SERBIAN ARCHIVES OF MEDICINE Est.1872

Address: 1 Kraljice Natalije Street, 11000 Belgrade, Serbia
☎ +381 11 4092 776, Fax: +381 11 3348 653
E-mail: office@srpskiarhiv.rs, Web address: www.srpskiarhiv.rs

Paper Accepted* **ISSN Online 2406-0895**

Original Article / Оригинални рад

Zorana Arizanović[1,†], Svetlana Vujović[1,2], Miomira Ivović[1,2], Milina Tančić-Gajić[1,2], Ljiljana Marina[1,2], Miloš Stojanović[1,2], Dragan Micić[1,2]

Hot flush values of gonadotropins and estradiol in the menopause

Вазомоторне вредности гонадотропина и естрадиола у менопаузи

[1] Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, Belgrade, Serbia
[2] School of Medicine, University of Belgrade, Serbia

Received: March 3, 2016
Revised: January 24, 2018
Accepted: January 29, 2018
Online First: February 13, 2018
DOI: https://doi.org/10.2298/SARH160303012A

* **Accepted papers** are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy edited and/or formatted in the publication house style, and the text may be changed before the final publication.
Although accepted papers do not yet have all the accompanying bibliographic details available, they When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

† **Correspondence to:**
Zorana Arizanović
Clinic for Endocrinology, Diabetes and Metabolic Diseases, 13 Dr Subotića Street, 11000 Belgrade, Serbia
E-mail: **drzarizanovic@gmail.com**

Hot flush values of gonadotropins and estradiol in the menopause

Вазомоторне вредности гонадотропина и естрадиола у менопаузи

SUMMARY

Introduction/Objective Hot flashes are one of the first clinical symptoms of the menopause. Mechanism of hot flashes is still not fully understood. Changes in concentrations of circulating follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen and other hormones can lead to thermoregulatory dysfunction.

The aim of this study was to examine the association between dynamic changes in concentrations of sex hormones and the presence of vasomotor symptoms in menopausal women.

Methods The study involved 36 subjects divided into two groups: in first group there was 24 women with hot flushes, BMI 26.16±3.42 kg/m^2, in control group was 12 women, BMI 26.82±3.89 kg/m^2. Data on the presence of hot flashes were based on history data. Venous blood samples were collected for analyses of FSH, LH, prolactin, estradiol, progesterone, and testosterone, sex hormone binding globulin, dehidroepiandrosteron sulfate, thyroid stimulating hormone and thyroxin. During the subjective feeling of hot flushes three blood samples during the day and night were collected to determine the mean levels of FSH, LH and estradiol in women with hot flushes.

Results Women with hot flashes had significantly higher prolactin (389.58±123.69mIU/L to 258.19±122.00mIU/l, p<0.01) and dehidroepiandrosterone sulfate (3.60±2.49nmol /L vs 1.88±1.27nmol/L,p<0.05) and lower mean values of FSH during hot flushes during the day (107.18±39.11IU/L vs 69.08±28.84IU/L, p<0.01) and night (104.57±38,06IU/L vs 60.72±21.89IU/L, p<0.01).

Conclusion Women with hot flushes have significantly lower mean FSH levels during hot flushes during the day and night than control group.

Keywords: hot flushes, menopause, sex hormones

САЖЕТАК

Увод/Циљ Валунзи представљају један од првих клиничких симптома менопаузе. Механизам настанка валунга још увек није у потпуности разјашњен. До терморегулаторне дисфункције доводе промене у концентрацијама циркулишућег фоликулостимулишућег хормона (ФСХ), лутеинизујућег хормона (ЛХ), естрадиола и других хормона.

Циљ ове студије био је да испита повезаност динамске промене концентрације полних хормона и присуства вазомоторних симптома у жена у менопаузи.

Методе Испитивањем је обухваћено 36 жена подељених у две групе: у првој су биле 24 жене са валунзима, индекса телесне масе (ИТМ) 26,16±3,42 кг/м^2, а у контролној групи 12 жена без валун-га, ИТМ 26,82±3,89кг/м^2. Подаци о присуству валунга су добијени на основу анамнезних података. У крви су одређене базне вредности хормона: ФСХ, ЛХ, пролактина, естрадиола, прогестерона, тестостерона, глобулина који везује полне стероиде, дихидроепиандростерон сулфата, тиреостимулишућег хормона и тироксина. Испитаницама са валунзима је узимана крв за одређивање вредности ФСХ, ЛХ и естрадиола током субјективног осећаја валунга у три узорка током дана и током ноћи.

Резултати Жене са валунзима су имале значајно виши пролактин (389,58± 23,69 мИЈ/Л према 258,19±122,00 мИЈ/Л; *p*<0.01) и дихидроепиандростерон сулфат (3.60±2.49 нмол /Л према 1.88± 1.27нмол/Л, *p*<0.05) а ниже средње вредности ФСХ током валунга током дана (107,18±39,11 ИЈ/Л према 69,08±28,84ИЈ/Л; п<0,01) и током ноћи (104,57±38,06ИЈ/Л према 60,72±21,89 ИЈ/Л; *p*<0,01).

Закључак Жене са валунзима су имале значајно ниже средње вредности ФСХ током валунга током дана и током ноћи у односу на контролну групу.

Кључне речи: валунзи, менопауза, полни хормони

INTRODUCTION

Hot flashes are one of the first clinical symptoms of the menopause, causing considerable distress and reducing quality of life of women [1]. They occur in climacterium in 4 out of 10 women over the age of 40 years. A recent review of the worldwide medical literature reported that the mean prevalence of vasomotor symptoms among women aged 40to 64 was 57% [2]; about 20% women described them as unbearable [3].

Hot flashes are characterized by the sudden appearance of redness of the face and trunk, a sense of unbearable heat, excessive and rapid sweating. Feeling of the heat wave usually starts from the chest and spreads to the neck and face. It is accompanied, sometimes, by chills and profuse sweating,

as well as reducing superficial skin temperature by 0.2 ° C. In most women, hot flashes are associated with sleep disturbances, anxiety, tension, depression, lack of concentration and reduced sexual desire, which leads to the overall decrease of quality of life.

The mechanism of hot flashes is still not fully understood. Since the central temperature is normal before the occurrence of hot flashes, it looks like that hot flush starts with a reduction in the sensitivity of the central thermostat in the hypothalamus, leading to the activation of mechanisms for heat dissipation and changes in the functioning of the autonomic nervous system. The results of these changes are: the vasodilatation of skin blood vessels, redness and sweating. Number of factors can lead to thermoregulatory dysfunction, such as changes in concentrations of estradiol (E_2), follicle stimulating hormone (FSH), luteinizing hormone (LH), serotonin, norepinephrin, calcitonine gene binding proteine and neuropeptid Y [4-6].

The aim of this study was to determine the association between the concentrations of sex hormones and the presence of vasomotor symptoms in menopausal women, as well as forming a group of women with hot flushes and high risk for cardiovascular diseases with the main purpose of preventing cardiovascular diseases.

METHODS

This study involved thirty-six women hospitalized in Clinic for endocrinology, diabetes and metabolic diseases, Clinical Center Serbia. The study group was consisted of twenty-four women with hot flushes, with average age of 51.83±4.48 years, with body mass index (BMI) 26.16 ±3.42 kg/m^2. In control group there were twelve women without hot flushes with average age of 57.17 ± 2.66 years and BMI 26.82±3.89 kg/m^2. The presence of the menopause was determined on the absence of menstrual cycles during the period of one year, estradiol levels lower than 50 pmol/L and FSH levels higher than 40 IU/L. Data on the presence of hot flashes are based on history data. They had no hypertension. Other diseases were excluded.

Anthropometric parameters were measured in patients: body height (BH), body mass (BM) and body-mass index (BMI). Venous blood samples were collected for analyses of follicle stimulating hormone, luteinizing hormone, prolactin, estradiol, progesterone, testosterone, sex hormone binding globulin (SHBG), dehidroepiandrosterone sulfate (DHEAS), thyroid stimulating hormone (TSH) and thyroxine (T4). Three blood samples during the day and night were taken from patients with hot flushes during the subjective feeling of hot flushes. Since that hot flushes occur more than three times a day, it was necessary to take blood samples for hormone analyses in the moment of the occurring of hot flushes. The time of sampling was defined by individual occurrence of hot flushes in menopausal women.

Plasma FSH (The ImmuChem FSH-CT IRMA kit, ICN Biomedicals, Inc., CV 2.6%), LH (The ImmuChem hLH IRMA kit, ICN Biomedicals, Inc., CA, USA, CV 2.4%), prolactin (The ImmuChem kit, ICN Pharmaceuticals INC, Canada, CV 7.0%), estradiol (ESTR-US-CT Cisbio, Bioassays, CV

2.8%), progesterone (PROG-CTRIA kit, proizvođača CIS Bio International, France, CV 3,5%) testosterone (TESTO-CT2, Cisbio International, CV 3.1%), SHBG (SHBG-RIACT, Cisbio International, France, CV 3.6%), DHEAS (ImmuChem^{TM} kit, ICN Biomedical INC, USA, CV 8,9%), TSH (IRMA hTSH kit, INEP Zemun, Serbia, CV 2,1%), T4 (RIA T4 kit, INEP Zemun, Serbia, CV 7,5%) were measured by radioimmunoassay.

Statistical analysis were conducted using the statistical program SPSS version 15 for Windows, having established that the data have a normal distribution. Statistically significant difference was considered when the significance level was less than 0.05 ($p<0.05$) and highly significant when the significance level was less than 0.01 ($p<0,01$).

Informed content was obtained from all patients. The study was approved by Ethic Committee of the Clinical Center of Serbia.

RESULTS

The mean age for the group with hot flushes was 51.83±4.48 years versus 57.17±2.66 years for the group without hot flushes. There was no statistically significant difference in BMI and age of menopause between groups of women.

Women with hot flushes had significantly more hot flashes during the day compared to night (6.75 to 4.5, $p<0.01$). They also had significantly higher prolactin (389.58±123.69 mIU/L vs. 258.19±122.00 mIU/L, $p<0.01$) and DHEAS (3.60±2.49nmol/L vs 1.88±1.27nmol/L, $p<0.05$) than women without hot flashes. There was no significant differences in concentrations of FSH, LH, estradiol, testosterone, SHBG, T4 and TSH between the two groups (Table 1).

Table 1. Baseline hormonal levels in women with and without hot flushes.

Hormones	Women with hot flushes	Women without hot flushes	*p*
FSH (IU/L)	64.80±16.44	68.00±33.41	0.70
LH (IU/L)	31.73±12.79	30.54±14.15	0.80
Prolactin (mlU/L)	389.58±123.69	258.19±122.00	0.01
Estradiol (pmol/L)	31.68±14.52	33.24±9.76	0.74
Progesterone (nmol/L)	4.44±2.35	2.05±1.57	0.01
Testosteron (nmol/L)	1.28±0.71	1.15±0.55	0.58
DHEAS (nmol/L)	3.60±2.49	1.88±1.27	0.03
SHBG (nmol/L)	56.13±37.78	73.96±38.64	0.19
T4 (nmol/L)	110.13±12.09	104.25±6.42	0.20
TSH (mlU/L)	2.02±1.64	1.10±0.63	0.08

FSH-follicle stimulating hormone; LH-luteinizing hormone; DHEAS-dehydroepiandrosterone sulfate; SHBG- sex hormone binding globulin; T4-thyroxin; TSH-thyroid stimulating hormon.

Table 2. Mean values of sex hormones during the day and night episodes of hot flushes.

Mean values	Women with hot flushes	Women without hot flushes	*p*
FSH day (IU/L)	69.08±28.84	107.18±39.11	0.01
FSH night (IU/L)	60.72±21.89	104.57±38.06	0.01
LH day (IU/L)	28.70±16.35	32.91±13.06	0.44
LH night (IU/L)	26.33±11.87	32.44±11.75	0.15
E_2 day (pmol/L)	28.17±10,06	30.63±13.56	0.54
E_2 night (pmol/L)	24.69±12.86	30.09±12.35	0.24

FSH-follicle stimulating hormone; LH-luteinizing hormone; E_2-estradiol

Table 2 shows the relationship between the mean values of sex hormones measured three times during the hot flashes in women with hot flushes

and mean values of sex hormones, taken randomly three times during the day and night, in women without hot flashes. Women with hot flushes had significantly lower mean concentrations of FSH during the day compared to controls (69.08±28.84 IU/L vs. 107.18±39.11 IU/L, $p<0.01$) and during the night (60.72±21.89 IU/L vs. 104.57±38.06 IU/L, $p<0.01$). Women with hot flushes had lower mean estradiol and LH levels during the day and night than women without hot flushes, but none of this differences were statistically significant.

DISCUSSION

Hot flashes occur as a result of changes in the concentrations of sex hormones that happen during the menopause. Elevated concentrations of FSH and LH accelerate the depletion of number of follicles and reduce the synthesis of estrogen and inhibin [7], leading to vasomotor instability and emergence of hot flushes [8, 9]. As estrogens are essential for normal reproductive function, low concentrations of estrogen lead to reduction in the number of ovulatory cycles and reduced fertility. In addition, low concentrations of estrogen and inhibin are not capable to overcome the negative feedback of FSH and LH, leading to even greater increase in the concentration of these hormones [10].

This study has shown that there is no difference in levels of FSH and LH in women with and without hot flashes. Dhanoya et al. [11] report that the higher FSH levels were significantly associated with the experience of hot flushes and Mitchell et all [12] showed that hot flush severity was significantly associated with higher FSH and lower estrone levels. Meldrum et al.[9] indicated that LH levels increased significantly during hot flashes. Other studies have shown that there was no difference between the concentrations of FSH or LH in women with and without hot flashes, [13-17], which was also obtained in this study.

Despite this studies, FSH and LH are not thought to be the crucial etiological hormones involved in hot flashes. Many investigators hypothesize that changes in endogenous estrogen levels are the primary etiological factor for hot flushes [18,19]. This hypothesis is suported indirectly by studies that show that estrogen replacement therapy reduces the number and intensity of hot flushes [19] and directly by a few studies that show that women with hot flushes have lower estradiol levels than women without them [8,18,20, 21], which was confirmed in this study, as well. Woods et al. [22] showed in their study that women with high severity hot flushes have lower levels of estrogen and higher levels of FSH. There are also studies that show no correlation between serum estradiol levels in women with hot flashes and women without them [13, 14, 16, 17, 23, 24].

After reviewing the literature, this is the first study measuring mean values of FSH, LH, and estradiol during the day and night episodes of hot flashes. In order to achive more accurate levels, hormone values were measured three times during the day hot flush episodes and three times during the night hot flush episodes, and average value was then calculated. It was found that the mean daily and nocturnal value of FSH were lower in women with hot flashes, which can be explained by the fact

that women without hot flashes were slightly younger than those without them. Women with hot flushes had lower mean estradiol and LH levels during the day and night than women without hot flushes, but none of this differences were statistically significant.

Whereas most studies have focused on the roles of FSH, LH and estrogens in the etiology of hot flushes, a few studies suggest that other sex horomones may be involved. Gallicchio et al. [21] and Ratka et al. [25] found that progesterone levels were lower in the group of women with hot flashes than women without them, which this study did not confirm. As the levels of progesterone in both groups were anovulatory, this difference is not clinically relevant.

In this study, we obtained that women with hot flushes had higher prolactin levels than the women without them, although the concentrations were in the normal value range. This could be explained by the fact that women who have hot flashes are more sensitive and vulnerable due to more activated symphatetic system. Similar results where shown in the study by Lambrionoudaki et all. [14]

Menopause is associated with a reduction in the overall synthesis of testosterone. Also, levels of androstenedione, DHEA and its conjugate, DHEA-S decline during the menopause. This study has shown that the levels of DHEAS were higher in women with hot flushes, while

no difference in testosterone levels was found between these two groups. Overlie et al. [8] showed in their study that women with hot flushes have more androstenedione leves, but there was no difference in testosterone and DHEA-S levels between women with and without hot flashes. Lambrinoudaki et all [14] found no difference in testosterone and androstenedione levels between women with and without hot flushes; Kaya et all. [24] showed that there was no difference in testosterone and DHEA-S levels, and similar results were shown by Ratka et al. [25].

Low SHBG levels are correlated with increased risk of cardiovascular diseases [26]. Several studies found no differences in the SHBG levels between women with and without hot flashes [13, 14], which was confirmed in this study. Randolph et al. [27] found that women with hot flushes have higher SHBG levels than women without hot flashes.

Although Overlie et al. [8] showed that women with hot flushes have higher TSH levels, the mechanisam that links the elevated TSH levels with the development of hot flashes is not yet known. Since TSH affects the metabolism of many hormones, including estradiol, we might assume that TSH increases metabolism of estradiol leading to hipoestrogenism. It is possible that TSH has a direct effect on the thermoregulatory center in the brain and enabling the emergence of hot flashes. Other studies found no difference in the concentration of TSH in women with and without hot flashes [14,17, 24], and the same result is demonstrated by our study.

CONCLUSION

According to our knowledge, thist is the first study with hormone levels measured during hot flushes. We have found that women with hot flushes have significantly lower mean FSH levels during

the day and night compared to control group. They also had lower mean LH and estradiol during the day and night, although none of this differences were statistically significant. There was not statistically significant difference between basal levels of FSH, LH and estradiol between these two groups of women.

Future studies should focus on dynamic assessment of hormone levels, meaning that they should be measured in shorter periods of time during the episodes of hot flushes in order of getting more precise data.

REFERENCES

1. Sturdee DW. The menopausal hot flush--anything new? Maturitas. 2008; 60(1): 42–9.
2. Makara-Studzińska MT, Kryś-Noszczyk KM, Jakiel G. Epidemiology of the symptoms of menopause - an intercontinental review. Prz Menopauzalny. 2014; 13(3): 203–11.
3. Skurnick JH, Weiss G, Goldsmith LT, Santoro N, Crawford S. Longitudinal changes in hypothalamic and ovarian function in perimenopausal women with anovulatory cycles: relationship with vasomotor symptoms. Fertil Steril. 2009; 91(4): 1127–34.
4. Shanafelt TD, Barton DL, Adjei AA, Loprinzi CL. Pathophysiology and treatment of hot flashes. Mayo Clin Proc. 2002; 77(11): 1207–18. Erratum in: Mayo Clin Proc. 2004; 79(8): 1088.
5. Whiteman MK, Staropoli CA, Benedict JC, Borgeest C, Flaws JA. Risk factors for hot flashes in midlife women. J Womens Health (Larchmt). 2003; 12(5): 459–72.
6. Rapkin AJ. Vasomotor symptoms in menopause: physiologic condition and central nervous system approaches to treatment. Am J Obstet Gynecol. 2007; 196(2): 97–106.
7. Richardson SJ, Senikas V, Nelson JF. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. J Clin Endocrinol Metab. 1987; 65(6): 1231–7.
8. Øverlie I, Moen MH, Holte A, Finset A. Androgens and estrogens in relation to hot flushes during the menopausal transition. Maturitas. 2002; 41(1): 69–77.
9. Meldrum DR, Defazio JD, Erlik Y, Lu JK, Wolfsen AF, Carlson HE, et al. Pituitary hormones during the menopausal hot flash. Obstet Gynecol. 1984; 64(6): 752–6.
10. Burger HG. The endocrinology of the menopause. Maturitas. 1996; 23(2): 129–36.
11. Dhanoya T, Sievert LL, Muttukrishna S, Begum K, Sharmeen T, Kasim A, et all. Hot flushes and reproductive hormone levels during the menopausal transition. Maturitas. 2016; 89: 43–51.
12. Mitchell ES, Woods NF. Hot flush severity during the menopausal transition and early postmenopause: beyond hormones. Climacteric. 2015; 18(4): 536–44.
13. Tuomikoski P, Ylikorkala O, Mikkola TS. Menopausal hot flashes and insulin resistance. Menopause. 2012; 19(10): 1116–20.
14. Lambrinoudaki I, Augoulea A, Armeni E, Rizos D, Alexandrou A, Creatsa M, et al. Menopausal symptoms are associated with subclinical atherosclerosis in healthy recently postmenopausal women. Climacteric. 2012; 15(4): 350–7.
15. Akiyoshi M, Kato K, Owa Y, Sugiyama M, Miyasaka N, Obayashi S, et al. Relationship between estrogen, vasomotor symptoms, and heart rate variability in climacteric women. J Med Dent Sci. 2011; 58(2): 49–59.
16. Hoikkala H, Haapalahti P, Viitasalo M, Väänänen H, Sovijärvi AR, Ylikorkala O, et al. Association between vasomotor hot flashes and heart rate variability in recently postmenopausal women. Menopause. 2010; 17(2): 315–20.
17. Hautamäki H, Piirilä P, Haapalahti P, Tuomikoski P, Sovijärvi AR, Ylikorkala O, et al. Cardiovascular autonomic responsiveness in postmenopausal women with and without hot flushes. Maturitas. 2011; 68(4): 368–73.
18. Erlik Y, Meldrum DR, Judd HL. Estrogen levels in postmenopausal women with hot flashes. Obstet Gynecol. 1982; 59(4): 403–7.
19. Bachmann GA. Vasomotor flushes in menopausal women. Am J Obstet Gynecol. 1999; 180(3 Pt 2): S312–6.
20. Wilbur J, Miller AM, Montgomery A, Chandler P. Sociodemographic characteristics, biological factors, and symptom reporting in midlife women. Menopause. 1998; 5(1): 43–51.
21. Gallicchio L, Miller SR, Kiefer J, Greene T, Zacur HA, Flaws JA. Risk factors for hot flashes among women undergoing the menopausal transition: baseline results from the Midlife Women's Health Study. Menopause. 2015; 22(10): 1098–107.

22. Woods NF, Cray L, Mitchell ES, Herting JR. Endocrine biomarkers and symptom clusters during the menopausal transition and early postmenopause: observations from the Seattle Midlife Women's Health Study. Menopause. 2014; 21(6): 646–52.
23. Gordon JL, Rubinow DR, Thurston RC, Paulson J, Schmidt PJ, Girdler SS. Cardiovascular, hemodynamic, neuroendocrine, and inflammatory markers in women with and without vasomotor symptoms. Menopause. 2016; 23(11): 1189–98.
24. Kaya C, Cengiz H, Yeşil A, Ekin M, Yaşar L. The relation among steroid hormone levels, lipid profile and menopausal symptom severity. J Psychosom Obstet Gynaecol. 2017 ;38(4): 284–91.
25. Ratka A, Miller V, Brown K, Jenschke M, Simpkins J. Association of various dimensions of hot flashes with systemic levels of gonadal steroids. Exp Biol Med (Maywood). 2009; 234(4): 395–402.
26. Karim R, Hodis HN, Stanczyk FZ, Lobo RA, Mack WJ. Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. J Clin Endocrinol Metab. 2008; 93(1): 131–8.
27. Randolph JF Jr, Sowers M, Bondarenko I, Gold EB, Greendale GA, Bromberger JT, et al. The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. J Clin Endocrinol Metab. 2005; 90(11): 6106–12.

