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THE ROLE OF SERUM AND FOLLICULAR FLUID CYTOKINES IN SUCCESSFUL RESULTS OF IN VITRO FERTILIZATION PROCEDURE

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The purpose of the study was to find out the association between cytokines of serum and follicular fluid and successful results of an in vitro fertilization procedure. A total of 131 patients were included in the study. They were grouped based on the outcome of the in vitro fertilization procedure. The cytokines in the serum and follicular aspiration fluid obtained during the process of oocyte pick-up were measured. There was no significant difference in cytokines levels in serum and follicular fluid in patients with and without success results of in vitro fertilization procedure. IL-1β (p=0.851), IL-6 (p=0.994) in follicular aspiration fluid and IL-1β (p=0.266), IL-5 (p=0.365) and IL-6 (p=0.377) were different in serum, but the differences were not statistically significant (p>0.05). Our study suggests the nonsignificant association between some cytokines in serum and follicular fluid and successful results of in vitro fertilization procedure, but it is necessary to conduct the further studies for more detail results.

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

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РОЛЬ ЦИТОКІНІВ СИРОВАТКИ І ФОЛІКУЛЯРНОЇ РІДИНИ В УСПІШНИХ РЕЗУЛЬТАТАХ ПРОЦЕДУРИ ЕКСТРАКОРПОРАЛЬНОГО ЗАПЛІДНЕННЯ

Метою дослідження було з'ясувати зв'язок між цитокінами сироватки та фолікулярної рідини та успішними результатами процедури екстракорпорального запліднення. Усього до дослідження був включений 131 пацієнт. Вони були згруповані з урахуванням результатів процедури екстракорпорального запліднення. Вимірювали цитокіни в сироватці та фолікулярній аспіраційній рідині, отриманій у процесі забору ооцитів. Достовірної різниці у рівні цитокінів у сироватці крові та фолікулярній рідині у пацієнток з успішними результатами процедури екстракорпорального запліднення і без неї виявлено не було. IL-1β (p=0,851), IL-6 (p=0,994) у фолікулярній аспіраційній рідині та IL-1β (p=0,266), IL-5 (p=0,365) та IL-6 (p=0,377) у сироватці відрізнялися, але відмінності були статистично значущими (p>0,05). Наше дослідження передбачає наявність певного зв'язку між деякими цитокінами в сироватці та фолікулярній рідині та успішними результатами процедури екстракорпорального запліднення, але для отримання більш детальних та точних результатів необхідно провести подальші дослідження.

Ключові слова: вагітність, репродуктивні технології, імунологічні маркери, жіноча безплідність.

Infertility affects 48.5 million couples globally. It is defined clinically as failure to conceive after 12 months or more of regular unprotected sexual intercourse [8]. Prevalence of primary infertility was 1.9 % and secondary infertility 10.5 % [9]. WHO estimates that these figures go up 2.5 fold using an epidemiological definition of infertility [14].

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 [11]. A normal balance of different parts of the immune system is necessary for the proper development of follicles. Cytokines promote proper oocyte maturation, timely rupture of follicles, and neoangiogenesis, indirectly contributing to the supply of oxygen, nutrients, and substrates for subsequent steroidogenesis [7, 10]. Some cytokines, such as IL-6, also plays an important role in follicle development. Regarding folliculogenesis, cytokines regulate cell proliferation or differentiation and follicle survival or atresia [5, 13]. The central role of cytokines suggests that any modulation during follicle development and oocyte maturation may have a significant impact on the development of physiological conditions for fertilisation. Pro-inflammatory cytokines are crucial in the maturation process of the ovarian follicle, in addition to the process of embryo implantation. Immune imbalances have a negative impact on the prognosis of the effectiveness of IVF and possibly on natural fertilization [3, 6].

Thus, stadying the role of various cytokines in the procedure of in vitro fertilization may ultimately help in establishing an individual approach to the fertility treatment.

The purpose of the study was to investigate the cytokines levels in the serum and follicular aspiration fluid in women undergoing in vitro fertilization procedure.

Materials and methods. The study was carried out in the Reproductive Department of Caspian International Hospital in the period of 2020-2022. Totally 131 women, who were not able to achieve pregnancy through natural methods and undergoing IVF procedure, were included in the study. Patients were grouped and analyzed based on IVF procedure outcomes: IVF positive (IVF (+)) or negative (IVF (–)). Female Infertility and other factors: Tube-Over reserve, Gender selection or Male Factor-Azoospermia, Unexplained Cause also were assessed. All procedures were 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. Cytokines were measured in the serum and follicular aspiration fluid obtained during the process of oocyte pick-up (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^{0}C until used. Standard ELISA kits (Thermofisher) were used to measure IL-1β, TNF-a, IFN-y, IL-4, IL-5, IL-6, and IL-7 using STAT FAX 303 PLUS instrument in Caspian international Hospital Laboratory.

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. Infertility was observed in 37.4 % (n=49) 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.

Among the infertility factor the most common was person factors 45.0 % (n=59). Ovarian Factor was detected in 26 (19.8 %) of patients, tube factor – in 13 (9.9 %), unexplained reason – in 19 (14.5 %), gender selection – in 4 (3.1 %) and infertility depends on both sides – in 10 (7.6 %) of women.

According to the results of Hysterosalpingogram (HSQ), tubes were open in 118 (90.1 %) of patients, were closed in 13 (9.9 %) of all women, involved in the study.

The pregnancy outcomes were as following: no transfer – in 7 (5.3 %), no pregnancy – in 60 (45.8 %), presence of pregnancy – in 56 (42.7 %), menstrual cycle cancelled – in 8 (6.1 %) of patients.

Patient markers were analyzed based the success of IVF. The cytokines were quantified in these patients' follicular aspiration fluid and serum. The levels of cytokines follicular aspiration fluid were different depends on success of IVF procedure (Table 1).

Table 1

The levels of cytokines, depends on IVF Success Status (Follicular Aspiration Fluid)

Parameters		Group			
		IVF not successful (–) **(n=8)**	IVF successful (+) **(n=76)**	Total	***P***
IL-1β pg/ml	*Mean±SD*	1381.18±2474.95	1009.07±1597.7	1045.37±1687.89	***[a]0.851***
TNF-α pg/ml	*Mean±SD*	95.1±109.11	102.64±251.45	101.9±240.87	***[a]0.453***
IFN-γ pg/ml	*Mean±SD*	75.49±136.83	51.91±116.49	54.21±117.88	***[a]0.325***
IL-4 pg/ml	*Mean±SD*	153.49±292.7	128.1±231.87	130.57±236.46	***[a]0.424***
IL-5 pg/ml	*Mean±SD*	145.13±272.32	115.67±216.25	118.55±220.53	***[a]0.295***
IL-6 pg/ml	*Mean±SD*	119.63±240.59	69.36±134.91	74.26±147.08	***[a]0.994***
IL-7 pg/ml	*Mean±SD*	192.76±359.57	150.7±291.35	154.8±296.37	***[a]0.502***

Mann Whitney's U Test/Values are for pg/ml.

IL-1β, IL-6 and IFN-γ measurements were different in the follicular aspiration fluid of patients with IVF successful results and without them. The level of IL-1β in women with successful IVF was lower than in patient with IVF (–) (1009.07±1597.7 pg/ml vs 1381.18±2474.95 pg/ml, respectively). The similar results were found out in IL-6 levels (69.36±134.91 pg/ml vs 119.63±240.59 pg/ml, respectively) and IFN-γ levels (51.91±116.49 pg/ml vs 75.49±136.83 pg/ml, respectively). However, the differences were not statistically significant ($p>0.05$). The other cytokines had practically the same levels in compared groups ($p>0.05$).

The cytokines levels in the patient serum demonstrated similarities with follicular aspiration fluid results (Table 2).

Table 2

The levels of cytokines, depends on IVF Success Status (Serum)

Parameters		Groups			
		IVF not successful (–) (n=8)	IVF successful (+) (n=76)	Total	***p***
IL-1β pg/ml	*Mean±SD*	1274.75±2517.2	817.02±1594.09	860.61±1687.86	***[a]0.266***
TNF-α pg/ml	*Mean±SD*	56.63±60.86	77.58±148.02	75.58±141.95	***[a]0.766***
IFN-γ pg/ml	*Mean±SD*	75.54±164.86	49.49±135.75	51.97±137,85	***[a]0.402***
IL-4 pg/ml	*Mean±SD*	161.84±305.18	111.03±210.52	115.87±219.38	***[a]0.424***
IL-5 pg/ml	*Mean±SD*	173.73±362.55	105.99±226.11	112.44±240.17	***[a]0.365***
IL-6 pg/ml	*Mean±SD*	133.36±302.95	71.94±149.45	77.79±168.08	***[a]0.377***
IL-7 pg/ml	*Mean±SD*	144.15±249.3	125.36±244.62	127.15±243.61	***[a]0.915***

Mann Whitney's U Test/Values are for pg/ml.

Despite on presence of some differences in levels of IL-1β (1274.75±2517.2pg/ml in IVF (–) group vs 817.02±1594.09 pg/ml in IVF (+) group), IL-5 (173.73±362.55pg/ml in IVF (–) group vs 105.99±226.11 pg/ml in IVF (+) group) and IL-6 (133.36±302.95pg/ml in IVF (–) group vs 71.94±149.45 pg/ml in IVF

(+) group), these were not significantly different (p>0.05). On the opposite, the level of TNF-α was lower in patient with fail of IVF (56.63±60.86pg/ml in IVF (–) group vs 77.58±148.02 pg/ml in IVF (+) group).

As in the follicular aspiration fluid, the other cytokines measured in serum had practically the same levels in women with and without success of IVF procedure (p>0.05).

In our study, we investigated several cytokines in the serum and follicular liquid, in women undergoing IVF. Patients were grouped based on whether there was a positive IVF result. 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 serum 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]. 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].

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]. Our results were similar: the IL-1β levels in follicular fluids demonstrated some differences without statistical significance (p=0.851).

In our study, we also observed a reduced level of IL-6 in in follicular aspiration fluids in patients with IVF negative results. Among cytokines, such as IL-4, IL-6 and IL-10 which are primarily secreted by Th2 cells, IL-4 and IL-10 can cooperate with Treg cells in the regulation of immune balance to ensure a conducive environment for embryo implantation and maintenance of pregnancy [3, 4]. Zhang Y, et al indicated the changes of IL-4 and IL-10 and their ratio in patients with recurrent implantation failure [15]. But we did not revealed differences of levels of IL-4 in our study.

According to some studies, TNF-α could induce positive regulatory effects on trophoblast invasion at low concentrations [2, 15]. In contrast, high TNF-α concentrations can inhibit endometrial decidualization and promote apoptosis in trophoblasts, leading to embryo implantation failure or pregnancy loss [5]. In our patients there were not significant differences in levels of TNF-α both in group IVF (+) and group IVF (–), but interestingly that in serum the level of this cytokine were lower in women in case of IVF failure.

Alhilali MJS, et al (2019) in their study found out that IL-5 in follicular fluid as a negative predictor of the intracytoplasmic sperm injection outcome [1]. We revealed the higher levels of IL-5, but in serum, not in follicular fluid.

Studies mentioned above demonstrate an association between cytokines and success in IVF procedure and pregnancies. 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

There are some differences in levels of cytokines in serum and follicular aspiration fluid between women with and without successful results of IVF procedure.

1. IL-1β (p=0.851), IL-6 (p=0.994) in follicular aspiration fluid were different in groups IVF (+) and IVF (–).

2. IL-1β (p=0.266), IL-5 (p=0.365) and IL-6 (p=0.377) were different in serum, but the differences were not statistically significant (p>0.05).

However, absence of statistically significance of data required necessity of further studies.

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PROGNOSIS OF DISABILITY IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

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105 patients with relapsing-remitting multiple sclerosis aged 18 to 49 were examined. The degree of disability was assessed using the Expanded Disability Status Scale. Neuropsychological examination was performed to determine the level of personal and reactive anxiety on the Spielberger-Khanin scale and depression on the Beck scale. Magnetic resonance imaging of the brain was performed and brain atrophy indices were calculated, namely, the bicaudal and Sylvian sulcus indices. The ability of the studied clinical and paraclinical indicators to predict the progression of disability in multiple sclerosis was assessed using ROC analysis. Areas under the ROC curve had excellent prognostic characteristics for indices of atrophy, levels of anxiety and depression about significant neurological manifestations (EDSS≥3.5 points) and disability in patients with relapsing-remitting multiple sclerosis. According to the data of the multiple logistic regression analysis, it was established that the degree of subcortical atrophy according to the BCR index is a statistically significant independent factor affecting the appearance of significant neurological manifestations, according to the adjusted odds ratio, purified from the influence of other factors (OR=26.2; 95 % CI 3.5–198.3), the level of depression on the BDI scale (OR=97.2; 95 % CI (3.4–2744.0). A significant protective effect of disease-modifying therapy was confirmed (OR=0.006; 95 % CI 0.001–0.045).

Key words: multiple sclerosis, atrophy, disability, prognosis, course.

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ПРОГНОЗУВАННЯ ІНВАЛІДИЗАЦІЇ ПРИ РЕЦИДИВУЮЧЕ-РЕМІТУЮЧОМУ РОЗСІЯНОМУ СКЛЕРОЗІ

Обстежено 105 хворих на рецидивуюче-ремітуючий розсіяний склероз у віці від 18 до 49 років. Ступінь інвалідизації оцінювали за шкалою Expanded Disability Status Scale. Проводили нейропсихологічне обстеження з визначенням рівня особистісної та реактивної тривожності за шкалою Спілбергера-Ханіна та рівня депресії за шкалою Бека. Проводили магнітно-резонансну томографію мозку та розраховували індекси атрофії мозку, а саме – бікаудальний та індекс Сильвієвої борозни. Здатність вивчених клінічних та параклінічних показників прогнозувати прогресування інвалідизації при розсіяному склерозі оцінювали за допомогою ROC-аналізу. Площі під ROC-кривою мали відмінні прогностичні характеристики для індексів атрофії, рівнів тривожності та депресії щодо значних неврологічних проявів (EDSS≥3.5 балів) й інвалідизації у хворих на РРРС. За даними множинного логістичного регресійного аналізу встановлено, що статистично значущими самостійними факторами впливу на появу значних неврологічних проявів, згідно скоригованих, очищених від впливу інших чинників, відношення шансів є ступінь підкоркової атрофії за індексом BCR (ВШ=26.2; 95 % ДІ 3.5–198.3), рівень депресії за шкалою BDI (ВШ=97.2; 95 % ДІ (3.4–2744.0). Підтверджено значний захисний ефект прийому хворобо-модифікуючої терапії (ВШ=0.006; 95 % ДІ 0.001–0.045).

Ключові слова: розсіяний склероз, атрофія, інвалідизація, прогнозування, перебіг.

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Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) in which inflammation and demyelinating disease occur even in the early stages of the disease [3].

There are 4 types of MS course: relapsing-remitting (RRMS), secondary progressive, primary progressive and progressive-relapsing. RRMS is the most common one [6].