

Plasma urotensin-2 as a marker for menstrual irregularities in women: a clinical study

D. Cetin¹, M. Yayla¹, Y. Baykus², R. Deniz²

¹Department of Pharmacology; ²Department of Gynecology & Obstetrics; Kafkas University Faculty of Medicine, Kars (Turkey)

Summary

Purpose of Investigation: The objectives of this study were to evaluate urotensin 2 (UT-2) serum levels, a potent vasoconstrictor, in women with irregular menstrual irregularities and to determine its course in irregular menstrual cycles for the first time in the literature. It aims to determine the possible physiological roles of UT-2 by comparing the hormonal changes occurring in women with menstrual irregularities and their UT-2 levels. **Materials and Methods:** In this study, 120 patients with irregular menstruation was collected on day 3 of the menstrual cycle. Patients were divided into four groups: oligomenorrhea, hypomenorrhea, polymenorrhea, and menorrhagia/ menometrorrhagia. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), prolactin (PRL), and UT-2 levels were measured in serum. **Results:** Serum FSH, LH, and TSH levels were not significantly different between the groups. In this study a correlation test between UT-2 and PRL levels was carried out. This resulted in a statistically inverse correlation between increased UT-2 and decreased PRL levels among the groups being shown. **Conclusion:** It is interesting to note that the levels of urotensin in this study were higher in women with menstrual irregularities than in healthy individuals and that serum PRL levels in women with menstrual irregularities were lower than those in the control group, while still remaining within normal range.

Key words: Menstruation; Irregularity; Women; Urotensin 2; Prolactin.

Introduction

Irregular menstruation, an important public health problem, leads to serious problems both in a social sense and in terms of the state budget. The normal menstrual cycle is accompanied by bleeding, with an average total bleeding amount of 50 ml, which is completed within 21 to 35 days and lasts for an average of five days. Increased or more frequent menstrual bleeding is defined as abnormal uterine bleeding. Terms such as oligomenorrhea, polymenorrhea, menorrhagia, menometrorrhagia, and hypomenorrhea are used to describe abnormal uterine bleeding [1, 2].

The causes of abnormal uterine bleeding can be examined under two titles of organic and endocrinological causes. Endocrinological causes: irregular bleeding is most often due to anovulation [3, 4]. In the absence of postovulatory progesterone, ovarian estradiol production stimulates endometrial proliferation until bleeding occurs. Such bleeding where there is no other organic pathology other than anovulation is termed “dysfunctional uterine bleeding” (DUB) [5, 6]. While the most common cause of DUB is anovulation, it can also be seen in ovulatory patients. It is thought that the defects in corpus luteum function are responsible for this. Insufficient progesterone production of the corpus luteum or inadequate progesterone receptor number and function in the endometrium are thought to be responsible for this condition, called luteal phase insuffi-

ciency. The causes of premenstrual staining or early onset of menstruation are the same. Hormonal causes in anovulatory patients with abnormal bleeding should be investigated [7]. Serious bleeding can occur in women with hypothyroidism. Although the main cause is unknown, an alteration of hormonal metabolism and anovulation are suggested as possible mechanisms [8, 9]. As in adenomas with increased prolactin (PRL) or growth hormone secretion, primary pituitary diseases can also lead to anovulation. Increased PRL secretion is frequently seen in patients with secondary amenorrhea in particular [10]. It is known that elevated PRL or hyperprolactinemia (a serum PRL level of 35 and above) causes menstrual irregularities. The resulting menstrual irregularities result more in lack of menstruation rather than frequent menstruation. In the case of hyperprolactinemia, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) suppression is also seen [11].

Organic causes however are known as leiomyomas and endometrial pathologies, pregnancy complications, coagulation disorders, infections, endometriosis, contraceptive methods, and malignancy [2].

The pathophysiology of menstrual irregularity has not been fully elucidated in clinical trials conducted to this day. Stress, weight loss or gain, systemic diseases, and drug intake are other causes of menstrual irregularity [7]. It is known that in organisms under stress, many biochemical and physiological events experience changes. Changes in

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the endocrine system in particular cause hormonal imbalances [12].

Urotensin 2 (UT-2) is the most potent vasoconstrictor identified to date. UT-2 is synthesized in many tissues of the body outside of the vascular beds and is found in the central nervous system in particular among other tissues [13]. UT-2 is also known as a somatostatin analogue and is synthesized in the same gene locus as somatostatin. It is known that UT-2, a cyclic peptide, plays an important role in the pathophysiological processes that develop with stress. Therefore, it is thought that it may play an important role on the endocrine system in the release of hormones such as growth hormone (GH), thyroid stimulating hormone (TSH) and prolactin [14]. However, how UT-2 affects the endocrine system is not exactly known and it has not been assessed before. The objective of this study was to evaluate a potent vasoconstrictor, UT-2 serum levels, in women with irregular menstrual irregularities, and to determine its course in irregular menstrual cycles. It aims to determine the possible physiological roles of UT-2 by comparing the hormonal changes occurring in women with menstrual irregularities and their UT-2 levels.

Materials and Methods

In this study, the blood of 120 patients who presented to Kars Kafkas University Training and Research Hospital Obstetrics and Gynaecology Clinic with irregular menstruation was collected on the third day of the menstrual cycle, the follicular phase. The study was approved in a meeting of the Kafkas University Faculty of Medicine Noninvasive Clinic Researches Ethical Committee (2016/01/03).

Patients whose menstrual irregularities were determined after the evaluation of the patients by a specialist physician were divided into four groups (oligomenorrhea, hypomenorrhea, polymenorrhea, menorrhagia / menometrorrhagia), and evaluated.

Oligomenorrhea: menstruation occurs in intervals of over 35 days. It is usually associated with anovulation. Polymenorrhea: menstruation that occurs regularly in cycles of less than 21 days. It is usually associated with anovulation. Hypermenorrhea (menorrhagia): prolonged and profuse menstruation. Intervals are regular. Menometrorrhagia: menstruation that occurs at irregular intervals. Amount and duration of menstruation varies. Hypomenorrhea: menstrual bleeding in the form of light staining. Intervals are regular, duration is normal or decreased [1, 2]. In the present study, the authors did not evaluate patients with menstrual irregularity due to any pathological cause such as polycystic ovary syndrome, uterine malignancies, and hyperprolactinemia. One hundred twenty patients were included in this study. In addition, as a healthy control group of 30 patients without menstrual irregularities had blood drawn for comparison in this study. The data evaluation form was collected with data prepared by using the face-to-face interview technique following verbal approval from the patients and their families.

Routine tests (FSH, TSH, LH, and PRL) were performed on the blood samples taken at the outpatient clinic, this data was obtained from the hospital information system and statistically analysed. Blood was placed in biochemistry test tubes with EDTA was centrifuged at 4,000 rpm for 10 minutes at +4°C. It was stored at -80°C until analysis. UT-2 levels of each sample were measured

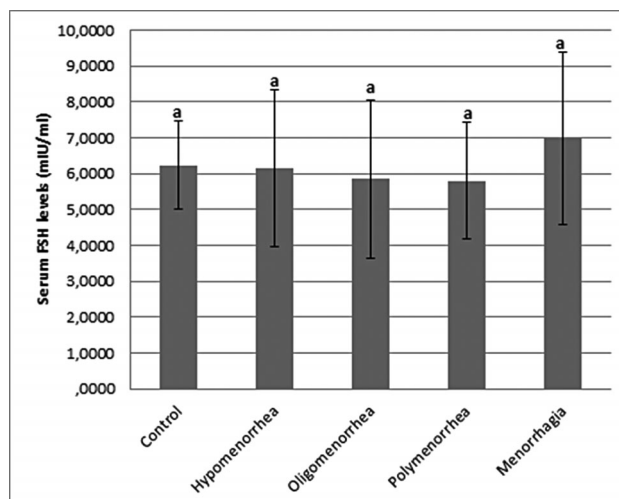


Figure 1. — Serum FSH levels of menstrual irregularity women (mIU/ml). Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups ($p < 0.05$).

with a high-sensitivity human ELISA kit with two repeats. In total, one kit covers 80 sample (plus 16 standard) measurements.

Data obtained from the study was analysed by one way ANOVA and post hoc duncan's multiple comparison test using the SPSS statistical program and standard deviation (SD) and p values were determined for the related fields. In this study, urotensin and PRL levels were compared by pearson correlation test.

Results

In this study, FSH levels were first evaluated. Women with menstrual irregularities were first initially assessed for transition to menopause (ovarian reserve) and then to pathophysiology of irregularity. The most important markers of the ovarian reserve is the FSH level measured on the third day of menstruation. FSH levels in the control group of this study are 5-7 mIU/ml, which is clinically appropriate (Figure 1). At the same time, the authors evaluated the FSH levels of the oligomenorrhea, hypomenorrhea, polymenorrhea, and menorrhagia groups in comparison with the control group and it was found to be statistically insignificant. In this case it is thought that the ovarian reserve of the individuals in the groups with irregularity is normal, and that it may be originating from a different pathophysiological condition.

Another important parameter of the menstrual cycle, LH levels, was also evaluated in this study (Figure 2). Like FSH levels, the LH levels were low in both control groups and groups with irregularity. On the third day of menstruation, decreased FSH level together with low LH level is considered normal.

TSH levels, another hormone that may cause menstrual irregularities, were evaluated among all groups in this study

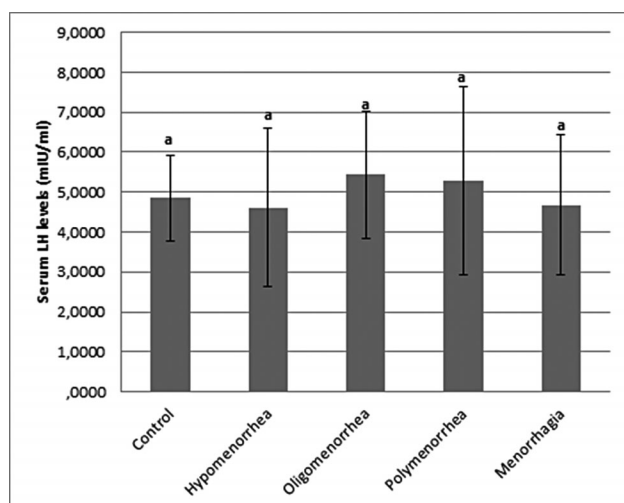


Figure 2. — Serum LH levels of menstrual irregularity women (mIU/ml). Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups ($p < 0.05$).

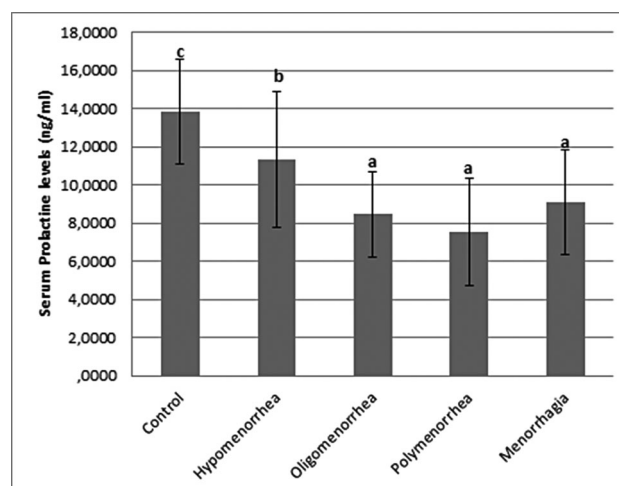


Figure 4. — Serum PRL levels of menstrual irregularity women (ng/ml). Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups ($p < 0.05$).

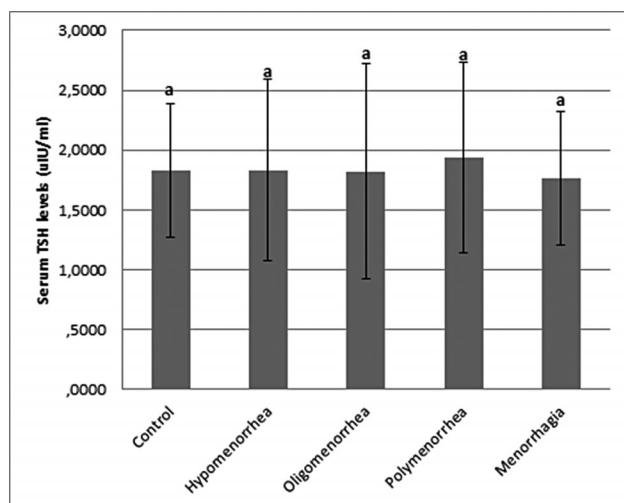


Figure 3. — Serum TSH levels of menstrual irregularity women (uIU/ml). Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups ($p < 0.05$).

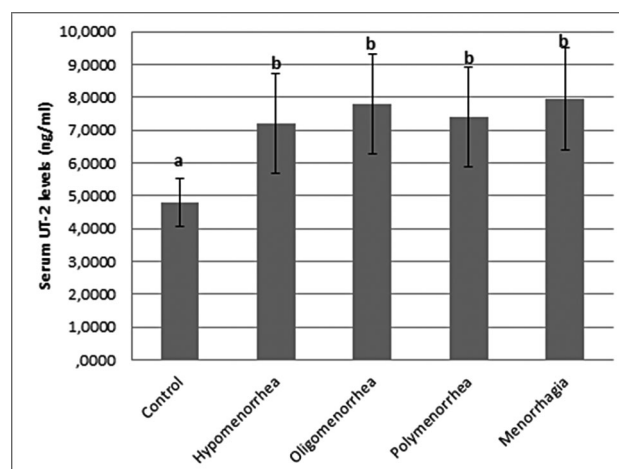


Figure 5. — Serum UT-2 levels of menstrual irregularity women (ng/ml). Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups ($p < 0.05$).

(Figure 3). No significant difference was observed between oligomenorrhea, hypomenorrhea, polymenorrhea, and menorrhagia groups when compared to the control group. While FSH, LH, and TSH caused some cases of menstrual irregularities, it is generally thought that other reasons for irregularities are considered.

Levels of another important hormone, PRL, were assessed in this study (Figure 4). Clinically, it is considered normal that the level of PRL is between about 0-30 ng/ml.

It is known that menstrual irregularity develops in hyperprolactinemic patients with high PRL levels. However, because of the low incidence of hyperprolactinemia, blood was not able to be collected clinically and this could not be included in the study. The mean PRL level, which was found to be 13.8 ng/mL in the control group, was found to be significantly lower in the hypomenorrhea group. The levels of PRL in the oligomenorrhea, polymenorrhea, and menorrhagia groups were found to be significantly lower

than the control and hypomenorrhea groups. The PRL levels being normal, however, lower than the control group, suggests that it may have an important role in the development of menstrual irregularity.

Finally, in this study, the authors evaluated the levels of UT-2, an endogenous peptide that contributes to the pathophysiology of many diseases. The levels of UT-2, which was found to be around 4.7 ng/ml in the control group of this study, were significantly increased compared to the control group in the oligomenorrhea, hypomenorrhea, polymenorrhea, and menorrhagia groups. The presence of low PRL levels along with high levels of UT-2 in groups with menstrual irregularity when compared with the control, suggests that UT-2 may have a significant role in menstrual irregularity (Figure 5).

Finally, in this study a correlation test between UT-2 and PRL levels was carried out. This resulted in a statistically inverse correlation between increased UT-2 and decreased PRL levels among the groups.

Discussion

During the follicular phase of the normal menstrual cycle, LH levels show a sudden rise to peak at the end of the phase. The FSH level is slightly wavy to form a lower peak at the end of the phase. As the level of estradiol suppressing FSH is low on the third day of menstruation, a more accurate idea can be obtained regarding the hormones that dictate ovarian reserve such as FSH. As estradiol increases, a fake low FSH level is observed. LH is also low in this period. As a result, the low level of FSH on the third day of menstruation is expected in a healthy woman. While there are cases of menstrual irregularities caused by FSH and TSH, it is generally thought that there are irregularities for other reasons [15]. In the present study, FSH, LH, and TSH levels on the third day of menstruation were similar in all groups and there was no statistically significant difference.

Unlike other anterior pituitary hormones controlled by hypothalamic releasing factors, PRL secretion is under tonic inhibition of prolactin inhibitory factors (PIF), the most important of which is dopamine. PRL release is caused by stimulation of several factors including vasoactive intestinal peptide (VIP), thyroid releasing hormone, (TRH), and gonadotropin releasing hormone (GnRH). In hormonal imbalances, an increase in PRL can be caused: among them, inadequacy of hormones secreted in the thyroid gland (hypothyroidism) are a relatively common cause of PRL elevation. The mechanism can be explained as follows. Inadequate thyroid hormones cause increased secretion of TRH from the hypothalamus. Since the TRH secretion is also a stimulator for PRL, the increased TRH secretion will lead to hyperprolactinemia. Hyperprolactinemia manifests itself with amenorrhea galactorrhea, infertility, decreased libido, and habitual abortus [16]. Pa-

tients with normoprolactinemia were included in the study. However, interestingly, compared with healthy individuals, the levels of PRL in the oligomenorrhea, hypomenorrhea, polymenorrhea, and menorrhagia groups were found to be statistically lower while being in the physiologic range. In a clinical trial, premenstrual and follicular phase PRL levels were assessed and in parallel with this study; follicular phase PRL levels were found to be lower than in healthy adults [17]. In another study, the levels of PRL in oligomenorrhea patients were statistically lower than those who had regular menstruation [18].

Human UT-2 is obtained from prepro-U-II, a large precursor molecule. The prepro U2 encoded by a gene on the 1p36 chromosome also has two variants, one with 124 amino acids and the other with 139 amino acids [19, 20]. It is the strongest endogenous vasoconstrictor in the body, one- to two-fold more potent than endothelin I. However, the clinic effect is around 30% of the response to endothelin. This is probably due to the number of receptors in the target organ. The effects on the vascular bed of UT-2 vary according to the type and size of the vessels and may show different characteristics in humans and animals. Studies suggest that UT-2 has a biphasic effect on the peripheral vascular system [21, 22]. Studies have shown that some arteries of rats have different vasoconstrictor responses to UT-2. When continuous depolarization and L-type calcium channel effect is present, arterial vasospasm can be facilitated in vascular pathophysiologic processes such as subarachnoid haemorrhage and hypertension [23].

UT-2 and UT-2 receptors are also excreted in high amounts in the heart and large arteries. There is a variety of evidence that they may have many roles on the physiology and pathophysiology of the cardiovascular system [24-26]. The effects of UT-2 on the cardiovascular system varies depending on the type of species, the type of vascular bed, the concentration of the receptor, and the dose [27]. It is suggested that UT-2 has an endothelium-dependent vasodilator and endothelium-independent vasoconstrictor effect, and that net effect may depend on the balance between the two [28]. However, it has been understood that there are other physiological roles that go far beyond the regulation of vascular tone. Blood levels have been shown to increase in essential hypertension, coronary artery disease, congestive heart failure, ischemic cardiomyopathy, diabetes mellitus renal failure, portal hypertension due to liver cirrhosis, and eclampsia [21, 29, 30]. In studies carried out on rats, UT-2 receptors have been shown to be found in brain regions including the olfactory bulb, hippocampus, thalamus, hypothalamus, epiphyseal gland, tegmentum, pituitary gland, pons, medulla oblongata and spinal cord [31].

In another study, urantide, an urotensin receptor antagonist, has been shown to alleviate rectal bleeding [32]. It is also believed that the urotensinergic system is upregulated due to the subarachnoid haemorrhage and that the increased level of UT-2 leads to increased subarachnoid haemorrhage

as a result of increasing vasospasm [33]. In the present study, high levels of UT-2 were found in the oligomenorrhea, hypomenorrhea, polymenorrhea, and menorrhagia when compared with the healthy group. However, it is not clear whether increased serum level of UT-2 is directly related to menstrual irregularity. It may be related to both strong vasoconstriction and other pathophysiologic effects of UT-2.

There are many different studies about urotensinergic systems and PRL levels; however findings are not consistent with related studies. In vitro studies have shown that high levels of UT-2 inhibit PRL release [34]. However, in another study on rats, human UT-2 was delivered into the central ventricle at a concentration higher than physiological level and in certain regions of the brain dopamine metabolite (DOPAC_HVA) / dopamine ratio was measured, and it was found to be lower in the hippocampus, which plays a major role in PRL release. According to this finding, with the lower catabolism of dopamine in the hippocampus and the high PRL inhibiting effect, it is expected that the level of PRL in the periphery is decreased. However, following UT-2 being given to the brain, it has been found that PRL and TSH levels are increased according to the dose. PRL, the release of which is controlled through many mechanisms, being high, is thought provoking. In the study in question, the increased TSH levels are thought to have indirectly increased PRL. Studies carried of UT-2 and PRL in fish and rats showing different findings suggest that the impact of UT-2 cannot be predicted according to species. For this reason, studies carried out on humans provide more clear information [35].

Conclusion

It is interesting to note that the levels of urotensin in this study were higher in women with menstrual irregularities than in healthy individuals, and that serum PRL levels in women with menstrual irregularities were lower than those in the control group, while still being within normal range. However, it would be wrong to arrive at a definite opinion about the physiopathological importance of this condition. Therefore, more detailed preclinical and clinical studies are needed.

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Corresponding Author:
D. ÇETİN, M.D.
Kafkas University, Faculty of Medicine
Department of Pharmacology
Kars 25240 (Turkey)
e-mail: damlacetin.erz@gmail.com