

THE EFFECT OF POSTMENOPAUSAL HORMONE REPLACEMENT THERAPY ON THE NUMBER AND THE ACTIVATION OF T-CELL SUBPOPULATIONS AND ON THE NUMBER OF B CELLS AND NATURAL KILLER CELLS

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SUMMARY

Objective: The aim of this study is to determine the changes in circulating T-cell subpopulations, B cells and natural killer cells under continuous combined postmenopausal hormone replacement therapy.

Design: Thirty postmenopausal women were administered estradiol valerate 2 mg/d and norethisterone acetate 1 mg/d for three months. Immunophenotyping with flow cytometry was performed before and 3 months after hormone replacement therapy.

Setting: University Hospital

Patients: Thirty postmenopausal women

Intervention: Thirty postmenopausal women were administered estradiol valerate 2 mg/d and norethisterone acetate 1 mg/d for three months.

Main outcome measure: T cell subset numbers and activation, B cell and natural killer cell number

Results: The mean percentage of T cells (CD3+), cytotoxic T cells (CD8+), helper T cells (CD4+) did not significantly change after 3 months of hormone replacement therapy. The mean percentage of B (CD19+) cells significantly increased after 3 months of hormone replacement therapy ($p=0.01$). The mean percentage of active T cell (CD3+HLA-DR+) and natural killer cells (CD16+CD56+CD3-) decreased significantly after 3 months of hormone replacement therapy ($p<0.05$).

Conclusion: Hormone replacement therapy is associated with altered immune parameters. In this study, women taking hormone replacement therapy had decreased number of active T cells and natural killer cells.

Key words: immune system, postmenopausal hormone replacement therapy

ÖZET

Postmenopozal Kadınlarda Hormon Replasman Terapisinin T Hücre alt Grupları ve Aktivasyonu ile B Hücre ve Doğal Öldürücü Hücre Sayısı Üzerine Etkisi

Amaç: Bu çalışmanın amacı; postmenopozal sürekli kombine hormon replasman tedavisi alan hastalarda dolaşımdaki T hücre altgrupları, B hücreler ve doğal öldürücü hücrelerdeki değişiklikleri belirlemektir.

Planlama: Otuz postmenopozal kadına, 3 ay 2 mg/d östradiol valerate ve 1mg/d noretisteron asetat verildi. Hormon replasman tedavisi öncesinde ve üç ay sonrasında flow sitometri ile immünoflow sitometri yapıldı.

Ortam : Üniversite Hastanesi

Hastalar : Otuz postmenopozal kadın

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Girişim : Otuz postmenopozal kadına, 3 ay 2 mg/d östradiol valerate ve 1mg/d noretisteron asetat verildi.

Ölçülen parametreler: T hücre alt grupları, aktivasyonu, B hücresi ve NK hücresi sayıları

Bulgular: Hormon replasman tedavisinden 3 ay sonra, T hücrelerin (CD3+), sitotoksik T hücrelerin (CD8+), yardımcı T hücrelerin (CD4+) ortalama yüzdeleri arasında anlamlı değişiklik yoktu. Hormon replasman tedavisinden 3 ay sonra, B (CD19+) hücrelerin ortalama yüzdeleri anlamlı olarak yüksekti ($p=0.01$). Hormon replasman tedavisinden 3 ay sonra, active T hücre (CD3+HLA-DR+) ve doğal öldürücü hücrelerin ortalama yüzdeleri anlamlı olarak düştü ($p<0.05$).

Sonuç: Hormon replasman tedavisi immün parametrelerdeki değişiklikler ile ilişkilidir. Bu çalışmada, hormon replasman tedavisi alan kadınlarda active T hücrelerin ve doğal öldürücü hücrelerin sayısı azalmıştır.

Anahtar kelimeler: immün sistem, postmenopozal hormon replasman tedavisi

INTRODUCTION

The improvements of postmenopausal disorders after hormone replacement therapy is a well-known entity. Aging is generally associated with impaired immune responses measured by most indices either in vitro or in vivo⁽¹⁾. Aging is associated with a progressive increase in the number of circulating natural killer cells together with a decreased lytic activity per cell⁽²⁾. In addition, decreased numbers of both CD4+ and CD19+ cells and poor T-cell proliferative responses and high CD8+ percentages in 86-92 years old individuals were found to be associated with increased mortality in subsequent years⁽³⁾.

In patients with premature ovarian failure immunologic dysregulation, including decreased natural killer cell activity⁽⁴⁾, reduced CD4+ to CD8+ ratio⁽⁵⁾, and elevated numbers of CD8+ and natural killer lymphocyte subpopulations⁽⁶⁾ was demonstrated. And also, a lowered CD4+ to CD8+ ratio was reported in young individuals with estrogen deficiency due to gonadal dysgenesis or hypothalamic pituitary failure⁽⁷⁾.

In this study, we investigated the changes in T-cell subpopulations, B cell and natural killer cell numbers under continuous combined hormone replacement therapy in peripheral blood from postmenopausal women.

MATERIALS AND METHODS

This study consisted of 30 postmenopausal women who visited our clinic for hormone replacement therapy. The participating postmenopausal women suffered from hot flushes, sweating, sleeplessness and nervousness and sought medical relief. The diagnosis of menopause was confirmed on the basis of amenorrhea for a year as well as a serum FSH level of >40 mIU/mL and E2 level

of <20 pg/mL. The study program was approved by the ethics committee of our hospital, and each participant provided signed informed consent after receiving and extensive explanation of the examination. All of the participating women were free of recent infection and obvious clinical immunologic diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and Hashimoto thyroiditis. The postmenopausal women had not received hormone supplementation for at least six months before the study.

The hormone replacement regimen was composed of continuous usage of estradiol valerate 2 mg/d and norethisterone acetate 1 mg/d (Kliogest, Novo Nordisk). Peripheral venous blood was obtained on the day before and 3 months after hormone replacement therapy. The aspirated blood was collected in glass tubes containing ethylenediaminetetraacetic acid. To test whether there were any deviation in the proportions of T cells, B cells and natural killer cells and in the activation of T cells in peripheral blood, we performed immunophenotypic analysis by flow cytometry as described by Yang et al⁽⁸⁾. Monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) or with phycoerythrin (PE) were obtained (Becton Dickinson, San Jose, CA). Blood samples were incubated with monoclonal antibodies at 4°C for 30 minutes and then were washed twice in phosphate-buffered saline containing 2% fetal calf serum and 0.1% sodium azide. These samples were fixed with 0.5% paraformaldehyde. Immunofluorescence and dual-color flow cytometric analyses were performed using a FACScan cytofluorimeter (Becton Dickinson) with computer interface to software (Hewlett-Packard Consort 32; Becton Dickinson) for full-list-mode data storage, recovery, and analysis.

The following combinations of monoclonal antibodies

were used: anti-CD45 FITC/CD14 PE (LeucoGate), anti-CD3 FITC (T cells), anti-CD19 PE (B cells), anti-CD4 FITC (T helper cells), anti-CD8 PE (T cytotoxic cells), anti CD16 CD56 FITC (natural killer cells), anti HLA-DR FITC (active T cells).

LeucoGate was used to measure the proportion of lymphocytes in the sample being studied without any scatter gates. The gate was set around the lymphocytes (CD45+CD14-) to exclude other cells from analysis. The Simultest control (mouse IgG1 FITC+IgG2a PE) was used for background control. The doublets (ie, 2 cells either stuck together or very close in space) were strictly excluded from the calculation. In each cell suspension, 10.000 events acquired for gated lymphocytes were measured.

Paired student t test was used to compare differences before and at the end of the hormone replacement therapy. P<0.05 was considered statistically significant.

RESULTS

The mean age of the postmenopausal women was 50±3.3 (ranged 45 to 58 years) and the mean time since menopause was 4.9±3.3 (ranged 1 to 15 years) years. The results of immunophenotyping of various lymphocyte subpopulations are summarized in Table I. The results of immunophenotyping of T cells are summarized in Table II.

Table I: Various lymphocyte subpopulations in postmenopausal women before and after hormone replacement therapy

Percentage of lymphocytes	Before HRT	3 Mo after HRT	P value
T cells (CD3+)	65.5±8.4	67.6±8.3	0.12
B cells (CD19+)	8.9±2.0	10.4±3.0	0.01
NK cells (CD16+,CD56+, CD3-)	19.73±8.2	11.36±5.1	<0.001

Values are normal mean±standart deviation, Mo:Month, HRT: Hormone replacement therapy, NK: natural killer

Table II: T lymphocyte subpopulations in postmenopausal women before and after hormone replacement therapy

Percentage of T lymphocytes	Before HRT	3 Mo after HRT	P value
T helper cells (CD4+)	38.6±5.5	38.3±4.3	0.69
T cytotoxic cells (CD8+)	29.1±5.2	30.4±4.7	0.2
CD4+ to CD8+ ratio	1.38±0.4	1.28±0.24	0.13
Active T cells (CD3+, HLA-DR+)	5.4±3.2	4.06±2.5	0.045

Values are normal mean±standart deviation, Mo:Month, HRT: Hormone replacement therapy

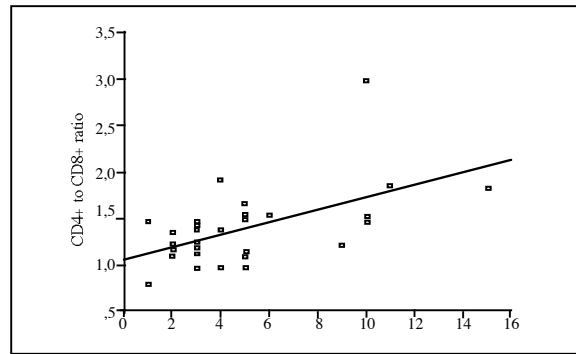


Figure 1: The graphic shows the positive correlation of CD4+ to CD8+ ratio with time since menopause (p<0.05)

The mean percentage of T cells (CD3+), cytotoxic T cells (CD8+), helper T cells (CD4+) did not significantly change after 3 months of hormone replacement therapy. The mean percentage of B (CD19+) cells significantly increased after 3 months of hormone replacement therapy (p=0.01) (Figure 2). The mean percentage of active T cell (CD3+HLA-DR+) and natural killer cells (CD16+CD56+CD3-) decreased significantly after 3 months of hormone replacement therapy (p<0.05) (Figure 3 and 4).

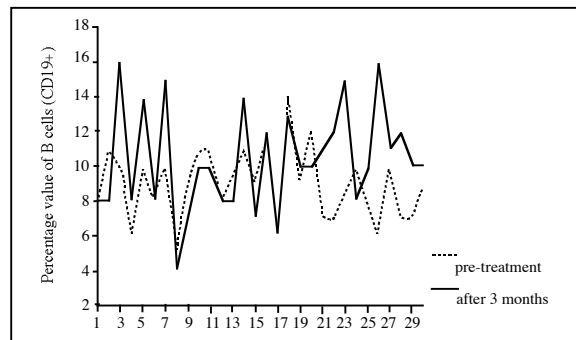


Figure 2: The percentage values of B cells (CD19+) before and 3 months after treatment with sequential combined hormone replacement therapy.

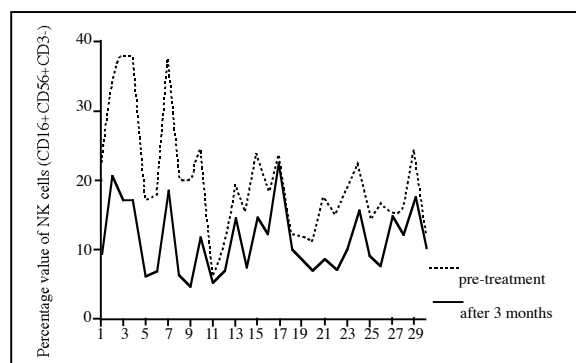


Figure 3: The percentage values of NK cells (CD16+CD56+CD3-) before and 3 months after treatment with sequential combined hormone replacement therapy.

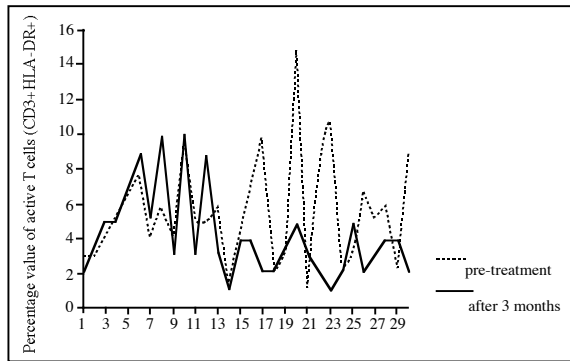


Figure 4: The percentage values of active T cells (CD3+HLA-DR+) before and 3 months after treatment with sequential combined hormone replacement therapy.

CONCLUSION

The capability of cells of the immune system to synthesize and respond to molecules classically designated as protein or peptide hormones has strengthened the concept of an integrated endocrine and immune system⁽⁹⁾. In the present study, the T-cell population did not change but the number of active T cells (CD3+ HLA-DR+) were decreased in postmenopausal women after hormone replacement therapy incompatible with previous findings^(8,10,11). Active T cell population may change in response to many environmental factors so that the observed change may not be meaningful.

Natural killer cells represent a third distinct functional population of lymphocytes in addition to T and B cells. In contrast to T cells, natural killer cells have direct HLA-unrestricted cytotoxicity against several benign and malignant target cells without the requirement of previous sensitization. Accordingly, natural killer cells play an important role in immunosurveillance to protect the body from viral infections and tumor development. In this study the number of natural killer cells decreased significantly after 3 months of hormone replacement therapy.

In the study of Yang et al⁽⁸⁾ natural killer cell cytotoxicity and the T-cell subpopulations of CD3+ CD25+ and CD3+HLA-DR+ were increased significantly after 6 months of postmenopausal continuous combined hormone replacement therapy. Brunelli et al⁽¹²⁾ investigated the effects of hormone replacement therapy on 10 healthy postmenopausal women on days 0, 8, 21, and 28 during the first month of treatment. CD4+CD45RO+ cells were found to be significantly reduced on day 8. CD56+ cells and

CD8+CD11b+ cells were decreased on day 21 and recovered basal level on day 28. Natural killer cell function was transiently increased on day 8 and greatly reduced on day 21. Fahlman et al⁽¹³⁾ investigated the effects of long-term hormone replacement therapy on selected indices of resting immune function in postmenopausal women. Those postmenopausal women taking hormone replacement therapy showed significantly higher lymphocyte proliferation and significantly lower natural cell-mediated cytotoxicity compared to controls.

Kamada et al⁽¹⁴⁾ reported a significant decrease in naive T cells and an increase in memory activated T cells occurred at late postmenopause (≥ 30 years postmenopausal period) compared to those at early postmenopause (≤ 10 years) ($P < 0.05$). Also, the percentage of lymphocytes in women on hormone replacement therapy was significantly higher than in untreated women at late postmenopausal stage ($P < 0.05$).

In elderly subjects the capacity for antibody production is depressed. This immunosenescence state of humoral immunity is associated with the occurrence of autoimmune disorders involving CD5+ B (B-1) cells. Since estrogen is capable of stimulating the production of autoantibodies, this sex steroid hormone may be a contributing cause of the higher incidence of autoimmune diseases in women. The effect of hormone replacement therapy on B cell subsets was examined to establish whether the administration of gonadal hormones influence humoral immunity in postmenopausal women⁽¹⁴⁾. In late postmenopausal women (≥ 30 years postmenopausal period), the proportion of B-2 cells was significantly reduced ($p < 0.01$) compared to those of premenopausal and perimenopausal women. Hormone replacement therapy induced a significant ($p < 0.01$) increase in the percentage of B-2 cells, while that of B-1 cells remained unchanged. Hormone replacement therapy did not affect autoantibody production. Hormone replacement therapy may retard the progress of immunosenescence by increasing the production of B-2 cells⁽¹⁵⁾.

In conclusion, postmenopausal hormone replacement therapy is currently being prescribed for prevention of a variety of conditions including osteoporosis, cardiovascular disease, stroke, and Alzheimer's disease yet hormone replacement therapy is often associated with altered immune system parameters. In this study,

women taking hormone replacement therapy had decreased number of active T cells and natural killer cells. Further longitudinal studies on postmenopausal women who have been investigated both before and during treatment are necessary to determine the impact of these alterations have on disease or disease prevention in long-term users.

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