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Quantitative serum determination of CD3, CD4, CD8, CD16, and CD56 in women with primary infertility: The role of cell-mediated immunity

Primer infertilitesi olan kadınlarda CD3, CD4, CD8, CD16 ve CD56'nın kantitatif serum tayini: Hücre aracılı bağışıklığın rolü

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Abstract

Objective: Cellular adaptive immunity plays an essential role in the etiology of primary infertility. This study aimed to measure the T-lymphocyte subpopulations and natural killer (NK) cells in infertile women compared with healthy ones.

Materials and Methods: From January to September 2021, we conducted this cross-sectional study among women with primary infertility, and healthy women were referred to Isfahan Fertility and Infertility Center affiliated with Najafabad University of medical sciences in Isfahan, Iran for immunological investigations. For each person, we determined quantitative serum measurements of CD3, CD4, CD8, CD4/CD8, CD16, CD56, and CD56+16.

Results: This study included one hundred and fifty-one infertile women with a mean age of 31.4±4.7 years and 46 healthy women with a mean age of 31.5±3.4 years. Compared to the controls, immunophenotyping findings in infertile patients revealed a significant drop in CD8 T cells [p=0.01, 95% confidence interval (CI) 0.53 to 4.57] and the percentage of CD 56 NK cells (p=0.005, 95% CI 0.74 to 4.03) in infertile patients.

Conclusion: Despite having a normal quantity of CD3 T cells, infertile women had lower CD8 T cells and CD56 NK cells than the controls. More studies are needed to confirm the role of cell-mediated assessments as a screening test in patients with primary infertility.

Keywords: Infertility, cellular immunity, flow cytometry, female infertility, NK cells, T cells

Öz

Amaç: Hücresel adaptif immünite, primer infertilite etiyolojisinde önemli bir rol oynar. Bu çalışma, infertil kadınlarda ve sağlıklı kadınlarda T-lenfosit alt popülasyonlarını ve doğal öldürücü (NK) hücrelerini ölçmeyi ve bunların iki grup arasında kıyaslanmasını amaçlamaktadır.

Gereç ve Yöntemler: Bu kesitsel çalışmayı Ocak-Eylül 2021 tarihleri arasında İran'ın İsfahan kentindeki İsfahan Tıp Bilimleri Üniversitesi'ne bağlı İsfahan Doğurganlık ve Kısırlık Merkezi'ne immünolojik incelemeler için sevk edilen primer infertilitesi olan kadınlar ve sağlıklı kadınlar üzerinde gerçekleştirdik. Her kişide CD3, CD4, CD8, CD4/CD8, CD16, CD56 ve CD56+16'nın kantitatif serum ölçümlerini yaptık.

Bulgular: Bu çalışmaya yaş ortalaması 31,4±4,7 yıl olan 115 infertil kadın ve yaş ortalaması 31,5±3,4 yıl olan 46 sağlıklı kadın dahil edildi. Kontrollerle karşılaştırıldığında, infertil hastalarda immünofenotipleme bulguları, CD8 T hücrelerinin sayısında [p=0,01, %95 güven aralığı (GA) 0,53 ila 4,57] ve CD 56 NK hücrelerinin yüzdesinde (p=0,005, %95 GA 0,74 ila 4,03) önemli bir düşüş ile uyumluydu.

Sonuç: Normal miktarda CD3 T-hücresine sahip olmalarına rağmen, infertil kadınlarda kontrollere göre daha düşük CD8 T hücreleri ve CD56 NK hücreleri vardı. Primer infertilitesi olan hastalarda bir tarama testi olarak hücre aracılı değerlendirmelerin rolünü doğrulamak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Kısırlık, hücresel bağışıklık, flow sitometri, kadın infertilitesi, NK hücreleri, T hücreleri

PRECIS: The rate of peripheral CD8 T cells and CD56 NK cells significantly decreased in women with infertility compared to the controls in our study.

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Introduction

Primary infertility (PI) is defined as a woman's inability to become pregnant after one year of regular intercourse without using contraception methods, as well as normal results for semen analysis, ovulation tests, and tubal patency. Based on a World Health Organization report, PI affects around one-fourth of all couples worldwide. According to a new theory, PI can be caused by immunological disorders, including a deficiency in the humoral or cellular immune systems[1-4].

Cell-mediated immunity is an immune response that does not involve antibodies but involves the production of T-lymphocytes and the activation of macrophages and natural killer (NK) cells. It secretes various cytokines in response to an antigen or immunogen[5,6].

CD3 is a characteristic marker for recognizing T cells, T-cell receptors, and a ubiquitous T-cell receptor complex member. CD3 T cells can be divided into two predominant types by the expression of surface molecules of CD4 ($CD4^+$ or T-helper cells) and CD8 ($CD8^+$ or cytotoxic T cells). $CD4^+$ or T-helper cells (TH) may be differentiated into two main categories: TH_1 cells, which produce interferon-gamma and lymphotoxin-alpha, and TH_2 cells, which produce IL-4, IL-5, and IL-13. As a third group, T helper 17 cells (TH17), which secrete IL-17, were found. $CD8^+$ or cytotoxic T-cells generally produce interferon-gamma; they may also be differentiated into two main categories: TC_1 cells, TC_2 cells, and newly discovered TC_{17} that secretes Interleukin 17[6,7].

NK cells are granular lymphocytes with NK- specific CD antigens, CD16 and CD56, and a potent source of interferon-gamma. Peripheral NK cells and uterine NK cells are two types of NK cells. Peripheral NK cells circulate in the bloodstream, but uterine NK cells lack the same destructive ability as peripheral NK cells[8,9].

Infertility is a serious problem that can ruin a couple's life. Although the interest in considering the disturbance of immunologic factors for the occurrence of PI has recently increased, there are limited reports of cell-mediated immunity in the serum of infertile females. This study measured the serum levels of CD3, CD4, CD8, CD16, and CD56 in infertile females compared with the control group in Isfahan, Iran.

Materials and Methods

From January to September 2021, this cross-sectional study involved voluntary females with PI aged 18 to 42 who were referred to Isfahan Fertility and Infertility Center, affiliated with Najafabad University of medical sciences in Isfahan, Iran, for immunological investigations. PI diagnosis was based on the lack of abnormality in the semen analysis and the normal assessment of ovulation and fallopian tubes in the women. After the approval of the study protocol by the Ethics Committee of Najafabad University of Medical Sciences (IR.IAU.NAJAFABAD.REC.1399.119), written informed consent was obtained from the patients.

The women were excluded if they had at least one successful pregnancy, uncontrolled diabetes, uncontrolled hypertension, autoimmune diseases, malignancy, or an immune deficiency disorder. Women who had previously had an abortion were included if it had been at least a year since the abortion. Forty-eight unrelated healthy women with a history of having at least two children aged from 18 to 42 years were selected as the control group by the non-probability Quota sampling method from the same geographic region.

A questionnaire was designed to collect information about age, duration of the marriage, and the number of abortions. Peripheral blood samples were collected aseptically by venipuncture into ethylenediaminetetraacetic acid collection tubes from all individuals in the case and control groups for quantitative serum determination of CD3, CD4, CD8, CD4/CD8, and CD16, CD56, CD56+16. According to the manufacturer's instructions, a minimum of 5 mL of whole blood is required for the immunophenotypic analysis. Analysis was performed using a FACS Canto II flow cytometer and FACS Diva software (BD Biosciences). Each lymphocyte subpopulation count was expressed in absolute value and as a percentage of total lymphocytes. An example of gating for standard proliferation analysis of lymphocytes in a woman with PI using flow cytometry is shown in Figure 1.

Statistical Analysis

The data were analyzed using SPSS Statistics (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.); correlations were significant if the p-value was less than 0.05. Continuous variables were presented as the mean ± standard deviation. Chi-square, independent sample t-test, and Fisher's exact test were used to determine the mean and compare the mean of CD markers in patients with PI and the control group. The association between independent variables was also assessed 95% confidence interval (CI).

Results

One hundred and fifty-one infertile women with a mean age of 31.44±4.72 years, the age range of 20 to 44 years, and 46 healthy women with a mean age of 31.50±3.39 years, the age range of 22 to 39 years were included in this study. There were no significant differences in age between the two groups (p-value=0.8). All patients were from Isfahan province, central Iran.

The mean period of infertility was 4.7±3.5 years (range: 2 to 16 years) in infertile females. Among enrolled women, 61 (40.4%) had never experienced pregnancy, while 90 (59.6%) had at least one unsuccessful pregnancy. Table 1 represents the mean percentile of T-cell-mediated markers in infertile women compared with control healthy women; the level of CD8 between these two groups was significantly different. The comparison of the mean percentile of natural killer cell markers in infertile women with the control group is shown in Table 2.

Discussion

There are a few studies on the role of T-cell and NK cell marker changes in women with PI. In this study, the immunologic profile showed a significant difference in the percentage of CD 8-T cells and CD 56 NK cells of females with PI without a successful pregnancy and those who had. Compared to the control group, there were no differences in the percentages of CD4 T cells and CD 16 NK cells in patients with infertility.

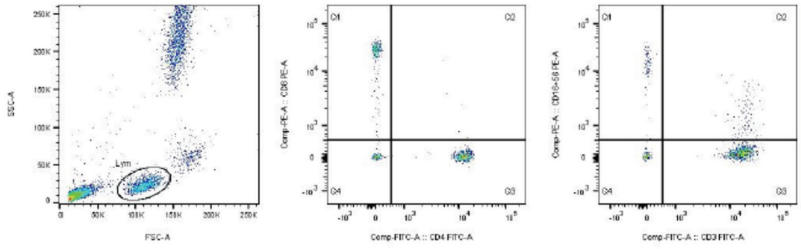
Russell et al.[10] reported focal perivascular aggregation of CD8 T cells in the endometrium of most women with recurrent reproductive failure. Bczkowski and Kurzawa[11] showed that CD4 and CD8-positive cells did not significantly differ in patients treated with intracytoplasmic sperm injection compared with control fertile patients. Lachapelle et al.[12] found that the percentage of endometrial CD8 T-lymphocytes was significantly decreased in cases with recurrent miscarriage. The analysis of the presence of CD8 T cells seems to be a somewhat controversial issue, as our study showed decreased CD8 T cells in patients with PI. Different studies used local endometrial tissue samples to analyze CD8 T cells, whereas our research used peripheral blood. According to Chernyshov et al.[13], no changes in CD8 T lymphocytes were found between endometrial and peripheral blood. All the discrepancies cannot be attributed to the lack of a standardized CD4 measurement location.

The level of CD56 NK cells in women with PI was considerably lower than in controls, confirming that NK cells play a key role in human reproductive performance. According to a recent meta-

Table 1. Comparison of mean percentile of T- cell mediated markers in infertile women with control healthy women

Cell markers	Group	n	Mean	Standard deviation	Minimum	Maximum	95% Confidence Interval		p-value
							Lower	Upper	
CD3, %	case	151	66.14	7.13	34	89	-.1.172	4.587	0.23
	control	46	67.84	7.13	48	78			
CD4, %	case	151	42.01	7.87	22.5	61	-0.464	4.67	0.09
	control	46	44.14	6.84	32	56.5			
CD8, %	case	151	24.01	6.33	3	38	0.533	4.568	0.01*
	control	46	26.56	5.11	17	38.4			
CD4 to 8 ratio	case	151	1.89	0.86	0.8	6.9	-0.447	0.079	0.16
	control	46	1.71	0.50	0.9	3.2			

*: Statistical significant



Immunophenotyping by flow cytometry

CD Marker	Description	Gate	Results		Reference value	
			Relative count (%)	Absolute count (cell/mm³)	Relative count (%)	Absolute count (cell/mm³)
CD3	Pan T cell	Lym	79.4	2717	58-86	550-2202
CD4	T helper cell	Lym	54.67	1871	32-64	365-1437
CD8	T cytotoxic cell	Lym	30	1027	13-40	145-846
CD4:CD8	Th/Tc Ratio		1.8		≥0.9	
CD3-/CD56+	NK cells	Lym	4.76	163	3.5-23	57-611
CD3+/CD56+	NKT cells	Lym	3.47		0.9-15	
CD3-/CD16+	NK cells	Lym	3			
CD3-/CD16+CD56	Total NK cells	Lym	7	239	4-25	57-611
CD3+/CD16+CD56	NKT cells	Lym	10.2		1-15	

Figure 1. Example of gating for standard prolifration analysis of lymphocytes in a woman with primary infertility using flow cytometry

Table 2. Comparison of mean percentile of natural killer cell markers in infertile women with control healthy women

Antibodies	Group	n	Mean	Standard deviation	Minimum	Maximum	95% Confidence Interval		p-value
							Lower	Upper	
CD16, %	Case	151	10.18	5.30	3	31	-0.959	2.394	0.40
	Control	46	10.9	4.03	3	27.6			
CD56, %	Case	151	8.19	5.40	1	31	0.735	4.032	0.005*
	Control	46	10.34	4.00	3	27			
CD16+56, %	Case	151	10.28	6.38	0.8	33	-1.852	2.141	0.82
	Control	46	10.43	4.46	2	28			

*: Statistical significant

analysis of studies that looked at peripheral NK cell levels, the percentage of NK cells in the blood is much higher in women who have recurrent abortions than in those who don't[14]. However, a systematic review by Tang et al.[15] reported that the prognostic value of measuring peripheral NK cell parameters remains unclear, and more studies are needed to confirm the role of NK cell measurement as a predictive test for screening in infertility. Chernyshov et al.[13] revealed that the level of NK cells was higher in the endometrium samples compared to peripheral blood. It's expected that blood NK cells aren't currently used as a diagnostic test in women with PI, and measuring uterine NK cells will probably be a better option.

Conclusion

Our results revealed that despite the normal $CD3^+$ T cells, the rate of CD8 T cells and CD56 NK cells significantly decreased in women with infertility compared to the controls. There is insufficient evidence to recommend T- cell and NK cell testing of peripheral blood as a screening test in patients with PI.

Ethics

Ethics Committee Approval: Approval of the study protocol by the Ethics Committee of Najafabad University of Medical Sciences (IR.IAU.NAJAFABAD.REC.1399.119).
Informed Consent: Written informed consent was obtained from the patients.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.S., S.M., S.I., M.M., Design: B.S., S.M., S.I., M.M., Data Collection or Processing: B.S., S.M., S.I., M.M., Analysis or Interpretation: B.S., S.M., S.I., M.M., Literature Search: B.S., S.M., S.I., M.M., Writing: B.S., S.M., S.I., M.M.
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References

1. Ray A, Shah A, Gudi A, Homburg R. Unexplained infertility: an update and review of practice. Reprod Biomed Online 2012;24:591-602.
2. Sadeghi MR. Unexplained infertility, the controversial matter in management of infertile couples. J Reprod Infertil 2015;16:1-2.
3. Smith S, Pfeifer SM, Collins JA. Diagnosis and management of female infertility. JAMA 2003;290:1767-70.
4. Alecsandru D, García-Velasco JA. Immunology and human reproduction. Curr Opin Obstet Gynecol 2015;27:231-4.
5. Brazdova A, Senechal H, Peltre G, Poncet P. Immune aspects of female infertility. Int J Fertil Steril 2016;10:1-10.
6. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ. Immunobiology: The immune system in health and disease. 5th edition. New York: Garland Science; 2001. Chapter 8, T Cell-Mediated Immunity.
7. Annunziato F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. J Allergy Clin Immunol 2015;135:626-35.
8. Abel Alex M, Yang Chao, Thakar Monica S, Subramaniam M. Natural killer cells: Development, maturation, and clinical utilization. Front Immunol 2018;9:1869.
9. Azargoon A, Mirrasouli Y, Shokrollahi Barough M, Barati M, Kokhaei P. The State of Peripheral Blood Natural Killer Cells and Cytotoxicity in Women with Recurrent Pregnancy Loss and Unexplained Infertility. Int J Fertil Steril 2019;13:12-7.
10. Russell P, Sacks G, Tremellen K, Gee A. The distribution of immune cells and macrophages in the endometrium of women with recurrent reproductive failure. III: Further observations and reference ranges. Pathology 2013;45:393-401.
11. Baczkowski T, Kurzawa R. Immunophenotypic profiles of peripheral blood lymphocytes on the day of embryo transfer in women undergoing in vitro fertilization. Folia Histochem Cytobiol 2007;45(Suppl 1):S73-7.
12. Lachapelle MH, Miron P, Hemmings R, Roy DC. Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. Altered profile and pregnancy outcome. J Immunol 1996;156:4027-34.
13. Chernyshov VP, Dons'koi BV, Sudoma IO, Goncharova YO. Comparison of T and NK lymphocyte subsets between human endometrial tissue and peripheral blood. Cent Eur J Immunol 2019;44:316-21.
14. Seshadri S, Sunkara SK. Natural killer cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis. Hum Reprod Update 2014;20:429-38.
15. Tang AW, Alfirevic Z, Quenby S. Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review. Hum Reprod 2011;26:1971-80.