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A.F. Safarova
Caspian International Hospital, Baku, Azerbaijan

ASSOCIATION OF IL-1B AND IFN-Г AND SUCCESSFUL PREGNANCY IN WOMEN UNDERGOING IN VITRO FERTILIZATION PROCEDURE

e-mail: mic_amu@mail.ru

In this study, we aimed to investigate the association between immunological markers and a successful pregnancy following an in vitro fertilization procedure. A total of 131 patients were included in the study. They were grouped based on the outcome or parameters of the in vitro fertilization procedure such as successful pregnancy and female infertility. CD3, CD4, CD8, CD19, CD16/56 in the serum and cytokines in the serum and follicular aspiration fluid obtained during the process of oocyte pick-up were measured. There was no significant difference in CD3, CD4, CD8, CD19, CD16/56 levels in patients based on pregnancy outcome. IL-1β and IFN-y measurements were different in serum of patients with pregnancy and without pregnancy (p=0.041 and p=0.037, respectively). IL-1β levels were also different in the follicular aspiration fluid of patients with pregnancy and without pregnancy (p=0.039). Our study suggests an association between some cytokines (IL-1β in serum and follicular liquid) and IFN-γ (in serum)) and successful pregnancy in in vitro fertilization procedure.

Key words: pregnancy, reproductive technologies, immunological markers, female infertility.

А.Ф. Сафарова

АСОЦІАЦІЯ IL-1B І INF-Г І УСПІШНОЇ ВАГІТНОСТІ У ЖІНОК, ЯКІ ПЕРЕНЕСЛИ ПРОЦЕДУРУ ЕКСТРАКОРПОРАЛЬНОГО ЗАПЛІДНЕННЯ

У цьому дослідженні ми поставили за мету вивчити зв'язок між імунологічними маркерами та успішною вагітністю після процедури екстракорпорального запліднення. У дослідження було включено загалом 131 пацієнта. Вони були згруповані на основі результатів або параметрів процедури екстракорпорального запліднення, таких як успішна вагітність та жіноча безплідність. Вимірювали CD3, CD4, CD8, CD19, CD16/56 у крові та цитокіни в сироватці та фолікулярній аспіраційній рідині, отриманій у процесі забору ооцитів. Достовірної різниці CD3, CD4, CD8, CD19, CD16/56 та HLA-DR у пацієнток залежно від результату вагітності не було виявлено. Показники IL-1β та IFN-y відрізнялися у сироватці крові пацієнток з вагітністю та без вагітності (p=0,041 та p=0,037 відповідно). Рівні IL-1β також відрізнялися у фолікулярній аспіраційній рідині пацієнток з вагітністю та без вагітності (p=0,039). Наше дослідження передбачає зв'язок між деякими цитокінами (IL-1β (у сироватці та у фолікулярній рідині) та IFN-γ (у сироватці)) та успішною вагітністю при процедурі екстракорпорального запліднення.

Ключові слова: вагітність, репродуктивні технології, імунологічні маркери, жіноче безпліддя.

Infertility is an increasingly observed condition among couples and affects the relationship and mental health. Nearly 25 % of couples in developing countries are affected by infertility, which is the inability to become pregnant after 1 year of routine unprotected intercourse [11]. In vitro fertilization (IVF) is a process whereby mature oocytes are collected from ovaries, fertilized by sperm in vitro, and transferred back to uterus. Recent developments in IVF may be important in overcoming infertility [6]. On the other hand, the rate of live births in IVF per round is relatively low (~27 %) [2]. To overcome low rate of success in IVF, multiple embryo transfers and oocyte retrievals are performed; however, this practice may increase emotional and economic burden [11].

One of the parameters affecting the success of IVF procedure is the preparation and receptivity of the endometrium and the interaction between the mother and fetus. Cytokines are immune regulators that modulate the interactions between the mother and fetus in uterus [10, 11]. Cytokines are immunomodulatory molecules mainly produced by $CD4^+$ T helper cells and include Th1 specific cytokines such as interferon gamma (IFN–γ), interleukin 2 (IL–2), and Th2 type cytokines such as IL–4 and IL–5. There are also pro-inflammatory cytokines such as IL–1, TNF-α, and IL–6 [3]. It has been shown that there is a crosstalk and interplay between the mother's immune system and the fetus. Both anti-inflammatory and proinflammatory pathways, mediated by cytokines, involve this crosstalk and embryo implantation [4, 9]. Cytokines such as IL–1β have been shown to affect the outcome and pregnancy rates in IVF procedure [5, 6]. In addition, Th1 and Th2 type cytokines were studied and their association with implantation success and failure, and pregnancy losses were investigated. Some studies found a negative association [7]. Therefore, cytokines may be utilized as biomarkers to predict the outcome of IVF procedure in terms of pregnancy.

The purpose of the study was to investigate the cytokines and immune markers in the serum and follicular liquid in women undergoing in vitro fertilization procedures and their association with in vitro fertilization outcomes and parameters such as pregnancy and infertility.

Materials and methods. A total of 131 patients, who were not able to achieve pregnancy through natural methods and undergoing IVF procedure, were included in the study between 2020 and 2022 in the Reproductive Department of Caspian International Hospital. Patients were grouped and analyzed based on 4 parameters from IVF procedure outcomes: a) IVF outcome, positive or negative; b) Pregnancy, positive or negative; c) Female Infertility, present or not present; and d) Other Factors, Tube-Over reserve-Gender selection or Male Factor-Azoospermia- Unexplained Cause. The study was carried out in accordance with the principles of the Declaration of Helsinki 2008. Informed consent forms were signed for all patients.

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. Thirty-six hours after hCG administration, an oocyte pick-up (OPU) was performed through transvaginal ultrasoundguided follicular puncture. Progesterone administration (50 mg, intramuscular) was started at the day of OPU. 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 were measured in the serum and follicular aspiration fluid obtained during the process of OPU. All samples were taken at the day of OPU from 84 patients. Samples were centrifuged at 1,000xg for 15 minutes and then stored at -80^0C until used. Standard ELISA kits (Thermofisher) were used to measure IL–1β, TNF–a, IFN–γ, IL–4, IL–5, IL–6, and IL–7 using STAT FAX 303 PLUS instrument in Caspian international Hospital Laboratory.

Markers on peripheral blood mononuclear cells (PBMCs) were determined and quantified by flow cytometry using specific diagnosis kits (BD Biosciences) using a FACScan instrument (Becton Dickinson, FACScan) in ATU Immunological Laboratory. CD3, CD4, CD8, CD19, CD16/56, and HLA–DR markers were quantified in 50 patients. Briefly, antibody was added in whole blood, vortexed and incubated. After adding the lysing solution, cells were spun, washed, fixed and taken for 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 distributed quantitative variables. Statistical significance was accepted as $p<0.05$.

Results of the study and their discussion. It was observed that 20.6 % (n=27) of the cases participating in the study had a history of pregnancy. Of the cases, 48.1 % (n=13) with a pregnancy history were pregnant once, while 51.9 % of the cases (n=14) were observed to have pregnancy 2 times or more.

Infertility was observed in 37.4 % (n=49Gjghfdbkf) of the cases participating in the study. IVF history was observed in 39.7 % (n=52) of the cases. IVF numbers of cases with IVF ranged from 1 to 7, with a mean of 2.04±1.52.

Patient markers were analyzed based the success of IVF. Markers on PBMCs such as CD3, CD4, CD8, CD 19, CD16/56, and HLA–DR were compared in patients with or without successful IVF. There was no significant difference in these markers in patients based on IVF status ($P>0.05$). In addition, cytokines were quantified in these patients' sera and follicular aspiration liquid. There was no significant difference in these markers based on IVF status ($P>0.05$).

Patient markers were analyzed based on the pregnancy status. Markers on PBMCs such as CD3, CD4, CD8, CD 19, CD16/56, and HLA–DR were compared in patients with or without pregnancy (Table 1).

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 and follicular aspiration liquid. 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). IL–1β level was different in the follicular aspiration fluid of patients with pregnancy and without pregnancy ($p=0.039$) (Table 2).

Patient markers were analyzed based on the infertility status. Markers on PBMCs such as CD3, CD4, CD8, CD 19, CD16/56, and HLA–DR were compared in patients with (n=17) or without infertility (n=33). There was no significant difference in these markers in patients ($p>0.05$). Cytokines were also quantified in these patients' sera (infertility (+), n=33; infertility (–), n=51) and follicular aspiration liquid (infertility (+), n=32; infertility (–), n=50), there was no significant difference in these markers in patients based on infertility status ($p>0.05$).

Patients were 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. Markers on PBMCs such as CD3, CD4, CD8, CD 19, CD16/56, and HLA-DR and cytokines in the patient sera and follicular aspiration fluid were not significantly different (p>0.05).

Table 1

Comparisons of PBMCs markers in women based on pregnancy outcome

		Group			
		Pregnancy (-) (n=20)	Pregnancy (+) (n=23)	Total	***p***
CD3	*Mean±SD*	73.6±9	72.57±7.53	73.05±8.16	***[b]0.684***
	Median (MinMax)	73 (59-90)	73 (57-87)	73 (57-90)	
CD4	*Mean±SD*	47.4±4.39	46.17±7.71	46.74±6.34	***[b]0.534***
	Median (MinMax)	47 (39-55)	47 (27-60)	47 (27-60)	
CD8	*Mean±SD*	37.35±6.31	36±6.88	36.63±6.58	***[b]0.508***
	Median (MinMax)	38 (23-47)	37 (18-46)	37 (18-47)	
CD19	*Mean±SD*	10.4±3.1	11.39±3.09	10.93±3.1	***[b]0.301***
	Median (MinMax)	10 (6-21)	11 (7-18)	11 (6-21)	
CD16/56	*Mean±SD*	16.65±7.01	15.04±5.79	15.79±6.36	***[b]0.415***
	Median (MinMax)	15 (8-28)	15 (6-27)	15 (6-28)	
HLA DR	*Mean±SD*	10.7±4.18	9.7±5.24	10.16±4.75	***[b]0.496***
	Median (MinMax)	11.5 (2-17)	8 (2-23)	9 (2-23)	

Notes: [b]Student's t-test/ Values are for cells/ml.

Table 2

Comparisons of cytokines in women based on pregnancy outcome

		Group			
		Pregnancy (-) (n=42)	Pregnancy (+) (n=34)sdawq	Total	***p***
IL-1β	*Mean±SD*	816.96±1474.8	817.09±1753.0	817.02±1594.09	***[a]0.041****
	Median (MinMax)	373.6 (9.2–7536)	329.2 (17.8–8785)	351.5 (9.2–8785)	
TNF-α	*Mean±SD*	73.39±138.23	82.75±161.26	77.58±148.02	***[a]0.545***
	Median (MinMax)	40.5 (3.3–866.6)	46 (12.5–843.7)	43.3 (3.3–866.6)	
IFN-γ	*Mean±SD*	45.2±117.15	54.78±157.4	49.49±135.75	***[a]0.037****
	Median (MinMax)	18 (0.7–721.8)	14.6 (6–750.4)	16 (0.7–750.4)	
IL-4	*Mean±SD*	110.74±201.92	111.39±223.76	111.03±210.52	***[a]0.264***
	Median (MinMax)	47.6 (1.2–926)	39.5 (24.3–996.4)	45 (1.2–996.4)	
IL-5	*Mean±SD*	109.11±219.04	102.14±237.81	105.99±226.11	***[a]0.642***
	Median (MinMax)	41 (3.2–937.9)	41.8 (21.6–1094)	41.6 (3.2–1094)	
IL-6	*Mean±SD*	76.9±156.69	65.82±142.08	71.94±149.45	***[a]0.573***
	Median (MinMax)	24.1 (4.5–659.2)	24.8 (16.3–703.6)	24.5 (4.5–703.6)	
IL-7	*Mean±SD*	126.5±239.03	123.96±254.98	125.36±244.62	***[a]0.766***
	Median (MinMax)	53.7 (2.5–1255)	54.6 (34.8–1221)	54.2 (2.5–1255)	

Notes: [a]Mann Whitney's U Test / Values are for pg/ml. *p<0.05

In our study, we investigated several cytokines in the serum and follicular liquid, and immune markers on PBMCs in women undergoing IVF. Patients were grouped based on whether there was a positive IVF result or whether there was pregnancy. As a result, reduced IL–1β levels were found in the serum and follicular liquid obtained from women who were pregnant at the end of IVF procedure. In addition, serum IFN–γ was lower in women who were pregnant at the end of IVF procedure compared to women who were not pregnant.

Implantation of blastocyst to endometrium is mediated through interactions between hormones, cytokines, and adhesion molecules. The cyclical changes in the endometrium tissue affects the expression of factors such as cytokines and therefore, the timing of sample collection is of critical importance [3]. In our study, samples were obtained at the day of oocyte pick-up. In various studies, different time points were used varying from cycle day 3 to cycle day 28 and IL–1β levels were measured. Some studies demonstrated a positive association between IVF outcomes and pregnancies and IL–1β levels. 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 [6]. In another study with 76 women, IL–1β measurements were performed in endometrial secretions obtained prior to oocyte collection during IVF procedure and IL–1β levels were significantly higher in women with successful chemical pregnancies. However, the difference in the IL–1β levels did not reach to

significance in case of clinical pregnancy (p=0.06) [5]. IL–1β levels were measured at the day of embryo transfer in 44 women and were found to be higher in women with clinical pregnancy [1]. Sequeira et al. showed that IL–1 in maternal serum and cell culture medium of developing embryos on IVF day 3 were significantly higher in women with successful implantation [12].

There are also studies demonstrating a negative association between IL–1β and IVF pregnancy outcome. In an investigation with 307 women undergoing IVF, serum IL–1β was elevated on cycle days 24 and 28 in women with ectopic pregnancy [8].

In our study, we also observed a reduced level of IFN–γ, a Th1 type cytokine, in the serum of women who were pregnant after IVF procedure compared to women who were not pregnant as a result of IVF procedure. In a similar manner, PBMCs were found to have higher ratios of IFN-gamma:IL–4 and TNF-alpha:IL–4 at oocyte retrieval day in women with a history of recurrent failed IVF treatment. Supporting our finding, Kuroda et al. showed increased Th1/Th2 cell ratio in women with multiple implantation failure cycles and pregnancy losses [7].

Studies mentioned above demonstrate an association between IL–1β signaling pathway and pregnancies in IVF procedure. However, some data show a negative association, while others show a positive association. There may be several reasons behind the contrasting results. First, immunological pathways and their crosstalk are complex processes, and these interactions and levels of cytokines change during the cycle. The time point at which the serum was obtained is critical and may affect the results. Second, some studies focus on measurements in the in vitro culture medium. Others focus on measurements in the serum or endometrial secretions. These methodological differences may have a role in the different outcomes of studies.

Conclusions

1. There was no significant difference in CD3, CD4, CD8, CD19, CD16/56 levels in patients based on pregnancy outcome.

2. IL-1β and IFN-y measurements were different in serum of patients with pregnancy and without pregnancy (p=0.041 and p=0.037, respectively). IL-1β levels were also different in the follicular aspiration fluid of patients with pregnancy and without pregnancy (p=0.039).

Our study showed an association between IL–1β and successful pregnancy in the IVF procedure in both serum and follicular liquid. In addition, we found an association between serum IFN–γ and successful pregnancy in IVF procedure.

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