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

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

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Turkish Journal of Obstetrics and Gynecology published by Galenos Publishing House.

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 kadın ve sağlıklı kadın T-lenfosit alt populasyonlarını ve doğal öldürücü (NK) hücrelerini ölçümleri ve bununla iki grup arasında kıyaslanmasını amaçlamaktadır.

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

Sonuç: 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 immunofenotiplerle bulguları, CD8 T hücrelerinin sayısı [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 uyuşmuydu.

Anahtar Kelimeler: Kısırlık, hücresel başışıklık, 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.
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\(^1\). 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\(^2\).

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, cells, which produce interferon-gamma and lymphokinin-alpha, and TH cells, which produce IL-4, IL-5, and IL-13. As a third group, TH 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, cells, TC, cells, and newly discovered TC, that secretes Interleukin 17\(^4\).

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\(^3\).

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-

| Cell markers | Group | n  | Mean | Standard deviation | Minimum | Maximum | 95% Confidence Interval | p-value |
|--------------|-------|----|------|-------------------|---------|---------|-------------------------|---------|
| 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—0.67             | 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

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

Figure 1. Example of gating for standard proliferation 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                  | 0.40    |
|            | Control | 46 | 10.9  | 4.03              | 3       | 27.6    | 2.394                   |         |
| CD56, %    | Case  | 151| 8.19  | 5.40              | 1       | 31      | 0.735                   | 0.005*  |
|            | Control | 46 | 10.34 | 4.00              | 3       | 27      | 4.032                   |         |
| CD16+56, % | Case  | 151| 10.28 | 6.38              | 0.8     | 33      | -1.852                  | 0.82    |
|            | Control | 46 | 10.43 | 4.46              | 2       | 28      | 2.141                   |         |

*: 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.[19] 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.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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