Interleukin-20 levels during menopause and relation with obesity

Abstract

Objective. *We aimed to assess serum levels of interleukin (IL) -20 in menopausal patients and its relationship to nutritional status.* ***Methods.*** *A number of 175 women were included in the study and divided into five groups, as follow: control group, pre-menopause group, post-menopause group, surgical menopause group and chronic inflammation group. We evaluated IL-20 serum levels and hormonal profile for each patient including the main steroid sexual hormones (luteinizing hormone, follicle-stimulating hormone, 17β-estradiol, progesterone, dehydroepiandrosterone - DHEA and DHEA sulfate) using Quantakinine Human IL-20 Immunoassay kits, and ELISA sandwich method, respectively.* ***Results.*** *Serum IL-20 levels in women with post-menopause and surgical menopause are significantly lower than in fertile women. In the case of fertile women, IL-20 is positively associated with body weight and body mass index (BMI). However, levels of IL-20 do not show significant differences between women who are overweight and those with normal weight, from the same age group.* ***Conclusions.*** *Women with natural or surgical menopause present significantly lower levels of IL-20, by comparison to fertile women. In the present study a possible association between IL-20 serum levels and menopausal status was investigated. Serum levels of IL-20 showed an association with body weight and BMI in fertile women.*

Keywords: *menopause, interleukin, overweight, steroid hormones*

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Introduction

Menopause is a natural process in the life of every woman, being generally considered as an unpleasant process both from a physical and a psychological standpoint. The average age for the onset of menopause is 50 years, with wide variations spanning from 41 to 59 years old. As the average life expectancy has increased in industrialized countries, reaching up to 80 years of age, it is obvious that the life period spent by women after menopause has significantly increased, currently surpassing a third of a woman's life[1].

Once menopause has begun, estrogenic deficit is one of the determining factors for the increase of osteoporosis incidence, cardiovascular diseases, vasomotor disorders and cognitive dysfunction[2]. At the same time, menopausal transition is associated with a significant weight increase, through shifting of the metabolic status due to estrogenic deprivation[3]. Obesity has currently become a public health concern at international level[4], being associated with cardiovascular diseases and an increase in morbidity and mortality through this type of disorder[5].

The consequences of menopause on different organs, such as bone, blood vessels or adipose tissue, cannot be explained through a single mechanism. The human immune system is influenced by modifications in the endocrine system, and by sexual hormone levels, respectively.

At present there is a wealth of studies which report a systemic inflammation of reduced impact during menopause, an inflammation which seems to form the basis of a majority of disorders associated with menopause[6,7,8]. At the same time, with the onset of menopause, the shift in the inflammatory status is triggered by the spontaneous increase of proinflammatory cytokines, the most important and frequently studied being the interleukin (IL)-1, IL-6 and tumor necrosis factor α (TNF-α)[9].

IL-20 is a member of the IL-10 cytokine family, disposing of approximately 28% of the amino acid sequence identical to IL-10[10]. It is a pleiotropic cytokine mainly expressed by monocytes, epithelial and endothelial cells, having inflammatory, angiogenic and chemoattractant effects[11]. Although IL-20 is part of the 2nd class cytokines, alongside IL-10, with which it shares an amino acid sequence and which is one of the main anti-inflammatory cytokines, apparently IL-20 is involved in a series of disorders associated with chronic inflammation, such as obesity, psoriasis, rheumatoid arthritis or atherosclerosis[12]. It has been demonstrated that IL-20 and its receptors are expressed in the atherosclerotic plaques, and that it could be involved in the onset and evolution of atherosclerosis and cardiovascular diseases[13].

At present, there are no documented studies in which IL-20 level has been evaluated in relation to menopause. The objective of our study is to evaluate IL-20 serum levels in women with pre-, peri- and postmenopause and to investigate the relationship between IL-20 and the nutritional status of the patients. At the same time, we evaluated serum levels of the main steroid sexual hormones (luteinizing hormone (LH), follicle-stimulating hormone (FSH), 17β-estradiol (E2) progesterone (P), dehydroepiandrosterone (DHEA) and DHEA sulfate) and their relationship to IL-20 levels, having in the view that hormonal deprivation during menopause is strongly associated with an increase in the number of proinflammatory cytokines, as well as related disorders.

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Methods

Patients

The study was assessed between 1st February and 31st December 2011 at the "Dominic Stanca" Obstetrics and Gynecology Clinic from Cluj-Napoca (Romania).

A number of 175 patients were included in the study, and distributed into five groups as follows: **Group 1 (control group)** consisting of 35 healthy, non-pregnant women at the reproductive age (20-40 years); **Group 2 (pre-menopause group)** consisting of 40 healthy, non-pregnant women, with pre- and peri-menopause (46-53 years old), with normal menstrual cycle or without menstruation for up to 6 months, in which patients with amenorrhea for longer than 6 months were excluded; **Group 3 (postmenopause group)** consisting of 40 women with natural menopause (amenorrhea for at least 12 months) with ages in the 54-65 years range. Patients with surgical menopause or irradiation menopause were excluded, as well as patients with amenorrhea for less than 12 months. The exclusion criteria for all the patients of the first three groups were a chronic inflammatory status (diabetes mellitus, renal disorders, viral hepatitis, antiphospholipid syndrome, endometriosis, vulvovaginal papillomatosis, rheumatoid polyarthritis, psoriasis, sclerodermia, autoimmune diseases, manifest clinical infections), recent anti-inflammatory or corticosteroid treatment, local estrogenic treatment or hormonal substitution therapy; **Group 4 (surgical menopause group)** consisting of 35 women with surgical menopause, (total hysterectomy with bilateral adnexectomy or simply with bilateral adnexectomy) of at least 6 months regardless of age, from which patients with surgical menopause of at least 6 months were excluded; **Group 5 (chronic inflammation group)** consisting of 25 non-pregnant women at the reproductive age (between 20 and 40 years old) with disorders associated with inflammatory processes. Patients with natural or surgical menopause before the age of 40 were excluded, as well as those receiving local estrogenic treatment or hormonal substitution treatment within the past 12 months.

Patients were enrolled from "Dominic Stanca" Obstetrics and Gynecology Clinic and were included in the study taken into account all inclusion and exclusion criteria's.

Before being considered for the present study, informed consent from all the patients was obtained. The study was approved by The Ethics Committee of the "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca (Romania).

For each patient included in the study a form was filled out containing general and anthropometric data (weight, height), family history, personal pathological history, data concerning the age of menopause onset and its evolution, data regarding the symptoms obser-

Table 1 Descriptive statistics of variables considered for the study in the 5 groups

Variable	Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Age	Mean ± st. dev.	38.2±5.4	51.5±0.7	58.2±3.6	48.5±3.1	34.5±5.6
Weight	Mean ± st. dev.	69.5±14.2	72.0±4.6	75.3±13.5	73.4±8.7	61.7±5.8
BMI	Mean ± st. dev.	25.4±5.3	27.5±3.9	29.1±5.2	28.0±3.4	24.7±1.3
FSH	Mean ± st. dev.	2.22±1.91	12.3±17.5	61.7±30.4*	80.3±35.9	4.17±3.39
LH	Mean ± st. dev.	5.47±6.77	15.6±10.6	20.5±8.64	32.0±12.8	6.75±3.52
17beta E2	Mean ± st. dev.	22.7±31.1	60.8±133.0	0.115±0.411*	1.68±6.41	7.61±6.77
Progesterone	Mean ± st. dev.	4.04±6.39	1.39±4.50	0.137±0.301	0.225±0.276	2.98±3.74
DHEA	Mean ± st. dev.	12.4±5.81	9.05±4.08	8.56±6.37	11.3±7.96	10.8±4.35
DHEAS	Mean ± st. dev.	4.93±3.60	1.59±1.37	24.0±55.5	3.30±2.73	6.39±4.30
IL-20	Mean ± st. dev.	3.15±1.72	3.37±1.15	1.01±1.32*	2.34±1.77**	1.54±1.53

**p<0,01 compared to 1st Group; **p≤0,05 compared to 1st Group*

ved after the onset of menopause. Body mass index (BMI) was calculated as the ratio between weight (in kg) and square height (in m). Inflammatory status was evaluated using erythrocyte sedimentation rate (normal values between 1 and 13 mm/at 1 hour) and leukocytes count. About 5 ml of venous blood were taken from each patient in the morning, on an empty stomach, and then analyzed to obtain the full hemoleucogram. The blood was centrifuged, and the serum obtained was stored at -20°C for subsequent dosage.

IL-20 dosage

In order to evaluate IL-20 serum levels, two Quantakinine Human IL-20 Immunoassay cytokine kits were used (R&D Systems Inc., Minneapolis MN). Dosage was carried out using the serum samples stored at -20°C, through the ELISA sandwich method, according to the specifications provided by the producer.

Dosage of the steroid sexual hormones

Dosage of the steroid sexual hormones was carried out using the test samples stored at -20°C on ethylenediaminetetraacetic acid. Serum LH and FSH levels were evaluated through immune-enzymatic tests, using ELISA Sandwich method (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany).

Serum levels of 17β-E2, P, DHEA, and DHEAS were evaluated through classical ELISA immuno-enzymatic tests (NovaTec Immunodiagnostica GmbH, Dietzenbach, DRG Instruments GmbH, Marburg, respectively Germany).

Statistical analysis

Data are presented as standard deviation (SD) and median (1st quartile - 3rd quartile). We compared baseline data using a 'T' Student test for continuous variables. Pearson's simple correlation allowed studying the association between two variables. Statistical analyses were performed 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). The power of statistical tests used for our study groups allows a hypothesis acceptance/rejection threshold of 0.05.

Results

1. Anthropometric and hormonal characterization of the groups

The parameters considered for the study are presented in Table 1. The significantly low values of 17β-E2 and P are noted, for menopause groups, as well as the significant increase of FSH and LH.

2. Interleukin-20 and menopausal status

Regarding IL-20, a statistically significant drop was detected in the serum levels in women at postmenopause (3rd group), and in those with surgical menopause (4th group), compared with fertile women (1st group) and those at pre-menopause (2nd group).

3. Interleukin-20 and BMI

IL-20 serum levels were positively associated with BMI of fertile women with chronic inflammation ($p<0.001$) (5th group), and also in fertile women without chronic inflammation (1st group) but in a lower grade ($p=0.062$). Whatsoever this association is no longer maintained for pre-, peri- and postmenopausal women (2nd and 3rd groups). The relationship between IL-20 and BMI is presented in Table 2.

Discussion

As far as we know, this is the first study in which the modifications of the IL-20 serum levels in relation with menopause are investigated. Regarding a possible relationship, there is only one study[12] which sustained a good association between IL-20, overweight and obesity. IL-20 was first identified in the Expressed Sequence Tag database[14] and it is classified as belonging to the IL-10 general class alongside IL-10, IL-19, IL-22, IL-24, IL-26, IL-28 and IL-29[15,16]. Most of the studies conducted until present showed that Il-20 plays an important role in the pathogenesis of a series of diseases associated with chronic inflammation such as psoriasis[17], rheumatoid arthritis[18], atherosclerosis[19], chronic renal insufficiency[20]. The over-expression of IL-20 in mice proved lethal through poor skin formation[21] a potential role of IL-20 in angiogenesis was designated[22].

Table 2 Correlation between IL-20 and BMI in fertile women, pre-, peri- and postmenopausal women

Group	Correlation coefficient	p-value
Group 1 (control patients)	0.318	0.062
Group 2 (pre and perimenopausal women)	0.014	0.930
Group 3 (postmenopausal women)	-0.117	0.471
Group 4 (surgical menopause women)	-0.028	0.873
Group 5 (chronic inflammation women)	0.807	< 0.001

Furthermore, it was showed that Il-20 plays an important role in the differentiation of osteoblasts and osteoporosis progression[23,24], whilst its blockage could prevent bone loss during menopause[25].

All these studies revealed a proinflammatory role of IL-20, even when it shows identical sequences to IL-10[26]. Menopause represents a pro-inflammatory state, which induces the growth of several proinflammatory cytokines (IL-1, IL-6, TNFα)[9,27]. IL-20 is produced both by monocytes, as well as by non-immune tissues in inflammatory disorders[28].

Therefore, a growth of this cytokines serum levels would be expected after menopause, at the same time with the onset of menopause associated pathologies. In our study, evaluation of IL-20 serum values evidenced a statistically significant drop in women with natural and surgical menopause, compared to fertile women, which does not support the proinflammatory role of this cytokine during menopause. Conversely, it could be the case that it is not the absolute serum value of IL-20 which represents the proinflammatory state of this cytokine, rather the increased expression of the latter in certain tissues and organs, such as the synovial liquid in patients with rheumatoid polyarthritis[29] or skin, in cases of patients with psoriasis[30]. On the other hand, it is possible that the change in IL-20 levels may be in relationship with aging, and thus the drop in IL-20 levels observed in our study in relation with menopause could be related to woman's age and not with a specific pathology. Another study which analyzed the association between IL 10 gene polymorphisms and recurrent pregnancy loss (RPL) which could have an impact on menopausal period among Romanian women demonstrated a role for - 819 C/T IL10 promoter polymorphism in idiopathic RPL[31].

Previous studies have reported increased IL-20 serum levels in obese women as compared to women of normal weight, as well as a drop in these levels after weight loss[12]. Our findings are in accordance with this hypothesis, but only in the case of fertile patients, excluding those after menopause, in which this association is not present anymore. This lack of correlation between IL-20 values and BMI after menopause could be due to a drop in its serum levels during this period.

Conclusions

Our study shows a significant drop of IL-20 in postmenopause women compared to fertile women. IL-20 serum levels are positively correlated with BMI in fertile women, a correlation which is no longer maintained after menopause.

This is the first study in which the modifications of the IL-20 serum levels in relation with menopause is investigated, and further studies are necessary in order to establish if IL-20 triggers pro-inflammatory activity after menopause or if it is directly involved in the onset of diseases associated with menopause. ■

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