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The Impact of 17B Estradiol Level Variations on Blood Lymphocyte Counts Among Healthy Females

Sağlıklı Kadınlarda 17B Estradiol Düzeyi Değişikliklerinin Lenfosit Sayısı Üzerindeki Etkisi

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Abstract

Objective: To investigate the effects of 17β-estradiol (E2) levels on blood lymphocyte counts in women.
Methods: Blood samples were obtained from 428 healthy women. Serum E2 levels were measured using the solid-phase enzyme-labelled chemiluminescent method. Complete blood count was performed by a fully automated cell counter. White blood cell differential, including lymphocytes, was performed by volume, conductivity, and five-part scatter measurements (VCS technology). The participants were divided into four groups, according to the blood lymphocyte levels: <1000 cells/μL (Group A), 1001–2000 cells/μL (Group B), 2001–3000 cells/μL (Group C), and 3001–4000 cells/μL (Group D).
Results: The mean age of the participants was 37.03 years (between 15 and 66 years). E2 levels on admission in the study group ranged from <11 to 4264 pg/mL, and the median E2 level was 121.98. Twelve women had high levels of E2, as a result of the ovulation induction treatment. Total lymphocyte count varied between 0.9×10^3 to as high as 105×10^3 cells/μL, and the median lymphocyte count was 2.6×10^3 cells/μL. Lymphocyte–white blood cell ratio (LWR) was also studied in addition to lymphocyte levels. The lowest LWR value was 9.1 and the highest was 99.7. The medium E2 concentration was 40.3±11, 114±144, 117.2±128, and 192.2±595 in Groups A–D, respectively. The relationship between lymphocyte counts and LWR was positive, as expected. There were no statistically significant differences in lymphocyte counts either with age or E2 levels.
Conclusion: It is well established that estrogens affect lymphocyte functions in several ways. In the present study, we were unable to demonstrate a correlation between the E2 serum levels and blood lymphocyte counts in women.
Keywords: 17β-estradiol, lymphocyte, white blood cells

Öz

Amaç: Kadınlardaki 17β Estradiol (E2) düzeylerinin kan lenfositleri üzerine olan etkisini araştırmak.
Yöntemler: 428 sağlıklı kadından kan örnekleri elde edildi. Serum E2 düzeyleri, katı faz enzimiyle etiketlenmiş kemilüminesans yöntemi ile ölçüldü. Tam kan sayımı, tam otomatik hücre sayacı ile gerçekleştirildi. Beyaz kan hücresi diferansiyasyonu, lenfositler dahil olmak üzere, hacim, iletkenlik ve beş parçalı dağılım ölçümleri (VCS teknolojisi) kullanılarak gerçekleştirildi. Toplam lenfosit sayısına göre 4 grup oluşturuldu; <1000 hücre/μL (grup A), 1001-2000 hücre/μL (grup B), 2001-3000 hücre/μL (grup C), 3001-4000 hücre/μL (grup D).
Bulgular: Çalışmaya dahil olanların yaş ortalaması 37,03 (15-66 aralığında) olarak hesaplandı. Çalışma grubundaki hastaların başvuru anındaki E2 düzeyleri <11-4264 pg/mL arasında değişmekteydi, ortalama E2 düzeyi 121,98 idi. Ovülasyon indüksiyon tedavisi sonucunda 12 kadında E2 seviyeleri anormal yüksek saptandı. Toplam lenfosit sayısı, 0.9×10^3 ile 105×10^3 hücre/μL arasında değişmektedir ve medyanı 2.6×10^3 hücre/μL olarak hesaplanmıştır. Lenfosit düzeylerine ek olarak "Lenfosit/lökosit oranı" da (LWR) araştırılmıştır. En düşük LWR değeri 9,1, en yüksek değer 99,7 idi. Gruplardaki ortalama E2 seviyeleri sırasıyla 40,3±11, 114±144, 117,2±128 ve 192,2±595 idi (A'dan D'ye). Lenfosit sayısı ile LWR arasındaki ilişki beklendiği gibi pozitifti. Lenfosit sayısı ile ne yaş ne de E2 arasında gruplar arasında istatistiksel olarak anlamlı fark bulunamadı.
Sonuç: Östrojenlerin çeşitli mekanizmalarla lenfosit fonksiyonlarını etkilediği iyi bilinmektedir. Bu çalışmada, kadınlardaki serum E2 seviyeleri ile kan lenfosit sayısı arasında bir ilişki bulunamamıştır.
Anahtar sözcükler: 17 β estradiol, lenfosit, beyaz kan hücreleri

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INTRODUCTION

Estradiol (E2) is an important steroid hormone that has various effects on different tissues, while the main target is the female reproductive system. Three major estrogens, estrone, E2, and estriol, are synthesized primarily in the ovaries and placenta during pregnancy. E2 is the most common form found in non-pregnant women (1). E2 levels differ during the menstrual cycle, and E2 reaches its peak value at the end of the follicular phase (1, 2).

Lymphocytes, at the second rank of differential, are important members of white blood cells (WBCs), which govern the immune response. They play a role in antibody production and cell-mediated immune response; however, there is an increasing number of studies that investigate their unknown functions (3). The effect of E2 on lymphocyte count and functions has been investigated, and a negative correlation has been documented in some studies (4, 5). Autoimmune disorders are more common in women, which is probably due to estrogen-dependent mechanisms. Estrogens are claimed to cause thymic atrophy and suppress lymphocyte functions (6). Moreover, estrogen has various effects on splenocytes by regulating nitric oxide pathways, which is related to T-cell maturation (7). Estrogen analogues also enhance autoantibody production by B1 cells, particularly by increasing IgM synthesis (8).

There are several studies demonstrating the relationship between female sex hormones and immunomodulation. The number of WBC counts may be associated with altered estrogen levels in women. We have designed a preliminary study to understand the relationship between E2 levels and their effect on the lymphocyte count in peripheral blood.

METHODS

Samples were obtained, according to appropriate protocols, from 428 healthy women who were admitted to our clinic for several reasons. The stage of the menstrual cycle at that time was not considered. No pregnancy was noted among all the participants. The age at the time of blood sampling was noted separately. Blood samples were collected between 8:00 and 10:00 a.m. All participants underwent testing for complete blood count and serum 17-β E2 levels at the same time.

Venous blood samples were collected into K2-EDTA tubes and serum separator tubes (Beckton Dickonson and Company, NJ) for complete blood count and hormone assays, respectively. After centrifugation, the serum sample was removed. Serum E2 levels were measured by a solid-phase enzyme-labelled chemiluminescent method *(*IMMULITE 2000; Siemens Healthcare Diagnostic, UK); this is a competitive method wherein E2 in the sample competes with E2 conjugated with bovine ALP for the limited number of polyclonal anti-E2 antibodies. The signal is inversely proportional to the patient E2 level. Normal reference values for E2 were 27 - 400 pg/mL. Complete blood count was performed using a UniCel DXH800 fully automated cell counter (Beckman Coulter Inc, US). WBC differential, including lymphocytes, was performed by volume, conductivity, and five-part scatter measurements (VCS technology). This system integrates VCS technology with advanced computer algorithms to identify WBCs more accurately.

Four groups were formed according to the blood lymphocyte levels: Groups A–D within the defined levels: <1000 cells/μL (Group A), 1001–2000 cells/μL (Group B), 2001–3000 cells/μL (Group C), 3001–4000 cells/μL (Group D), respectively. Abnormal lymphocyte levels (>4001 cells/μL) were excluded from the study group. Median levels of E2 in each group were calculated separately.

Statistical Analysis

All the statistical analyses were performed using Version 17.3 Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA). Pearson correlation coefficient was used to compare all variables. We used the non-parametric Kruskal–Wallis test to determine whether differences (E2 and age) between the groups existed.

Ethics

The study was approved under the number "GO 16/371-13" by the Hacettepe University School of Medicine, Non-interventional Clinical Researches Ethics Board. We used the hospital computer system to collect the necessary data for this retrospective study; so written informed consent could not be obtained from all participants.

RESULTS

The mean age of study participants was 37.03±11. The youngest participant was 15 and the oldest 66 years old. Levels of E2 on admission in the study group ranged from 11 to 4264 pg/mL, and the median E2 level was 121.98 pg/mL (±25.2 pg/mL interquartile range IQR)). Twelve women had high levels of E2 (maximum physiological level is 450 pg/mL during the periovulatory phase), as a result of the ovulation induction treatment.

The median lymphocyte count of all participants was 2600 cells/μL (± 900 cells/μL IQR). The lymphocyte WBC ratio (LWR) was also studied in addition to lymphocyte levels. The reported lowest value of LWR was 9.1 and the highest was 99.7.

The median E2 levels were 40.3±11, 114±144, 117.2±128, and 192.2±595 pg/mL in groups A–D, respectively. There was a trend of an increase in lymphocyte counts with the increase in E2 levels (Table 1). We found that the E2 levels increased, but this was not statistically significant by age in Groups A–D. We also found the highest E2 level in Group D, which seems to be the result of an increased number of lymphocytes. There was no significant difference between the groups in terms of E2 and age (p=0.178 and p=0.826).

Correlations between all the parameters (age, lymphocyte level, LWR, and E2 level) were investigated using the Pearson correlation *test* (Table 2). The relationship between the lymphocyte count and LWR was positive, as expected. There were no significant differences in the blood total lymphocyte count neither with age nor E2 level.

The participants were separated into four groups according to their ages to determine the impact of age on the lymphocyte count (Figure 1). The highest mean lymphocyte count was detected among young individuals, whereas their mean E2 levels were 72 pg/mL. Menopause seems to be associated with low E2 levels, as expected, but there was no significant difference detected in this age group.

Table 1. Groups A–D defined according to lymphocyte counts, median estradiol level, and median age for each group

	Group A	Group B	Group C	Group D
	Lymphocyte count/uL <1000 (n=5)	Lymphocyte count/uL 1001–2000 (n=161)	Lymphocyte count/uL 2001–3000 (n=200)	Lymphocyte count/uL 3001–4000 (n=50)
Median Estradiol pg/mL	40.3±11	114±144	117.2±128	192.2±595
Median Age (Year)	38.6±11	36.9±11	36.7±11	38.1±11

Table 2. Pearson correlation test results (p and r values)

	17β-estradiol	Lymphocyte % (LWR)	Lymphocyte (absolute)	Age
17β-estradiol	-	p: 0.008 r: 0.096	p: 0.52 r: 0.083	p: 0.92 r: 0.071
Lymphocyte % (LWR)	p: 0.52 r: 0.096	-	p: 0.049 * r: 0.412	p: 0.38 r: 0.045
Lymphocyte (absolute)	p: 0.008 R=0.083	p: 0.049 *	- r: 0.412	p: 0.53 r: 0.036
Age	p: 0.92 r: 0.071	p: 0.38 r: 0.045	p: 053 r: 0.036	-

*Statistically significant

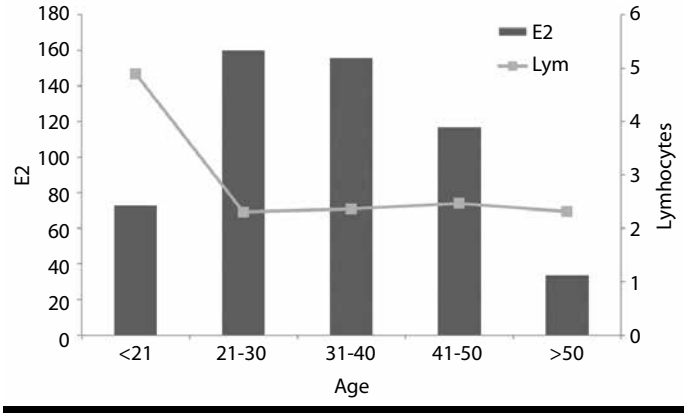

Figure 1. Median estradiol (E2) and lymphocyte levels in terms of age-dependent groups.

DISCUSSION

Estrogen has several functions on the nervous, immune, vascular, muscular, and endocrine systems in addition to its effect on the reproductive system (9). The incidence of autoimmune diseases is higher in females than in males. Estrogen hormones have important roles in both the adaptive and native immune systems, with different mechanisms. This well-known fact encourages investigators to clarify the effect of sex hormones on the immune system (10).

It is well established that estrogen influences the function of lymphocytes by several mechanisms in addition to their negative impact on blood levels. The effect of hormone replacement therapy on lymphocytes has been studied, and exogenous estrogen administration resulted in decreased T-cell levels and increased B-cell levels (2). We did not categorize our postmenopausal patients in terms of hormone replacement therapy due to limited data.

The T-cell differentiation and maturation steps in the thymus are complex and depend on multifactorial pathways. Gonadal hormones, estrogen and androgen, affect the bone marrow and thymus during lymphopoiesis. In addition, sex steroid hormones have been shown to decrease the thymus size and functions via complex mechanisms that result in thymic atrophy (6).

In the present study, we have shown that the median E2 levels were the highest in Group D, which may be due to increased lymphocyte counts. Previous studies have shown that postmenopausal women have decreased blood CD4+ and B lymphocyte levels. However, blood CD8+ cell counts have been found to be increased in these studies. Because our study was a descriptive study, we could not calculate CD4+ and CD8+ counts for all participants. We excluded values indicating abnormal lymphocyte counts (>4000 cells/μL) because they may be due to undefined infections, chronic inflammatory processes, or malignancies.

The main effect of sex hormones on the immune response is associated with the modulation of cytokine production via estrogen receptors (ER) (11). These nuclei-located peptides act like transcription factors if estrogen/estrogen agonists bind to these receptors (12). Estrogen regulates lymphopoiesis by binding surface *ERβ* on T cells and *ERα* on B cells dominantly. The different expression levels of ERs in several lymphoid tissues (thymus–bone marrow–liver) is the probable cause of different estrogen effects on the immune system (13).

The immunomodulatory function of estrogen has complex mechanisms on different tissues with various effects (14). The most important immunomodulatory effect is the increase in immunoglobulin synthesis (particularly IgG and IgM). However, testosterone suppresses immunoglobulin production, opposite to female sex hormones. It has been reported that E2 increases the levels of some cytokines, particularly IL-10, and immunoglobulin production alters due to these physiological reactions (15). The effects of sex hormones on inflammatory cytokines have been indicated in previous studies. Estrogen increases the production of some important mediators, such as IFN-γ, IL-4, and IL-12, with long-term treatment (14, 16).

It has been reported that estrogen stimulates T-cell-dependent immunity by activating gene expression on T and B lymphocytes. E2 enhances p-ERK 1/2, p-CREB, and p-Akt gene activations via an ER-dependent mechanism. It has also been shown that high levels of E2 cause an increase in superoxide dismutase, catalase, and glutathione peroxidase (11).

CONCLUSION

Estrogen affects the immune system via multifaceted mechanisms that have not yet been well documented. These preliminary data show that an increase within normal limits of E2 levels does not suppress blood lymphocyte counts among healthy women. Although we conducted our study carefully, there were some limitations. First, a small sample size does not reflect the entire women population. Second, E2 levels change during the menstrual period, which we could not clearly identify for each participant. Finally, it is not possible to claim that the blood lymphocyte count is affected only by the E2 levels. Further studies are necessary to clarify the complex relationship between estrogen and the immune response.

Ethics Committee Approval: Ethics committee approval was received for this study from the Hacettepe University Non-interventional Clinical Researches Ethics Board (No: GO 16/371-13).

Informed Consent: Written informed consent could not be obtained from all participants due to the retrospective design of study.

Peer-review: Externally peer-reviewed.

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