

# Proinflammatory and anti-inflammatory cytokine changes related to menopause

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## Abstract

**The aim of the study** was to determine menopause-related changes in serum levels of main proinflammatory and anti-inflammatory cytokines.

**Material and methods:** The study included 175 women, who were divided into 5 study groups (group 1 – fertile women; group 2 – pre- and perimenopausal women; group 3 – postmenopausal women; group 4 – surgically induced menopausal women; group 5 – women with chronic inflammatory pathology). We evaluated the serum levels of interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, IL-20 and of the tumour necrosis factor (TNF) $\alpha$  with the use of two multiplex cytokine kits. We also determined the serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), 17 $\beta$ -estradiol (17 $\beta$ -E2), progesterone (P), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) using sandwich ELISA.

**Results:** The serum level of IL-1 $\beta$ , IL-8 and TNF- $\alpha$  in women with natural menopause and in women with surgically induced menopause is significantly higher than in fertile women in the control group. In patients with surgically induced menopause and in women with natural menopause, IL-8 serum levels are similar to those seen in patients with chronic inflammatory diseases. There is a statistically significant decrease in serum levels of IL-20 in women with natural or surgical menopause than in fertile and premenopausal women.

**Conclusions:** Women in menopause have elevated levels of the key proinflammatory cytokines, i.e. IL-1 $\beta$ , IL-8 and TNF- $\alpha$  and low serum levels of IL-20 in comparison with fertile women.

**Key words:** cytokine, inflammation, interleukin, menopause, sex hormones.

## Introduction

Menopause is a turning point in every woman's life, the final episode of menstrual bleeding associated with cessation of the activity of ovarian follicle, resulting in the permanent cessation of menstruation. The onset of menopause is generally associated with a hormone deficiency, which is a contributory factor for the increased incidence of osteoporosis, cardiovascular diseases, vasomotor disturbances and cognitive deterioration [1]. Recent studies have established that the onset of menopause is associated with a low systemic inflammatory status, an inflammation manifested by increased serum levels of the key proinflammatory cytokines, interleukin (IL)-1, IL-6 or tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) [2, 3].

The relationship between hormonal declines associated with menopause and increased serum levels of proinflammatory cytokines is not yet fully understood. There are studies showing that postmenopausal women have elevated serum levels of proinflammatory cytokines, especially IL-6 and TNF- $\alpha$  [1, 2]. Also, a recent study demonstrated that elevated levels of IL-8 in premenopausal, perimenopausal and postmenopausal women and bilateral oophorectomized women with

severe hot flushes were significantly higher than those in women without hot flushes [4]. On the other hand, it has been shown that osteoporotic women have increased levels of proinflammatory and adipogenic cytokines at the level of the bone marrow supernatant fluid (BMSF) [5].

Conversely, anti-inflammatory cytokines are immunoregulatory molecules that control proinflammatory cytokine response and activity; their leading representatives are IL-1 antagonist receptor, IL-4, IL-10, IL-11 and IL-13 [6]. For example, IL-10 is a cytokine that is currently regarded as a potential therapy for inflammatory diseases involving T helper 1-type responses and it induces the differentiation of a subset of regulatory CD4+ T cells (Tr1). These cells were shown to inhibit Th1- and Th2-type inflammatory responses through the secretion of IL-10 [7].

All these studies suggest the involvement of proinflammatory cytokines in the pathology of most diseases associated with menopause, but none assess the relationship between proinflammatory and anti-inflammatory cytokines at the same time, thus characterizing a full cytokine profile in menopause. This study aims to evaluate proinflammatory and anti-inflammatory cy-

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tokine profile in relation to menopause and their relation to the hormonal status.

## Material and methods

### Subjects

The study was conducted between 01.02.2011 and 31.12.2011 in "Dominic Stanca" Obstetrics and Gynaecology Clinic, Cluj-Napoca, Romania.

The study included 175 patients admitted to the clinic, who were divided into five groups as follows: group I (control group) – 35 healthy non-pregnant women of reproductive age (aged 20-40 years old); group II (premenopausal women) – 40 healthy non-pregnant women in pre- and perimenopause (aged 46-53 years old), with regular menstruation or who had been without menstruation for no more than 6 months; group III (postmenopausal women) – 40 women in natural menopause (amenorrhea for at least 12 months) aged 54-65 years old, excluding patients with surgical or radiation induced menopause; group IV (surgically-induced menopause) – 35 women in surgically induced menopause (total hysterectomy with bilateral adnexectomy or simple bilateral adnexectomy) for at least 6 months, regardless of their age; none of the patients included in groups I-IV presented any acute or chronic inflammatory disease or were under recent corticosteroid treatment or under local oestrogen therapy or hormone replacement therapy; group V (chronic inflammation) – 25 non-pregnant women of reproductive age (aged 20-40 years old) with chronic inflammatory disease associated with low-grade systemic inflammation (psoriasis – 8 patients, systemic lupus erythematosus – 8 patients, antiphospholipid syndrome – 3 patients, alopecia areata – 2 patients, rheumatoid polyarthritis – 2 patients, scleroderma – 2 patients, endometriosis – 1 patient), excluding patients with natural or surgically induced menopause before the age of 40, patients under local oestrogen therapy or hormone replacement therapy in the past 12 months.

Before enrolment, the purpose of this study had been explained to all patients and their informed consent was received. The study was conducted under the Declaration of Helsinki. The study was approved by the Ethics Committee of the "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Data were collected for each subject included in the study in a form containing general and anthropometric data (weight, height), the heredo-collateral history, personal pathological history, and data on the age and onset of menopause, data on the symptoms that appeared after the onset of menopause. The body mass index (BMI) was calculated as the ratio between the weight (kg) and the squared height (in metres). 5 ml of venous blood was collected from each patient before breakfast, which was used to determine the complete

blood count. Blood was centrifuged and the serum obtained was stored at  $-20^{\circ}\text{C}$  for future determinations.

### Cytokine dosage

We used multiplex cytokine kits (Fluorokine MAP Human MultiAnalyte Kit; 2 pieces) in order to measure serum levels of 11 cytokines: IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, IL-20 and TNF- $\alpha$ . Dose measurements were performed with the use of a Luminex 200 system (Luminex Corporation, Austin, TX, USA) in accordance with the manufacturer's specifications (R&D Systems, Minneapolis, MN, USA). The sensitivity of the test was specified by the manufacturer (R&D Systems, Minneapolis, MN, USA) in the informative material included in the kits.

The average sensitivity of the test for IL-1 $\alpha$  was 0.24 pg/ml, and the intratest and intertest variation coefficients ranged from 6.0% to 6.6% and 9.3% to 14.3%, respectively. The average sensitivity of the test for IL-1 $\beta$  was 0.27 pg/ml, and the intratest and intertest variation coefficients ranged from 5.1% to 5.5%, and 7.4% to 10%, respectively. The average sensitivity of the test for IL-2 was 0.89 pg/ml, and the intratest and intertest variation coefficients ranged from 3.1% to 5.2%, and 9.7% to 13.2%, respectively. The average sensitivity of the test for IL-4 was 1.75 pg/ml, and the intratest and intertest variation coefficients ranged from 3.0% to 4.3%, and 9.4% to 15.9%, respectively. The average sensitivity of the test for IL-5 was 0.33 pg/ml, and the intratest and intertest variation coefficients ranged from 4.8% to 6.5%, and 4.5% to 9.5%, respectively. The average sensitivity of the test for IL-6 was 0.36 pg/ml, and the intratest and intertest variation coefficients ranged from 4.3% to 4.7%, and 5.9% to 7.9%, respectively. The sensitivity of the test for IL-8 was 0.39 pg/ml, and the intratest and intertest variation coefficients ranged from 4.6% to 7.8%, and 11.6% to 18.7%, respectively. The average sensitivity of the test for IL-10 was 0.13 pg/ml, and the intratest and intertest variation coefficients ranged from 5.2% to 6.4%, and 7.3% to 10.1%, respectively. The average sensitivity of the test for IL-17 was 0.39 pg/ml, and the intratest and intertest variation coefficients ranged from 3.6% to 5.3%, and 7.6% to 8.1%, respectively. The average sensitivity of the test for IL-20 was 2.63 pg/ml, and the intratest and intertest variation coefficients were 6.2% and 9%, respectively. The test for TNF- $\alpha$  revealed an average sensitivity of 0.6 pg/ml, with intratest and intertest variation coefficients that ranged from 3.7% to 4.8%, and 6.2% to 7.3%, respectively.

### Determination of steroid sex hormones

The steroid sex hormones were determined based on the samples stored at  $-20^{\circ}\text{C}$  on the EDTA according

to the technical specifications provided by the manufacturer.

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) serum values were evaluated using immunoenzymatic assays by sandwich ELISA, according to the specifications provided by the manufacturer (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany). The sensitivity of the tests was 0.22 mIU/ml in the case of LH, and 0.22 mIU/ml in the case of the FSH, while the coefficients of variation were  $\leq 9.21\%$  for intratest and  $\leq 7.91\%$  for intertest in the case of the LH, and  $\leq 5.1\%$  for intratest and  $\leq 7.6\%$  for intertest in the case of the FSH.

Serum levels of 17 $\beta$ -estradiol (17 $\beta$ -E2), progesterone (P) and dehydroepiandrosterone sulfate (DHEAS) were evaluated by competitive ELISA immunoenzymatic assays, according to the specifications provided by the manufacturer (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany). The sensitivity of the tests was 8.68 pg/ml for 17 $\beta$ -E2, 0.05 ng/ml for P, 0.03 mg/ml DHEAS. The coefficients of variation were  $\leq 9\%$  for intratests and  $\leq 10\%$  for intertest in the case of 17 $\beta$ -E2,  $\leq 4\%$  for intratests and  $\leq 9.3\%$  for intertests in the case of P, and  $\leq 5.7\%$  for intratests and  $\leq 9.6\%$  for intertests in the case of DHEAS.

To determine dehydroepiandrosterone (DHEA) we used ELISA competitive immunoenzymatic assay, in accordance with the manufacturer's specifications (DRG Instruments GmbH, Marburg, Germany). The sensitivity of the test was 0.108 ng/ml, with the coefficients of variation ranging between 3.84% and 6.92% in the case of the intratests, and 3.75% and 9.96% in the case of the intertests.

**Statistical analysis**

Data are presented as the group mean (SD) and median (1<sup>st</sup> quartile – 3<sup>rd</sup> quartile). We compared baseline data using a *t* test for continuous variables. Pearson's simple correlation allowed us to study the association between two variables. Statistical analyses were per-

formed using SPSS software (version 15.0, SPSS Inc, Chicago, IL) and STATA software (version 9.1, StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

**Results**

Table I presents all the parameters considered for the study. Significantly low values of 17 $\beta$ -E2 and *p* are noted, for menopause groups, as well as a significant increase in FSH and LH when compared to group 1 (controls) (*p* < 0.001).

Serum concentration of IL-20 was detectable in all women under the study. Serum concentrations of IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, IL-10, IL-17 and TNF- $\alpha$  were detected in 74.28%, 55.42%, 56.57%, 56.57%, 98.28%, 47.42%, 47.42%, respectively, that is 89.71% of the patients under the study. By contrast, serum concentrations of IL-1 $\alpha$  and IL-5 were detected in only 6.85% and 16.57%, respectively, of the patients.

IL-1 $\alpha$  showed undetectable levels in groups 1, 2, 3 and 4. In the case of fertile patients with chronic inflammatory diseases (group 5), 12 patients had detectable amounts of IL-1 $\alpha$  (mean (SD): 181.06 (42.98), median: 185.2), the remaining patients having values below the minimum detectable value. IL-5 was sporadically detected in groups 1, 2 and 5 and we could only find a single value in group 3 and no value in group 4. The data obtained on IL-5 could not be interpreted statistically.

As shown in Figure 1, IL-1 $\beta$  serum levels were significantly higher in patients with natural and surgically induced menopause compared with patients in the control group (mean 5.318, 3.479, and 2.317, respectively; *p* = 0.033, *p* = 0.05, and *p* = 0.014, respectively).

Regarding IL-2, IL-4 and IL-6, detectable values were present in groups 1, 2 and 5, while in groups 3 and 4, including women with natural and surgically induced menopause, their values were below the minimum detectable level. The results show that for all cytokines, the median serum levels showed no statistically significant differences between fertile patients and patients

**Tab. I.** Descriptive statistics of variables considered for the study

Variable	Calculated parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Age	Mean $\pm$ SD	38.2 $\pm$ 5.4	51.5 $\pm$ 0.7	58.2 $\pm$ 3.6	48.5 $\pm$ 3.1	34.5 $\pm$ 5.6
Weight	Mean $\pm$ SD	69.5 $\pm$ 14.2	72.0 $\pm$ 4.6	75.3 $\pm$ 13.5	73.4 $\pm$ 8.7	61.7 $\pm$ 5.8
BMI	Mean $\pm$ SD	25.4 $\pm$ 5.3	27.5 $\pm$ 3.9	29.1 $\pm$ 5.2	28.0 $\pm$ 3.4	24.7 $\pm$ 1.3
FSH	Mean $\pm$ SD	2.22 $\pm$ 1.91	12.3 $\pm$ 17.5	61.7 $\pm$ 30.4*	80.3 $\pm$ 35.9	4.17 $\pm$ 3.39
LH	Mean $\pm$ SD	5.47 $\pm$ 6.77	15.6 $\pm$ 10.6	20.5 $\pm$ 8.64	32.0 $\pm$ 12.8	6.75 $\pm$ 3.52
17 $\beta$ E <sub>2</sub>	Mean $\pm$ SD	22.7 $\pm$ 31.1	60.8 $\pm$ 133.0	0.115 $\pm$ 0.411*	1.68 $\pm$ 6.41	7.61 $\pm$ 6.77
Progesterone	Mean $\pm$ SD	4.04 $\pm$ 6.39	1.39 $\pm$ 4.50	0.137 $\pm$ 0.301	0.225 $\pm$ 0.276	2.98 $\pm$ 3.74
DHEA	Mean $\pm$ SD	12.4 $\pm$ 5.81	9.05 $\pm$ 4.08	8.56 $\pm$ 6.37	11.3 $\pm$ 7.96	10.8 $\pm$ 4.35
DHEAS	Mean $\pm$ SD	4.93 $\pm$ 3.60	1.59 $\pm$ 1.37	24.0 $\pm$ 55.5	3.30 $\pm$ 2.73	6.39 $\pm$ 4.30

\**p* < 0.001 compared to group 1

in pre-menopause. In contrast, fertile patients with associated chronic inflammatory diseases (group 5), have serum levels of IL-2, IL-4 and IL-6 that are significantly higher than in fertile and premenopausal patients without chronic inflammatory diseases. Table II presents mean IL-2, IL-4 and IL-6 levels.

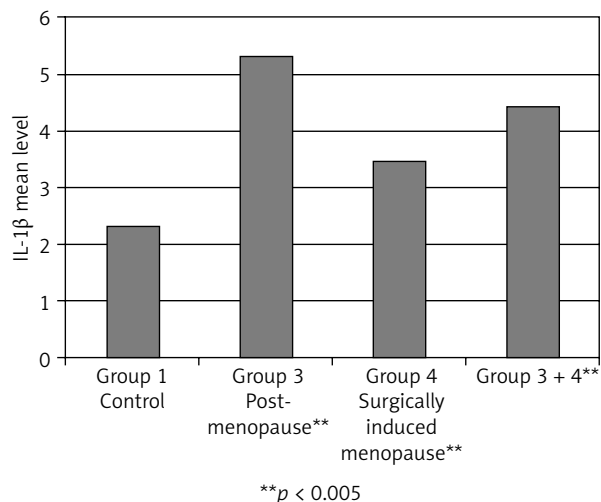
IL-8 serum levels were significantly higher in postmenopausal women and in women with surgically induced menopause compared to fertile women without chronic inflammatory disease ( $p < 0.001$ , and  $p = 0.045$ , respectively), while IL-8 serum levels in fertile patients without chronic inflammatory diseases were significantly lower than in those with inflammatory pathology ( $p = 0.02$ ). IL-8 serum levels in groups 3 and 4 – post-menopause and surgically induced menopause – are similar to those in group 5 – chronic inflammation. All these data are presented in Table III.

Table IV shows that there is no correlation between serum levels of IL-8 and  $17\beta$ -E2 or DHEA values in pre-, peri- and postmenopausal women and in patients with surgically induced menopause.

IL-10 and IL-17 had detectable values only in groups 1, 2 and 5, while in groups 3 and 4, their values were below the minimum detectable level. There were no statistically significant differences between the 3 groups for any of the two interleukins. Table V shows the serum levels obtained.

Regarding IL-20, we detected a statistically significant decrease in serum levels in postmenopausal women and with surgically induced menopause in comparison with those in fertile and premenopausal women (mean 1.001, 2.345, 3.153, and 3.371, respectively;  $p < 0.001$ , and  $p = 0.05$ , respectively). Figure 2 presents the mean IL-20 value among the 4 groups, the standard deviations and the  $p$ -values.

At the same time, Figure 3 shows that serum levels of TNF- $\alpha$  in postmenopausal women and in patients with surgically induced menopause were significantly higher compared with those in fertile women in the group control (mean 81.47, 107.03, and 49.39, respectively;  $p = 0.034$ ,  $p = 0.038$ , and  $p = 0.009$ , respectively).



**Fig. 1.** IL-1β levels in fertile women and in women with natural menopause or with surgically induced menopause

### Discussion

In our study we demonstrated changes in serum levels of 11 pro- and anti-inflammatory cytokines and changes occurring during the menopausal transition. IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, IL-20 and TNF- $\alpha$  serum levels were measured using a Luminox 200 system. Previous studies evaluated the possibility of detecting serum cytokines related to menopause, but with different limitations due to the small volume of the sample, or due to the small number of markers studied [8-11]. More recent studies using Luminox technology have managed to simultaneously evaluate a large number of cytokine markers, while also assessing the temporal reproducibility of these cytokines in serum samples [12-15].

In this study we showed that IL-1 $\beta$ , IL-8 and TNF- $\alpha$  serum levels are significantly higher in women with natural and surgically induced menopause than in fertile women in the control group. Moreover, in patients with natural and surgically induced menopause, IL-8 serum levels are similar to those seen in patients presenting chronic inflammatory diseases. A recent study confirmed the presence of elevated serum levels of IL-8 in

**Tab. II.** Comparison of serum levels of IL-2, IL-4 and IL-6 in study groups

Comparison between	IL-2			IL-4			IL-6		
	Mean (SD)	Median	$p$ -value	Mean (SD)	Median	$p$ -value	Mean (SD)	Median	$p$ -value
Group 1 vs. group 2	103.8 (5.99)	105.4	1.000	84.88 (1.468)	84.98	0.178	64.53 (5.119)	62.69	0.385
Group 1 vs. group 5	103.8 (5.99)	105.4	0.0004	84.88 (1.468)	84.98	0.014	64.53 (5.119)	62.69	0.039
Group 2 vs. group 5	104.7 (5.91)	105.4	0.0004	84.50 (0.707)	84.65	0.013	63.61 (3.685)	62.69	0.034
	187.3 (86.85)	171.5		133.5 (91.79)	98.32		91.73 (62.32)	68.85	

**Tab. III.** Comparison of serum levels of IL-8 depending in study groups

Compared groups	Mean	SD	p-value
Group 3 vs. group 1	233.0	226.5	< 0.001
	84.88	82.32	
Group 3 vs. group 2	233.0	226.5	< 0.001
	78.05	95.06	
Group 4 vs. group 1	148.0	162.0	0.045
	84.88	82.32	
Group 4 vs. group 2	148.0	162.0	0.029
	78.05	95.06	
Group 3, 4 vs. group 1, 2	193.4	202.3	< 0.001
	81.24	88.81	
Group 5 vs. group 1	152.3	121.0	0.020
	84.88	82.32	
Group 3 vs. group 4	233.0	226.5	0.063
	148.0	162.0	
Group 3 vs. group 5	233.0	226.5	0.067
	152.3	121.0	
Group 4 vs. group 5	148.0	162.0	0.907
	152.3	121.0	
Group 3, 4 vs. group 5	193.4	202.3	0.226
	152.3	121.0	

postmenopausal women presenting severe hot flushes compared with women without hot flushes; therefore, this cytokine may be involved in the occurrence and severity of hot flushes [4]. An association between serum levels of IL-8 and peripheral vasodilatation has also been reported [16]. Recent studies have suggested that IL-1 $\beta$  has a gene polymorphic role in the pathogenesis of osteoporosis in postmenopausal women, which is an independent risk factor for osteoporosis, but that IL-1 could participate in the primary reduction of bone mass in children [17-19]. On the other hand, it seems that TNF- $\alpha$  is involved in tumour-induced bone resorption and in non-tumour induced osteopenia [20].

We also found a statistically significant decrease in serum levels of IL-20 in women in menopause and in women in surgically induced menopause, than in fertile

**Tab. IV.** IL-8 association with 17 $\beta$ -E2 and DHEA

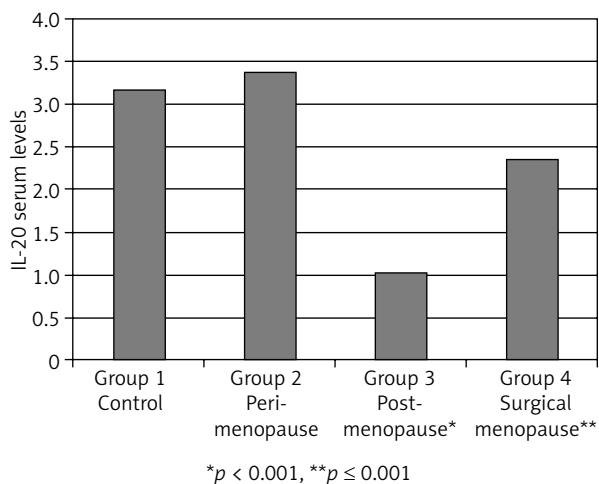
Group	Correlation between	Correlation coefficient	p-value
Group 2	IL-8 and 17 $\beta$ -E2	-0.223	0.166
	IL-8 and DHEA	0.034	0.835
Group 3	IL-8 and 17 $\beta$ -E2	-0.023	0.888
	IL-8 and DHEA	0.113	0.487
Group 4	IL-8 and 17 $\beta$ -E2	-0.213	0.219
	IL-8 and DHEA	0.001	0.996

and pre-menopausal women. It seems that IL-20 is involved in a number of diseases associated with chronic inflammation, such as psoriasis, rheumatoid arthritis and atherosclerosis [21]. It has been shown that IL-20 and its receptor are expressed in atherosclerosis plaques, which may be involved in the onset and progression of atherosclerosis and cardiovascular diseases [22]. At the same time, there are previous studies that reported elevated serum levels of IL-20 in obese women compared with normal weight women, and these levels decreased after weight loss [23]. Currently, there are no studies to investigate the changes in serum levels of IL-20 in relation to menopause. Our study does not support the pro-inflammatory effect of IL-20 after menopause, as the serum levels are lower compared to the genital activity period. On the other hand, the contradictory results could be due to different selection of patients and more studies are needed to clarify the IL-20 effect after menopause.

Regarding the other cytokines studied, except for IL-1 $\alpha$  and IL-5 whose values could not be interpreted statistically, IL-2, IL-4, IL-6, IL-10 and IL-17 were detected in groups 1, 2 and 5 in significant proportions, between 64% and 100% and were undetectable in groups 3 and 4. Failure to detect these cytokines in groups 3 and 4 could be a result of the use of regular assays for cytokine detection instead of high-sensitivity assays. In the case of IL-2, IL-4, IL-6, there was no statistically significant difference between fertile patients and premenopausal patients. In contrast, chronic inflammatory pathology-associated serum levels of IL-2, IL-4 and IL-6 were significantly higher than in women without chronic inflammatory diseases – in both fertile and premeno-

**Tab. V.** Comparison of serum levels of IL-10 and IL-17 in study groups

Comparison between	IL-10			IL-17		
	Mean (SD)	Median	p-value	Mean (SD)	Median	p-value
Group 1 vs. group 2	0.689 (0.809)	0.542	0.325	0.959 (1.125)	0.349	0.394
	8.129 (44.06)	0.542		1.245 (1.436)	0.349	
Group 1 vs. group 5	0.689 (0.809)	0.542	0.249	0.959 (1.125)	0.34	0.220
	31.75 (103.7)	0.709		55.85 (213.4)	0.998	
Group 2 vs. group 5	8.129 (44.06)	0.542	0.393	1.245 (1.436)	0.349	0.223
	31.75 (103.7)	0.709		55.85 (213.4)	0.998	

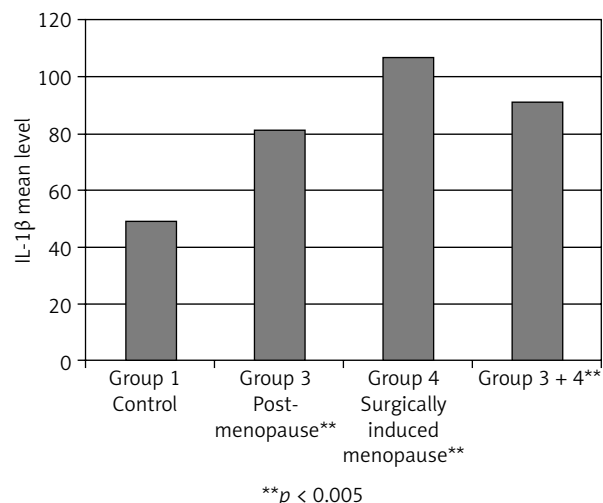


**Fig. 2.** The levels of IL-20 in fertile and in pre-, peri- and post-menopausal women

pausal women. At the same time, IL-10 and IL-17 do not show statistically significant differences between the 3 groups. Previous studies have reported an increase of IL-6 in menopause, this increase was present even in the absence of inflammatory diseases or trauma [2, 24, 25]. Also, recent studies show an increase in IL-2 and IL-6 in relation to menopause and their involvement in major depressive disorders [26, 27]. IL-4 and IL-10 are part of anti-inflammatory cytokines. There are studies showing an increase in inflammatory cytokines IL-4, IL-10 and IL-12, alongside TNF- $\alpha$ , after menopause, as a compensatory mechanism, by which these cytokines counteract to pro-inflammatory TNF- $\alpha$  [28]. As far as IL-17 is concerned, currently there are no studies to clarify its relation to menopause. Instead, there are numerous studies showing the pro-inflammatory role of IL-17 and its involvement in immune and autoimmune diseases, such as rheumatoid arthritis, asthma, lupus or antitumor immunity [29, 30]. Our study does not confirm nor deny the pro- and anti-inflammatory role after menopause of the above cytokines, due to the lack of serum levels in the groups including postmenopausal and surgically induced menopausal women.

Increased levels of pro-inflammatory cytokines and changes in serum levels of anti-inflammatory cytokines after menopause may be associated with monocytes and macrophages function, which is impaired due to oestrogen deficiency. The implications of these cytokines in the development and progression after menopause of important diseases such as osteoporosis, cardiovascular diseases, hot flushes or depressive syndrome, have been extensively studied, but there are still many gaps.

The main limitation of our study is lack of high-sensitivity assays usage instead of regular assays, as the former could probably offer the possibility to detect much smaller values of the cytokines studied and therefore small changes in their serum levels after men-



**Fig. 3.** Comparison of serum levels of TNF- $\alpha$  in patients with natural and surgically induced menopause compared with those in fertile patients

opause. However, the use of the multiplexed cytokine assay is a more effective alternative for the determination of several cytokines, by reducing the plasma volume required, the time needed, and by increasing sensitivity and reducing costs.

### Conclusions

Our study examined the changes that occur at menopause in the case of 11 pro-inflammatory and anti-inflammatory cytokines using the multiplexed cytokine assay. We have shown that IL-1 $\beta$ , IL-8 and TNF- $\alpha$  serum levels are significantly higher in women with natural and surgically induced menopause compared with those in fertile women in the control group, and that in fertile patients, without chronic inflammatory diseases, serum levels of IL-8 are significantly lower than in those with inflammatory pathology. By contrast, IL-8 serum levels in postmenopausal and surgically induced menopausal women are similar to those in women presenting chronic inflammatory pathology. We have also shown a statistically significant decrease in serum levels of IL-20 in women with menopause or surgically induced menopause than in fertile and premenopausal women. Further studies are needed to fully clarify all the discrete changes that occur in the serum levels of the key pro- and anti-inflammatory cytokines in relation to menopause.

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## Disclosure

Authors report no conflicts of interest.

## References

- Su HI, Freeman EW. Hormone changes associated with the menopausal transition. *Minerva Ginecol* 2009; 61: 483-489.
- Cioffi M, Esposito K, Vietri MT, et al. Cytokine pattern in postmenopause. *Maturitas* 2002; 41: 187-192.
- Yasui T, Uemura H, Tomita J, et al. Association of interleukin-8 with hot flushes in premenopausal, perimenopausal and postmenopausal women and bilateral oophorectomized women. *J Clin Endocrinol Metab* 2006; 91: 4805-4808.
- Ertek S, Cicero A. Impact of physical activity on inflammation: effects on cardiovascular disease risk and other inflammatory conditions. *Arch Med Sci* 2012; 8: 794-804.
- Pino AM, Rios S, Astudillo P, et al. Concentration of adipogenic and pro-inflammatory cytokines in the bone marrow supernatant fluid of osteoporotic women. *J Bone Miner Res* 2010; 25: 492-498.
- Benedusi V, Meda C, Della Torre S, et al. A lack of ovarian function increases neuroinflammation in aged mice. *Endocrinology* 2012; 153: 2777-2788.
- Wakkach A, Cottrez F, Groux H. Can interleukin-10 be used as a true immunoregulatory cytokine? *Eur Cytokine Netw* 2000; 11: 153-160.
- Gonzalez C, Cava F, Ayllon A, et al. Biological variation of interleukin-1beta, interleukin-8 and tumor necrosis factor-alpha in serum of healthy individuals. *Clin Chem Lab Med* 2001; 39: 836-841.
- Lang UE, Gallinat J, Danker-Hopfe H, et al. Nerve growth factor serum concentrations in healthy human volunteers: physiological variance and stability. *Neurosci Lett* 2003; 344: 13-16.
- Swiatecka D, Markiewicz LH, Wroblewska B. Experimental immunology: Pea protein hydrolysate as a factor modulating the adhesion of bacteria to enterocytes, epithelial proliferation and cytokine secretion – an in vitro study. *Centr Eur J Immunol* 2012; 37: 227-231.
- Ho GY, Xue XN, Burk RD, et al. Variability of serum levels of tumor necrosis factor-alpha, interleukin 6, and soluble interleukin 6 receptor over 2 years in young women. *Cytokine* 2005; 30: 1-6.
- Kaplan RC, Ho GY, Xue X, et al. Within-individual stability of obesity-related biomarkers among women. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 1291-1293.
- Ciebiada M, Kasztalska K, Gorska-Ciebiada M, et al. Basic research: Expression of IL-7 receptor in human peripheral regulatory T cells. *Arch Med Sci* 2013; 9: 555-560.
- Lee SA, Kallianpur A, Xiang YB, et al. Intra-individual variation of plasma adipokine levels and utility of single measurement of these biomarkers in population-based studies. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 2464-2470.
- Gu Y, Zeleniuch-Jacquotte A, Linkov F, et al. Reproducibility of serum cytokines and growth factors. *Cytokine* 2009; 45: 44-49.
- Wei M, Kuukasjärvi P, Laurikka J, et al. Relations of cytokines to vasodilatation after coronary artery bypass grafting. *World J Surg* 2003; 27: 1093-1098.
- Slotwinska SM. Cytokines and periodontitis. Part I: interleukin-1 and interleukin-1 receptor antagonist. *Centr Eur J Immunol* 2012; 37: 173-177.
- Chao TH, Yu HN, Huang CC, et al. Association of interleukin-1 beta (-511C/T) polymorphisms with osteoporosis in postmenopausal women. *Ann Saudi Med* 2010; 30: 437-441.
- Rusinska A, Chlebna-Sokol D. Evaluation of interleukin-1 and -6 in the etiopathogenesis of idiopathic osteoporosis and osteopenia in children. *Arch Immunol Ther Exp* 2005; 53: 257-265.
- Moffett SP, Zmuda JM, Oakley JJ, et al. Tumor necrosis factor-alpha polymorphism, bone strength phenotypes, and the risk of fracture in older women. *J Clin Endocrinol Metab* 2005; 90: 3491-3497.
- Hofmann SR, Rösen-Wolff A, Tsokos GC, et al. Biological properties and regulation of IL-10 related cytokines and their contribution to autoimmune disease and tissue injury. *Clin Immunol* 2012; 143: 116-127.
- Chen WY, Cheng BC, Jiang MJ, et al. IL-20 is expressed in atherosclerosis plaques and promotes atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 2006; 26: 2090-2095.
- Maiorino MI, Schisano B, Di Palo C, et al. Interleukin-20 circulating levels in obese women: effect of weight loss. *Nutr Metab Cardiovasc Dis* 2010; 20: 180-185.
- Kim OY, Chae JS, Paik JK, et al. Effects of aging and menopause on serum interleukin-6 levels and peripheral blood mononuclear cell cytokine production in healthy non obese women. *Age (Dordr)* 2012; 34: 415-425.
- Yasui T, Uemura H, Yamada M, et al. Associations of interleukin-6 with interleukin-1beta, interleukin-8 and macrophage inflammatory protein-1beta in midlife women. *Cytokine* 2008; 41: 302-306.
- Akyol S, Cinar SA, Purisa S, et al. Relationship between lymphocytes, IL2 and the hormones E2, LH, PRG and FSH in menopausal and postmenopausal women. *Am J Reprod Immunol* 2011; 66: 304-309.
- Liu Y, Ho RC, Mak A. Interleukin (IL)-6, tumour necrosis factor alpha (TNF- $\alpha$ ) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *J Affect Disord* 2012; 139: 230-239.
- Vural P, Canbaz M, Akgul C. Effects of menopause and postmenopausal tibolone treatment on plasma TNF $\alpha$ , IL-4, IL-10, IL-12 cytokine pattern and some bone turnover markers. *Pharmacol Res* 2006; 53: 367-371.
- Chiricozzi A, Guttman-Yassky E, Suárez-Fariñas M, et al. Integrative responses to IL-17 and TNF- $\alpha$  in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. *J Invest Dermatol* 2011; 131: 677-687.
- Kawaguchi M, Kokubu F, Fujita J, et al. Role of interleukin-17F in asthma. *Inflamm Allergy Drug Targets* 2009; 8: 383-389.