; Endometriosis, N.80; Congenital malformations of ovaries, fallopian tubes and broad ligaments, Q.50 (ICD-10), were included in the study between 2020 and 2022 in the Reproductive Department of Caspian International Hospital. The diagnosis' were confirmed according to NICE recommendations [17]. Patients were grouped and analyzed based on following parameters from IVF procedure outcomes: IVF outcome (positive or negative); pregnancy (positive or negative); infertility factors. In addition to general clinical studies (physical examination, gynecological examination, ultrasound), hysterosalpingography was performed to determine the condition of tubes.

IVF was performed according to standard clinical procedures. Briefly, ovulation induction was performed through injection of recombinant-follicle stimulating hormone (FSH) (Daily 225 IU) by starting from the third day of the menstrual cycle. Follicular growth and maturation were followed by serial vaginal sonography (Samsung sonoage). Human chorionic gonadotropin (hCG) (3300–10,000 IU) was administered after observing two follicles reaching a minimum mean diameter of 17 mm. 36 hours after hCG administration, an oocyte pick-up was performed through transvaginal ultrasound guided follicular puncture. Progesterone administration (50 mg, intramuscular) was started at the day of oocyte pick-up. Embryos with the best morphological appearance were transferred between day 3 and day 5. Ultrasound assessment of pregnancy was performed 4–5 weeks after embryo transfer.

Cytokines (IL-1β, TNF-α and IFN-ɣ) were measured in the serum. All samples were taken at the day of oocyte pick-up from 84 patients. Standard ELISA kits (Thermofisher) were used to measure cytokines using STAT FAX 303 PLUS instrument in Caspian international Hospital Laboratory.

CD16/56, and HLA-DR markers were quantified in 50 patients at the day of oocyte pick-up by flow cytometry using specific diagnosis kits (BD Biosciences) and a FACScan instrument (Becton Dickinson, FACScan) in Immunological Laboratory of Azerbaijan Medical University.

Statistical Analysis

NCSS (Number Cruncher Statistical System) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, and minimum and maximum) were used while evaluating the study data. The conformity of the quantitative data to the normal distribution was tested with the Shapiro-Wilk test and graphical examinations. A Student-t test was used for comparisons between two groups of normally distributed quantitative variables, and a Mann-Whitney U test was used for comparisons between two groups of non-normally dis-

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Table 1

Distribution of IVF related features in women undergoing IVF procedure

IVF related data	Measurement characteristics	n (%)
Female Infertility	Not present	82 (62.6%)
	Present	49 (37.4%)
Infertility Factor	Male factor	59 (45.0%)
	Ovarian factor	26 (19.8%)
	Tube factor	13 (9.9%)
	Unexplained reason	19 (14.5%)
	Gender selection	4 (3.1%)
	Infertility Depends on Male and Female factors	10 (7.6%)
IVF in anamnesis	Not present	79 (60.3%)
	Present	52 (39.7)
Hysterosalpingography	Fallopian tubes are passable	118 (90.1%)
	Fallopian tubes are blocked	13 (9.9)
Pregnancy Outcome	No transfer	7 (5.3%)
	No pregnancy	60 (45.8)
	Pregnancy	56 (42.7)
	Menstrual cycle cancelled	8 (6.1)

tributed quantitative variables. Statistical significance was accepted as $p<0.05$.

RESULTS AND DISCUSSION

According to anamnesis, 49 (37.4%) of total number of women observed had infertility. The rates of various factors and some important anamnestic information were presented in Table 1.

Mean infertility duration in our patients was 7.36±5.71 years (median-7; min-max: 0–30), mean IVF number in 52 women 2.04±1.52 (median-1; min-max: 1-7).

Patient immunological markers were analyzed based the IVF outcome (positive or negative). PBMCs markers (CD16/56, and HLA-DR) did not demonstrated statistical significant differences ($p>0.05$). There were not significant differences ($p>0.05$) in the levels of the cytokines too.

On the other hand, we analyzed the immunological parameters based the pregnancy status. CD16/56, and HLA-DR were compared in patients with or without pregnancy. There was no significant difference in these markers in patients based on pregnancy outcome ($p>0.05$). In addition, cytokines were quantified in these patients' sera. IL-1β and IFN-γ measurements were different in the sera of patients with pregnancy and without pregnancy ($p=0.041$ and $p=0.037$, respectively). But there are not statistical significant differences in levels of TNF-α between women with and without pregnancy (Table 2).

According to purpose of our studies, patients were also grouped based on infertility factors. One group (n=28) included patients with factors such as tubes over reserve and gender selection. The other group (n=48) included patients with factors such as male factor, azoospermia, and unexplained cause. CD16/56, and HLA-DR and IL-1β, TNF-α and IFN-γ in the women sera were not significantly different ($p>0.05$).

In vitro fertilization and its variants increasingly are used to treat nearly all causes of infertility. Fertility treatments are complex, and each ART cycle consists of several steps. If one of these steps is incorrectly applied, the stakes are high as conception may not occur. With this in mind, it is important that each step of the ART cycle is supported by good evidence from well-designed studies [18, 19].

Table 2

The levels of immunological parameters based IVF and pregnancy outcomes (Mean±SD)

Parameters	Groups					
	IVF (-) (n=7)	IVF (+) (n=43)	p	Pregnancy (-) (n=20)	Pregnancy (+) (n=23)	p
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
CD16/56 (cells/ml)	12.14±4.95	15.79±6.36	a0.188	16.65±7.01	15.04±5.79	b0.415
HLA DR (cells/ml)	9.14±4.02	10.16±4.75	a0.726	10.7±4.18	9.7±5.24	b0.496
	IVF (-) (n=8)	IVF (+) (n=76)		Pregnancy (-) (n=42)	Pregnancy (+) (n=34)	
	Median (min-max)	Median (min-max)		Median (min-max)	Median (min-max)	
IL-1β (pg/ml)	425.1 (191.6-7500)	351.5 (9.2-8785)	a0.266	373.6 (9.2-7536)	329.2 (17.8-8785)	0.041*
TNF-α (pg/ml)	40 (15.4-201.6)	43.3 (3.3-866.6)	a0.766	40.5 (3.3-866.6)	46 (12.5-843.7)	a0.545
IFN-γ (pg/ml)	19 (9.2-483.4)	16 (0.7-750.4)	a0.402	18 (0.7-721.8)	14.6 (6-750.4)	a0.037*

Note: IVF (+) – IVF procedure was successful; IVF (-) – IVF procedure was not successful; Pregnancy (+) – Pregnancy was present; Pregnancy (-) – Pregnancy was not present; [a] Mann Whitney U Test; [b]Student-t Test *$p<0.05$

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So, in our work, we investigated some markers of PBMCs in the blood serum and cytokines in women who underwent IVF.

Our results did not show statistically significant differences for CD16/56, and HLA-DR, which differs from the data of other authors presented in the literature.

Ho YK et al. (2020) used the determination of the percentage of peripheral CD16/56 natural killers (NK cells) in the early follicular phase on the 2nd–3rd day of the menstrual cycle in patients with repeated implantation failure (RIF) [20]. A total of 283 patients were examined with RIF consisting of at least 3 failed ART attempts and at least 2 high-quality embryo transfers. It is believed that NK cells make up 5–10% of peripheral blood lymphocytes and have the CD3–CD16/CD56+ phenotype. The authors concluded that a low percentage of peripheral CD16/CD56+ NK cells (≤10.6%) in the early follicular phase is a potential indicator of reduced pregnancy rates and implantation success in patients with RIF. Perhaps the absence of a difference in the values of this marker in our work is due to the fact that we analyzed absolute values, not percentages.

Kogan E. A. et al. (2020), observed 26 patients whose pregnancy occurred with IVF, to identify the structural and immunohistochemical features of the placentas and the placental sites after in vitro fertilization (IVF) with a donor egg (surrogate motherhood). The authors revealed that development of chronic inflammatory lesions in the perivascular areas related to an increase in the counts of HLA-DR positive cells and multinucleated trophoblastic giant cells [21]. But in our study there were not any differences in HLA-DR levels depend on IVF success.

Some studies demonstrated a positive association between IVF outcomes and pregnancies and IL-1β levels [22, 23]. In a clinical prospective study with 205 women, detectable IL-1β in the sera at the start of the IVF cycle was associated with positive IVF outcome and ongoing pregnancy, and IL-1β was shown to increase gradually in ongoing pregnancies [24]. Liang P. Y. et al. (2015) noted that the IFN-γ, IL-1β, IL-6 and IL-4 concentrations were higher in the RIF group compared with the women with a successful pregnancy in the first IVF/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) cycle group. The results suggested a shift toward a pro-inflammatory state in peripheral blood of the patients with RIF [25].

CONCLUSIONS

1. There were no significant differences in CD16/56 and HLA-DR levels in patients based on IVF and pregnancy outcomes and infertility factors.

2. IL-1β and IFN-ɣ levels were different in serum of patients with pregnancy and without pregnancy (p=0.041 and p=0.037, respectively), but there are no any differences in cytokines concentrations depends on IVF outcomes and infertility factors.

Our study showed that there is an association between serum levels of IL-1β and IFN-ɣ and successful pregnancy in the IVF procedure. We did not reveal the other relationships between clinical and immunological parameters in women underwent IVF procedure.

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