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Is it Possible to Predict the Onset of Side Effects in Patients Treated with Subcutaneous Buserelin?

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Buserelin acetate, a synthetic analog of gonadotropin-releasing hormone（GnRH）, is widely used in the treatment of endometriosis and uterine leiomyoma. This study sought to identify predictors of side effects of buserelin treatment. The medical records of 52 patients who received buserelin treatment were reviewed. The patients were divided into two groups based on the presence or absence of the common side effects of abnormal vaginal bleeding and climacteric symptoms, and compared in terms of age, height, weight, BMI, and basal gonadotropin level. Abnormal vaginal bleeding and climacteric symptoms were reported in 69.2% and 61.5% of the patients, respectively. Among patients who did not report these side effects, mean weight and mean BMI were significantly higher than those of patients who reported the side effects. Mean basal level of serum gonadotropins, the LH:FSH ratio, and patient menstrual history were not related to the incidence of these side effects. Those with *higher* weight and BMI are *less* likely to have buserelin effects, and those with *lower* weight and BMI are *more* likely to have these side effects. So, *lower* weight and BMI might be a predictors for two common side effects of monthly buserelin treatment.

Key words: GnRH analogue, uterine leiomyoma, endometriosis

INTRODUCTION

The hypothalamic decapeptide gonadotropin-releasing hormone（GnRH）plays an important role in the control of mammalian reproduction by regulating the synthesis and release of luteinizing hormone（LH）and follicle-stimulating hormone（FSH）from the anterior pituitary［1］. GnRH is released from the hypothalamus in a pulsatile manner that changes over the menstrual cycle［2］. The changes in GnRH pulse frequency have been shown to differently regulate LH and FSH［3］; that is, rapid GnRH pulse frequencies increase the secretion of LH, whereas slower frequencies decrease LH secretion and increase FSH secretion［4］. This pulsatile release of GnRH at an optimum pulse frequency and amplitude are required to maintain optimum gonadotrophic function, and continuous exposure of the gonadotropin-secreting cells to GnRH leads to desensitization and decreased LH and FSH secretion［5］.

On the basis of the pioneer studies by Knobil［5, 6］, the concept of medical suppression of ovarian function was developed. GnRH analogues were introduced in the early 1980s following developments in chemical synthesis. The synthesis and availability of long-acting GnRH agonists（GnRHa）resulted in their use as therapeutic agents for a variety of estrogen-dependent disorders such as uterine leiomyoma and endometriosis. Administration of GnRHa in females leads to rapid increases（flare-up）in the gonadotropins LH and FSH and in sexual steroids such as estradiol, followed by a down-regulation of GnRH receptors, with desensitization of sexual steroids to castration levels. Long-term GnRHa therapy induces several symptoms associated with hypoestrogenism, including abnormal vaginal bleeding, hot flashes, vaginal dryness, and reversible loss of vertebral trabecular bone［7］. Therefore, before starting GnRHa therapy, physicians are required to explain possible undesirable symptoms to patients.

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In this study, we reviewed the medical records of patients who underwent subcutaneous buserelin treatment at our institution in efforts to identify predictors of the side effects of such treatment.

PATIENTS AND METHODS

This study was conducted based on the declaration of Helsinki. We analyzed the medical records of 52 women who underwent treatment with subcutaneous buserelin (Suprecure MP 1.8, Mochida Pharmaceutical Co., Tokyo, Japan) from April 2004 to March 2007. Patients were excluded from the analysis if they were menopausal, had malignant disease, or complications directly attributable to other medications. Patients who underwent preoperative buserelin treatment for the purpose of diminishing tumor size or improving anemia were included.

The indications for buserelin treatment were uterine leimoyoma in 22/52 (42.3%) patients, endometriosis in 18/52 (34.6%), adenomyosis in 4/52 (7.3%), endometriosis and adenomyosis in 3/52 (5.8%), and endometriosis and leiomyoma in 2/52 (3.8%). Three additional patients underwent treatment for leiomyoma with adenomyosis, hypermenorrhea without organic disorder, and myelodysplastic syndrome, respectively. All patients had menstrual cycles. After providing adequate informed consent and considering alternative therapies, the patients received subcutaneous buserelin treatment. Buserelin acetate 1.8 mg was administered by subcutaneous injection in the abdomen or upper arm once a month, and the treatment was started within 5 days after the beginning of menstruation. Patient weight and height were measured and blood samples for measuring serum gonadotropin levels were taken before initial injection of buserelin. The total number of sequential doses differed among the patients, but was terminated at the sixth dose. Twenty-one of the 52 patients received maximal treatment. The presence or abnormal vaginal bleeding was based on patient reports and was defined as bleeding outside of the menstrual period or heavier than usual bleeding. Climacteric symptoms included hot flashes, headache, shoulder pain, sleep disturbance, and a feeling of anxiety. Patients with either vaginal bleeding or climacteric symptoms were defined as the side effect positive group and those without either were defined as the side effect negative group. The severity and frequency of these side effects were not considered. Plasma hormone measurement was performed in duplicate with a commercial chemiluminescence enzyme immunoassay (CLIA) kit (for LH and FSH) (SRL Inc., Tokyo, Japan), and the values and the ratios of these values were compared between groups.

We also evaluated the possible association between regular or irregular menses and the presence of the evaluated side effects. For this purpose, menstruation that occurred every 25-38 days was considered regular; all others frequencies or a lack of a consistent pattern was defined as irregular menstruation.

Differences were determined using an unpaired t-test. Comparison of the two groups was made using a Chi-squared test. A value of $P<0.05$ was considered statistically significant.

RESULTS

Association of patient characteristics with side effects

Among the 52 patients, 69.2% (36/52) experienced abnormal vaginal bleeding and 61.5% (32/52) experienced climacteric symptoms. Only 6 patients (11.5%) did not complain of any side effects (Table 1).

Abnormal vaginal bleeding mostly occurred between the first and second buserelin injections (24/36 patients, 66.7%). In 8 patients (22.2%), abnormal bleeding was observed until the third injection of buserelin and in 4 patients (11.1%) it continued until the fourth injection. Menstruation-like bleed-

Table 1. Appearance of side effects during buserelin treatment

	Abnormal bleeding (present)	Abnormal bleeding (absent)	No. of patients
Climacteric symptoms (present)	22	10	32
Climacteric symptoms (absent)	14	6	20
Total	36	16	52

ing was reported by 21 of the 36 patients (58.3%) up to the fourth injection, and another 4 (11.1%), 9 (25%), and 2 (5.6%) patients reported their abnormal bleeding as spotting, less than normal menstruation, and excessive normal menstruation, respectively (data not shown). Among the 32 patients who had climacteric symptoms, 15 (46.9%) felt their symptoms were slight, while 12 (37.5%) and 5 patients (15.6%) felt their symptoms were moderate and severe, respectively (data not shown).

Table 2 shows the correlations of different patient variables with the presence or absence of the treatment side effects examined, namely abnormal vaginal bleeding and climacteric symptoms. There was no significant difference between mean age or height and the presence of side effects. In contrast, mean weight and mean BMI were significantly higher in the groups without abnormal bleeding or climacteric symptoms compared to the groups with these respective side effects (all $p<0.05$). In addition, Chi-square tests were used to determine the significance of an association between the presence of side effects and personal background. Abnormal vaginal bleeding was reported by fewer patients in the group with a weight >50 kg or with a BMI >20.5 compared to the groups with a lower weight or lower BMI. Moreover, climacteric symptoms were reported by significantly fewer patients in the groups with a weight >52 kg or with a BMI >20.5, respectively (data not shown).

Mean basal level of serum gonadotropins and the LH:FSH ratio did not differ significantly between patients with or without abnormal bleeding and climacteric symptoms.

Menstrual history and side effects

Table 3 shows a comparison of regular and irregular menstruation among the patient groups. Among all 52 patients, 39 (75%) reported regular menstruation and 13 (25%) reported irregular menstruation.

Table 2. Patient variables according to presence or absence of adverse effects

	All patients	Abnormal bleeding		Climacteric symptoms	
		Present	Absent	Present	Absent
n	52	36 (69.2%)	16 (30.8%)	32 (61.5%)	20 (38.5%)
Age (years)	38.83±7.48	37.83±1.35	41.06±1.74	38.84±1.29	38.80±12.08
Height (cm)	158.18±8.28	157.99±1.17	158.63±1.48	157.76±1.32	158.87±13.35
Weight (kg)	52.87±2.75	50.78±1.03	57.59±2.39*	50.94±1.45	55.97±4.64*
BMI	21.12±1.61	20.33±0.32	22.89±0.91*	20.44±0.49	22.20±2.68*
LH (mIU/ml)	8.18±1.44	7.02±0.92	12.1±5.12	3.96±2.18	10.54±3.47
FSH	12.70±1.92	10.95±1.72	18.61±5.41	13.94±3.01	14.88±4.15
LH/FSH	0.74±0.07	0.76±0.07	0.69±0.19	0.69±0.08	0.87±0.12*

All values except those for n are expressed as mean ± standard deviation.
*Significant at $p<0.05$ (Student's t-test)
BMI, body mass index; LH, leutenizing hormone; FSH, follicle stimulating hormone

Table 3. Comparison of regular and irregular menstrual pattern in association with abnormal vaginal bleeding and climacteric symptoms

	Total	Abnormal bleeding		Climacteric symptoms	
		Present	Absent	Present	Absent
No. of patients	52	36	16	32	20
Regular menses	39 (75%)	27 (75%)	12 (75%)	24 (75%)	15 (75%)
Irregular menses	13 (25%)	9 (25%)	4 (25%)	8 (25%)	5 (25%)

The same percentages of patients with regular or irregular menstruation (75%/25%) were found in the groups with and without each examined side effect.

Incidence of side effects and maximal buserelin dose

The subgroup of 21 (of 52) patients who received the maximal dose of buserelin (6 doses) was analyzed separately because the patients who received the most doses would provide the best assessment of correlation between patient characteristics and the presence of side effects (Table 4). Only 2 patients of the 21 patients (9.7%) did not complain any adverse effects. Among these 21 patients, 15 (71.4%) had abnormal bleeding and 15 (71.4%) reported climacteric symptoms during their treatment.

Abnormal bleeding occurred between the first and second buserelin injections in 8 patients (8/15; 53.3%), and 4 (26.7%) and 3 (20%) patients reported this symptom until the third and fourth injections, respectively. Abnormal bleeding was not observed after the fourth injection. Eight of the 15 patients with abnormal bleeding (53.3%) observed menstruation-like bleeding during this period, and another 3 (20%), 6 (40%), and 1 (6.7%) patients reported their abnormal bleeding as spotting, less than normal menstruation, and excessive normal menstruation, respectively (data not shown). Among the 15 patients who had climacteric symptoms in this group, 7 (46.7%) felt their symptoms were slight, while 6 (40%) and 2 (13.3%) felt their symptoms were moderate and severe, respectively (data not shown).

Mean weight and mean BMI were significantly higher in patients without climacteric symptoms than in those with such symptoms (both $p<0.05$). By dividing the patients according to body weight and BMI, Chi-squared tests revealed that patients in the group with a weight >50 kg or with a BMI >20.5 were statistically less likely to have either side effect compared to groups with a lower weight or BMI group.

Neither the mean basal level of serum gonadotropins nor the LH: FSH ratio before buserelin treatment correlated with the absence or presence of the examined side effects.

DISCUSSION

The treatment of estrogen-dependent disorders such as uterine leiomyoma and endometriosis has

Table 4. Patient variables in the patients who received the maximal dose of buserelin

	Patients who received a maximal dose (6 treatments)	Abnormal bleeding		Climacteric symptoms	
		Present	Absent	Present	Absent
n	21	15(71.4%)	6 (28.6%)	15 (71.4%)	6 (28.6%)
Age (years)	42.05±1.81	41.07±2.22	44.5±3.21	41.73±1.97	42.83±4.32
Height (cm)	158.36±1.28	158.13±1.27	158.92±2.98	157.8±1.53	159.75±1.69
Weight (kg)	51.0±1.28	50.87±1.35	51.33±3.05	48.71±1.29	56.75±1.15*
BMI	20.33±0.43	20.34±0.46	20.3±0.99	19.56±0.43	22.24±0.32*
LH (mIU/ml)	8.18±1.44	8.51±1.89	7.33±3.04	8.20±2.12	8.24±2.12
FSH	12.70±1.92	12.55±3.46	23.68±10.40	13.42±3.69	19.52±9.03
LH/FSH	0.74±0.07	0.82±0.39	0.33±0.19	0.71±0.13	0.68±0.12

All values except those for n are expressed as mean ± standard deviation.

*Significant at $p<0.05$ (Student's t-test)

BMI, body mass index; LH, leutenizing hormone; FSH, follicle stimulating hormone

improved with the development of GnRHa therapy. Initial formulations in the form of nasal spray GnRHa were associated with low patient adherence because of complicated application procedures or patients forgetting to take their medication. In addition, absorption from the nasal mucosa was considered poor. As a result, new drug delivery systems were developed, such as a sustained-release GnRHa formulation in a microcapsule [8]. Higher adherence was obtained because of once-a-month administration without the inconvenience of the daily nasal spray. Due to its potent suppressive actions on pituitary function, GnRHa markedly lowers estrogen secretion, which has been associated with adverse events such as abnormal vaginal bleeding, estrogen deficiency symptoms, and loss of bone mineral density. Thus, GnRHa administration is usually limited to a 6-month duration [7].

Buserelin MP is a microcapsule produced by directly sealing buserelin acetate in polylactic acid-glycol acid (PLGA). Its release rate reaches the maximal level 1 h after initial administration, then promptly decreases and continues at a low level of 8.0–10.7 pg/ml for 1 to 4 weeks [9]. On the other hand, another low dose formulation of GnRHa is now available consisting of microcapsules produced by sealing leuprorelin acetate adsorbed on gelatin in PLGA in a dichromethane solution. The average size of these microcapsules is approximately 20μm, so they have an extremely stable, sustained effect [10]. As a result, leuprorelin has a higher biological activity than buserelin and tends to promptly down-regulate pituitary function and rapidly decrease serum E2 levels. Thus, compared with leuprorelin, buserelin MP needs a relatively longer period of time for patients to develop amenorrhea [11]. In addition, buserelin is thought to be associated with a lower incidence of hypoestrogenic symptoms such as abnormal bleeding or climacteric symptoms [12]. Nonetheless, in this study sample of patients receiving buserelin, 69.2% reported abnormal bleeding, which may have been due to a slow reduction in serum E2 levels, and 61.5% had climacteric symptoms.

We found that weight and BMI were significantly higher in patients who did not have abnormal bleeding or climacteric symptoms than in those who did. Even in the 21 patients who received the maximal 6 sequential buserelin treatments, 71.4% (15 cases) had abnormal bleeding and 71.4% (15 cases) had hypoestrogenic symptoms during their therapy. This association was also evident in patients who received more doses of buserelin (range 1-6 doses). The other examined characteristics-age, height, and gonadotropin levels-did not correlate with the absence or presence of the evaluated side effects. Since we did not obtain any evidence that the maximal dose of buserelin treatment increased the incidence of abnormal bleeding or climacteric symptoms, or that the severity of these symptoms was not correlated with the number of buserelin treatments, physical status might be a predictor for these side effects.

Speculating on the importance of physical status to predict the incidence of hypoestrogenic symptoms, one enzyme should be mentioned. Aromatase is the enzyme responsible for a key step in the biosynthesis of estrogens [13]; its principle action is to transform androstenedione to estrogen and testosterone to estradiol. Aromatase can be found in many tissues including the gonads, brain, adipose tissue, blood vessels, skin, and bone. Given that obesity is known to increase aromatase activity, women with higher weight and BMI may have a higher level of estrogen even in the presence of buserelin. However, as we could find no evidence in the literature of a correlation between aromatase activity and incidence of hypoestrogenic symptoms in primates, this remains no more than speculation at this stage.

We found no correlation between menstrual history and side effects from buserelin. We expected that women with irregular menstrual cycles would have a higher incidence of side effects, especially abnormal bleeding. This expectation was based on the belief that irregularity in the hypothalamic-pituitary gonadal axis probably potentiates menstrual irregularity in the presence of GnRHa. However, contrary to our expectation, the proportion of patients with regular menses to irregular menses was the same (75/25) regardless of the presence or absence of side effects. These observations suggest that menstrual history is not a predictor of buserelin side effects.

In conclusion, this retrospective study found that

abnormal bleeding and climacteric symptoms occurred in approximately 70% and 60%, respectively, of women who received buserelin treatment. Mean body weight and mean BMI were significantly higher in patients who did not suffer these side effects than in those who did. Patients with higher weight or higher BMI were statistically less likely to experience these symptoms than patients with lower weight or lower BMI, suggesting that these parameters might be a predictor for buserelin side effects. Menstrual cycle history (i.e., regularity vs. irregularity) did not correlate with the presence of these side effects.

REFERENCES

1) Stojilkovic SS, Catt KJ (1995) Expression and signal transduction pathways of gonadotropin-releasing hormone receptors. *Recent Prog Horm Res* 50: 161-205.

2) Crowley WF, Jr., Filicori M, Spratt DI, Santoro NF (1985) The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. *Recent Prog Horm Res* 41: 473-531.

3) Dalkin AC, Haisenleder DJ, Ortolano GA, Ellis TR, Marshall JC (1989) The frequency of gonadotropin-releasing-hormone stimulation differentially regulates gonadotropin subunit messenger ribonucleic acid expression. *Endocrinology* 125: 917-924.

4) Wildt L, Hausler A, Marshall G, Hutchison JS, Plant TM, Belchetz PE, Knobil E (1981) Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. *Endocrinology* 109: 376-385.

5) Knobil E (1981) Patterns of hypophysiotropic signals and gonadotropin secretion in the rhesus monkey. *Biol Reprod* 24: 44-49.

6) Knobil E (1980) The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res* 36: 53-88.

7) Comite F (1989) GnRH analogs and safety. *Obstet Gynecol Surv* 44: 319-325.

8) Okada H, Heya T, Ogawa Y, Shimamoto T (1988) One-month release injectable microcapsules of a luteinizing hormone-releasing hormone agonist (leuprolide acetate) for treating experimental endometriosis in rats. *J Pharmacol Exp Ther* 244: 744-750.

9) Kondo S, Takeda S, Suzuki E, Suetsugu K, Maeda T (1996) Form design of bucerelin acetate sustained release injection. *Jpn Pharmacol Ther* 24: 209-215.

10) Uemura T, Shirasu K, Hori H, Ooyama T, Onose R, Ishikawa M, Tsuchiya S, Minaguchi S (1992) Endocrinological study with leuprorelin acetate sustained release injection (TAP-144-SR) in endometriosis. *Jpn J Fertil Steril* 37: 572-579.

11) Takeuchi H, Kobori H, Kikuchi I, Sato Y, Mitsuhashi N (2000) A prospective randomized study comparing endocrinological and clinical effects of two types of GnRH agonists in cases of uterine leiomyomas or endometriosis. *J Obstet Gynaecol Res* 26: 325-331.

12) Makita K, Ishitani K, Ohta H, Horiguchi F, Nozawa S (2005) Long-term effects on bone mineral density and bone metabolism of 6 months' treatment with gonadotropin-releasing hormone analogues in Japanese women: comparison of buserelin acetate with leuprolide acetate. *J Bone Miner Metab* 23: 389-394.

13) Carreau S, Bourguiba S, Lambard S, Galeraud-Denis I, Genissel C, Levallet J (2002) Reproductive system: aromatase and estrogens. *Mol Cell Endocrinol* 193: 137-143.