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Immune effects of surgical menopause and estrogen replacement therapy in peri-menopausal women

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Abstract

The complex relationship between sex hormones and immune function suggests that sex hormone deficiency and estrogen replacement therapy (ERT) in post-menopausal women may have pleiotropic effects on immune function. For this reason, we aimed to investigate short-term effects of surgical menopause and ERT on immunity profile in peri-menopausal women. Seventeen healthy peri-menopausal women who were to undergo total abdominal hysterectomy and bilateral salpingo-oopherectomy (TAH + BSO) for uterine myoma were enrolled into this study. Three blood samples were collected from each patient: 1-day prior to surgery, 30 days after the operation (before ERT) and 30 days after transdermal ERT. The percentages of peripheral blood lymphocyte subpopulations, serum interleukin-4 (IL-4) and interferon-gamma (IFN-γ) concentrations were determined by flow-cytometry and ELISA, respectively. Following TAH + BSO, the percentage of $CD8^+$ cells was increased ($P < 0.001$) while the percentage of $CD19^+$ cells, serum IL-4, and IFN-γ concentration and the ratio of $CD4^+$ to $CD8^+$ cells were decreased ($P < 0.001$, $P < 0.001$, $P < 0.002$, and $P < 0.05$, respectively**)**. After ERT, this trend reversed and a decrease in the $CD8^+$ cells ($P < 0.001$), increase in the $CD19^+$ cells percentages ($P < 0.02$) and increase in serum IFN-γ concentration ($P < 0.002$) were observed. Although an increasing trend in the $CD4^+$ to $CD8^+$ ratio occurred by ERT, this was not significant. However, the decrease in the serum IL-4 concentration after TAH + BSO was not reversed by ERT. Hormone deficiency in post-menopausal women may cause an impaired immune response, and ERT can restore this phenomenon. Estrogen seems to have an important role in the regulation of immune function.

Keywords: Menopause; Estrogen replacement therapy; Lymphocyte; IL-4; IFN-γ

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1. Introduction

The increase in life expectancy during this century has resulted in the post-menopausal years constituting more than one-third of the lifespan of most women. Risks and benefits of post-menopausal estrogen replacement therapy (ERT) are still being discussed by a large number of investigators. A significant body of evidence suggests that the use of post-menopausal estrogen may have substantial beneficial effects in delaying atherosclerosis (Blum and Cannon, 1998), preventing bone loss (Rosen and Kessenich, 1997), delaying loss of cognitive function (Solerte et al., 1999), relieving menopause-associated hot flashes and mood changes, and reducing skin, and reproductive tract atrophy (Greendale et al., 1999). However, ERT is not completely without risk and can lead to potential detrimental effects, such as an increased risk of breast cancer, as most recently reported in Women's Health Initiative (Chlebowski et al., 2003) and Million Women Study (Beral, 2003).

The clinical literature suggests that estradiol plays a critical role in the regulation of immune function (Cutolo et al., 1996). The addition of estradiol to in vitro cultures of human lymphocytes enhances immunoglobulin secretion (Kanda and Tamaki, 1999), and in vivo treatment of mice with 17 β-estradiol causes an augmentation of antibody production against double-stranded DNA (Verthelyi and Ahmed, 1994). Similarly, both cellular and humoral immune responses have been found to be higher in hormone replacement therapy users than non-users (Porter et al., 2001). On the other hand, it has been suggested that elevated levels of estrogen make women susceptible to auto-immune diseases (Jansson and Holmdahl, 1998).

The complex relationship between sex hormones and the immune system suggests that sex hormone deficiency and ERT in post-menopausal women may have pleiotropic effects on immune function (Cutolo et al., 1995). There are relatively few studies investigating the effects of the menopause and hormone therapy on immunity profiles in humans. However, those studies are often cross-sectional (Cioffi et al., 2002), inconsistent due to differences in subject selection (e.g. differences in menopausal status or time since onset of menopause) and environmental factors (e.g. nutrition or alcohol intake etc) (Keller et al., 2001). Also, the effect of ERT on immunity profile has not so far been studied in surgically post-menopausal women. In this study, we aimed to investigate short-term effects of surgical menopause and transdermal ERT on immunity profile of peri-menopausal women who underwent total abdominal hysterectomy and bilateral salpingo-oopherectomy (TAH + BSO) for uterine myoma.

2. Materials and methods

Seventeen healthy peri-menopausal women who were to undergo TAH + BSO for uterine myoma were selected for this study. The subjects were selected from outpatients at the Division of Gynecology of Firat Medical Center in Elazig, Turkey, excluding those who had received estrogens during the three previous months and/or were affected by diabetes, thyroid or liver diseases, auto-immune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, vasculitis, myasthenia gravis, and multiple sclerosis), current or prior cancers and acute or chronic infection. They were not taking antihypertensive or antilipemic drugs, immunosuppressant, anti-rheumatic agents or antioxidant vitamins.

None were smokers. Intra-tracheal general anesthesia was chosen for anesthesia. In addition, all women in whom ERT was applied had a normal cervical cytology examination and mammography before inclusion in the study. All participants gave informed consent before participating to the study, and the study was approved by the local ethics committee.

Transdermal estradiol patches (17-β-estradiol, 50 μg per day once a week; Schering Alman Ilac ve Ecza Tic. Ltd. Sti. Istanbul, Turkey) were used for ERT. Blood samples were obtained following a standard clinical protocol at 9.00 a.m. from each subject by venepuncture, after an overnight fasting. The samples were taken from the subjects 1-day before surgery, 1-day before starting ERT (30 days after the operation), and on day 30th after the start of therapy. For immunophenotypic analysis, 2 ml of blood samples were collected in glass tubes containing ethylenediaminetetraacetic acid (EDTA), and analyzed within 1 h after collection. In order to test whether there were any deviations in the proportions of T, B, and NK cells in peripheral blood, we performed immunophenotypic analysis by flow-cytometry. The method has been described in detail previously (Ho et al., 1993a). Monoclonal antibodies (mAbs) conjugated with fluorescein isothiocyanate or with phycoerythrin (PE) were used (Becton Dickinson, San Jose, CA, USA). 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. A total of 10.000 gated cells were examined in each sample. After the immunophenotypic analysis, the $CD4^+$ to $CD8^+$ cell ratio was calculated.

The collected blood samples were centrifuged at 2500 g for 10 min at 4 °C. The serum layer was separated and frozen at −80 °C for cytokine analysis, which was performed in less than 2 weeks. Freezing/thawing cycles were avoided. The levels of interleukin-4 (IL-4) and interferon-γ (IFN-γ) were measured in serum using commercially available enzyme-linked immunosorbent assay kits (PeliKine® human IL-4 and IFN-$_\gamma$ ELISA kits, Amsterdam, The Netherlands). The sensitivities of the methods and kits were 0.2 and 1 pg/ml for IL-4 and IFN-γ, respectively. The range of these cytokine kits were 0–65 pg/ml for IL-4 and 0-450 pg/ml for IFN-γ. The assays were conducted according to the manufacturer's instructions, and absorbance measurements were made on an EL × 800 Universal Microplate Reader (Bio-Tek Instruments, USA) using WinSelect software. All measurements described above were carried out in duplicate due the to relatively small number of the sample population.

The data were analyzed using a non-parametric Wilcoxon Signed-Ranks Test, and expressed as mean ± S.D. It was considered that data were taken from the same patient and therefore linked. The level of significance was set at $P < 0.05$. All statistical analyses were performed with SPSS software package v. 9.05 from SPSS Inc., Chicago, IL, USA.

Table 1
General characteristics of the study group (mean ± S.D.)

Age (year)	49.2 ± 3.7
BMI (kg/m^2)	28.3 ± 2.7
Systolic BP (mmHg)	120 ± 15
Diastolic BP (mmHg)	85 ± 5

BMI: body mass index; BP: blood pressure.

Table 2
Immunophenotyping of lymphocyte sub populations (%) and serum cytokines (pg/ml) concentrations (mean ± S.D.) in peri-menopausal women

Immuno phenotypes and cytokines	Before TAH + BSO	After TAH + BSO	After ERT	*P*-value
$CD3^+$ (T-lymphocytes)	68.9 ± 12.6	69.8 ± 7.1	69.4 ± 6.0	NS
$CD4^+$ (T helper)	39.2 ± 10.8	41.9 ± 8.3	42.5 ± 6.4	NS
$CD8^+$ (T cytotoxic)	30.9 ± 8.5	35.2 ± 7.6	29.8 ± 7.9	1 vs. 2 (P <0.001) 2 vs. 3 (P <0.001)
$CD16^+$+$CD56^+$ (Natural killer cells)	10.0 ± 11.3	11.3 ± 7.3	14.5 ± 17.8	NS
$CD19^+$ (B-cells)	13.2 + 2.4	12.2 + 2.7	13.3 + 3.6	1 vs. 2 (P <0.001) 2 vs. 3 (P <0.02)
$CD25^+$ (Activated T-cells)	2.79 ± 0.80	3.08 ± 0.70	3.04 ± 0.67	NS
$CD14^+$ (Monocytes)	5.7 ± 1.5	5.8 ± 1.5	4.8 ± 1.5	NS
$CD4^+$ to $CD8^+$ cell ratio	1.54 ± 0.32	1.37 ± 0.28	1.50 ± 0.29	1vs. 2 (P <0.05) 2 vs. 3 NS
IL-4	2.5 ± 2.4	1.9 ± 2.3	1.8 ± 1.8	1 vs. 2 (P <0.001)
IFN-γ	8.2 ± 0.3	7.7 ± 0.7	8.2 ± 0.5	1 vs. 2 (P <0.002) 2 vs. 3 (P <0.001)

NS: not significant

3. Results

The ages, body mass indexes and blood pressures of the 17 patients who participated in the study are given in Table 1. The result of immunophenotyping of various lymphocyte sub populations and cytokine concentrations are summarized in Table 2. Percentages of $CD3^+$ (a T-lymphocyte marker), $CD14^+$ (monocyte marker) cells and $CD25^+$ (T-lymphocyte activation marker) in pre-operation, post-operation, and post-ERT samples were not changed.

Following TAH + BSO, the percentage of $CD8^+$ cells was increased while the percentage of $CD19^+$ cells (B-cells), serum IL-4 and IFN-γ concentrations and the ratio of $CD4^+$ to $CD8^+$ cells were decreased. After ERT, this trend reversed and a decrease in the $CD8^+$ cells, increase in the $CD19^+$ cell percentages, and increase in serum IFN-γ concentration ratio were observed. Although the reduced ratio of $CD4^+$ to $CD8^+$ cells was partially reversed, and an increasing trend in the $CD4^+$ cell to $CD8^+$ ratio occurred by ERT, this was not significant. However, the decrease in serum IL-4 concentration after TAH + BSO was not reversed by ERT.

4. Discussion

A bi-directional interaction between gonadal hormones and the immune system has been extensively investigated (Grossman, 1984; Olsen and Kovacs, 1996). Effects of estrogen are mediated by both estrogen α and β receptors (White and Parker, 1998). The estrogen receptors α and β have been found in a variety of murine and human immune cells, including thymocytes (Kohen et al., 1998), monocytes, B and T cells (Suenaga et al., 1998). Subjects with a sex hormone deficiency are reported to be susceptible to various kinds of immunologic impairments (Albrecht et al., 1996). There is also evidence that fertile women are more prone to auto-immune diseases than men, but this increased susceptibility disappears after menopause. This dual response in women has been attributed to the changes in gonadal hormones (Yang et al., 2000). To the best of our knowledge, changes in immunologic presentations after surgical menopause and ERT have not been elucidated in peri-menopausal women.

In the present study, an increase in $CD8^+$ cells and a decrease in the $CD19^+$ cell sub population, serum IL-4, and IFN-γ concentrations and reduced ratio of $CD4^+$ to $CD8^+$ cells after surgical menopause were observed. ERT reversed these trends, except for the serum IL-4 concentration and the $CD4^+$ to $CD8^+$ cell ratio. The increase in $CD8^+$ cell population and the decrease in the ratio of $CD4^+$ to $CD8^+$ cells in the surgical menopause cases are consistent with a previous observation in that $CD8^+$ cell percentages increased in women suffering premature ovarian failure (Pekonen et al., 1986). Furthermore, Ho et al. (1993b) reported an increased percentage of $CD8^+$ cells and reduced $CD4^+$ to $CD8^+$ cell ratio in premature ovarian failure cases, and that these deviations were restored by ERT. They suggested also that estrogen deficiency is the cause for the changes in the lymphocyte subsets. Since a decreased $CD4^+$ to $CD8^+$ cells ratio is considered as immunodeficiency (Parslow et al., 2001), we suggest that surgical menopause is accompanied by immunodeficiency, and hence post-menopausal ERT may partially prevent or restore this condition.

B-cells are the major lymphocyte sub population responsible for antibody production and maintenance of humoral immunity. It has been reported that B-cells decline by 40% with increasing age, and this reduction is associated with increased morbidity and decreased survival rate in elderly people (Huppert et al., 1998). The present findings showed that surgical menopause caused a decrease in the B-cell population, and ERT reversed this effect only after a month. Although we did not monitor these patients over a long period of time, it is seen that a menopause-related impairment in humoral immunity can be restored by ERT in short-term. Thus, we suggest that ERT may reduce B-cell related morbidity.

It has been suggested that IFN-γ is a key immunoregulatory cytokine secreted primarily by activated $CD4^+$ (T helper; Th), $CD8^+$, and NK cells (Ruemmele et al., 1998). IFN-γ mediates resistance against viral, intracellular bacterial, and protozoal infections. Our results revealed that serum IFN-γ levels decreased after surgical menopause, and ERT reversed these changes. Increased IFN-γ secretion by administration of estrogen to mice has also been shown (Karpuzoglu-Sahin et al., 2001a). Thus, it appears that estrogen may play a role via IFN-γ in the regulation of post-menopausal immunity.

Antigen-specific T-cell activation results in the differentiation of naive Th cells into Th1 and Th2 cells, based on the pattern of cytokine production and effectors function. Th1 cells produce IL-2, IFN-γ, and tumor necrosis factor-α. On the other hand, Th2 cells produce IL-4, IL-5, IL-10, and IL-13 (Mosmann and Coffman, 1989). In the present study, serum IFN-γ and IL-4 concentrations were decreased after menopause and ERT reversed the changes in serum IFN-γ, but not IL-4. In consistence with our findings, it has been reported that estrogen can modulate the secretion of IFN-γ without affecting secretion of IL-4 (Karpuzoglu-Sahin et al., 2001b). In addition, estrogen-induced elevations in IFN-γ might in turn inhibit an increase in serum IL-4 (Parslow et al., 2001). Therefore, we conclude that ERT may restore the decreased Th1 response in the post-menopausal women.

It may be argued that the effects of surgical stress could be responsible for the results of the menopausal condition. Previously, it has been reported that major surgery caused a transient immunosuppression, and normal function was restored after a few days (O'Flaherty and Bouchier-Hayes, 1999). Similarly, Ogawa et al. (2000) documented that such immunosuppressive effects of major surgery were restored within 2 weeks postoperatively, even after of large gastric cancer surgery. In addition, surgical stress induces a shift in Th1/Th2 balance towards Th2 responses, cell-mediated immunity is down-regulated, and antibody-mediated immunity is up-regulated after surgery (Decker et al., 1996; Ono and Mochizuki, 2000). In our study, samples were collected a month after the surgery, and the $CD19^+$ cell sub population and serum IL-4 (as Th2 response) concentrations were decreased. Thus, it appears that the findings observed here were a result of menopausal state rather than a reflection of surgical stress.

In summary, the results of the present study suggest that surgical menopause is accompanied by immunodeficiency as a result of decreased B-cell populations and serum IFN-γ levels. In addition, it appears that ERT can restore the changes in immune profile in peri-menopausal women. Thus, we confirm that estrogen has an important role in the regulation of immune functions. It should be noted that effects of surgical menopause and ERT on immune profiles were observed only for short-term (1-month) in the present study, and longer term studies on such patients need to be undertaken.

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