Original Article

17β estradiol / norethisterone acetate and estradiol valerate / norgestrel therapies in patients with dysfunctional uterine bleeding: The effects on estrogen and progesterone receptor levels and clinical response

Mevlüde Ayik[a], Metin Ingec[b], Necla Konar Ustyol[c,*], Cemal Gundogdu[d]

[a]*Department of Obstetrics and Gynecology, Kahramanmaraş State Hospital, Kahramanmaraş, Turkey*
[b]*Department of Obstetrics and Gynecology, Atatürk Universitiy Facullty of Medicine, Erzurum, Turkey*
[c]*Department of Obstetrics and Gynecology, Women and Children's Hospital, Van, Turkey*
[d]*Department of Pathology, Atatürk Universitiy Facullty of Medicine, Erzurum, Turkey*

Abstract. To compare estrogen receptor (ER) and progesterone receptor (PR) levels before and after estradiol valerate/norgestrel or 17β estradiol/norethisterone acetate therapy in dysfunctional uterine bleeding (DUB) and to examine the clinical response to these therapies.

The study was performed with 60 patients diagnosed with DUB. Patients were divided into two groups. One was given 17β estradiol / norethisterone acetate (group A) and the other estradiol valerate / norgestrel (group B). Pre- and post-treatment clinical parameters and ER and PR levels were measured.

Changes in ER levels following treatment were significant in both groups, while the change in PR levels was significant in the group B ($p<0.05$). Compared to the pre-treatment levels, an increase in hemoglobin-hematocrit values, decreased endometrial thickness and prolongation of menstrual cycle were observed in both groups ($p<0.05$). Furthermore, pre- and post-treatment bleeding was significantly shorter in group A ($p<0.05$).

Clinical responses obtained with hormonal preparates in the treatment of DUB are associated with decreases in ER and PR levels. A correlation can be established between determination of receptor level at the begining of treatment and clinical response.

Key words: Dysfunctional uterine bleeding, estrogen receptor, progesterone receptor

1. Introduction

Abnormal uterine bleeding is seen in approximately one in three women in the reproductive period. Diagnosis generally depends on level and time of bleeding (1). Abnormal uterine bleeding developing in the absence of any anatomical or systemic pathology is described as dysfunctional uterine bleeding. Diagnosis is made by excluding other gynecological or systemic diseases that may cause abnormal uterine bleeding (1-3). Dysfunctional uterine bleeding (DUB) may develop at any time between menarche and menopause and may be seen in anovulatory and ovulatory cycles (4,5).

*Corresponding Author: Necla Konar Ustyol, MD
Women and Children's Hospital Department of Obstetrics and Gynecology, Van, TURKEY
Mobile Phone: +90 505 451 14 14
Fax: +90 432 486 54 13
E-mail:necla.konar@hotmail.com
Received: 01.09.2014
Accepted: 06.01.2015

The aim in the treatment of DUB is the control of acute bleeding, prevention of repetition of abnormal uterine bleeding and prevention of long-term outcomes of prolonged estrogen exposure on the endometrium as a result of anovulation (6). Abnormal bleeding in ovulatory DUB can be controlled with nonsteroid anti-inflammatory drugs, antifibrinolytic agents, intrauterine devices containing progesterone or oral contraceptives (5). Oral contraceptives or cyclic progesterone are used in the treatment of anovulatory dysfunctional uterine bleeding (7).

Specificity of tissue reactions to sex steroid hormones depends on the presence of intracellular receptor proteins. Estrogen receptors (ERs) and progesterone receptors (PRs) are members of the steroid receptor family. In the follicular phase, ERs increase in the endometrial epithelium and stroma, decreasing after ovulation to below the level in the late luteal phase (8,9). PRs in the epithelium increase in the follicular phase and decrease in the luteal phase. However,

stroma remain at a high level until menstruation (8,10).

The purpose of this study was to compare estrogen and progesterone receptor levels before and after estradiol valerate/norgestrel or 17-beta estradiol/norethisterone acetate therapies in patients with DUB and to examine the clinical response to these therapies.

2. Materials and Methods

This study involved 60 patients attending the Atatürk University Faculty of Medicine, Obstetrics and Gynecology Clinic and diagnosed with DUB between 01.12.2007 and 01.12.2008. Patients aged 18-45, with no pregnancy, uterine myoma, endometrial polyp, neoplasia, infection, intrauterine lesion or systemic disease and not receiving hormonal therapy in the previous 6 months were included.

Patients were divided into two groups depending on order of presentation, even numbers being enrolled as Group A and odd numbers as Group B. Patients in Group A were given continuous triphasic hormone therapy [17β estradiol / norethisterone acetate, 2-2-1 / 0-1-0 (mg)] and those in Group B were given biphasic hormone therapy [estradiol valerate / norgestrel, 2-2 / 0-0.5 (mg)]. Patients were given instruction regarding noting their days of bleeding. Pre- and post-treatment hemoglobin-hematocrit values were measured, together with endometrial thickness using transvaginal ultrasonography. Endometrial specimens were collected prior to and after 3 months of treatment. Pathological specimens were evaluated histopathologically. Two 5 µ-thick sections were placed on adhesive slides. Estrogen and progesterone receptor proteins were investigated using the streptavidin-biotin method with Dako kits. Nuclear staining was regarded as positive for ER and PR expression. Receptor levels were divided into three groups on the basis of nuclear staining percentages, 0-25 (low), 26-75 (moderate) and 76-100 (high).

Statistical Analysis

Normal distribution of data was assessed using the Kolmogorov-Smirnov test. Normally distributed variables were compared using Student's T test, and the Matched T test was used for intragroup comparisons. Under nonparametric conditions, comparisons between groups were performed using the Mann-Whitney U test, and intragroup comparisons using the Wilcoxon test. Significance was set at $p<0.05$.

3. Results

Mean age,gravidity and parity values in Group A were 38.7±3.822, 3.33±1.63 and 3.33±1.63, respectively, and 39.17±3.26, 4.63±2.81 and 3.87±2.84 in Group B.

No difference was determined between the two groups in terms of pre-treatment hemoglobin-hematocrit, endometrial thickness or length of cycle ($p>0.05$). However, pre- and post-treatment duration of bleeding were shorter in the patients in Group A ($p<0.05$) (Table 1).

Pre-treatment ER levels were high in 15 (50%) of the 30 patients in Group A, but were only elevated in 2 (3.3%) patients after treatment. PR levels were high in 17 (56.7%) patients before treatment and in 12 (40%) after treatment. Pre-treatment ER levels were high in 25 (83.3%) patients in Group B, and in 6 (20%) patients after treatment. PR levels were high in 23 (76.7%) patients before treatment and in 12 (40%) after treatment (Table 2).

A significant decrease in ER levels was observed in both groups. ER levels increased in

Table 1. Pre-and post-treatment menstrual characteristics

	Group A n=30 (mean±SD)	Group B n=30 (mean±SD)	p
Pre-treatment hemoglobin (gr/dL)	10.5±1.5	9.8±1.3	0.11
Post-treatment hemoglobin(gr/dL)	12.27±1.51	11.70±1.26	0.09
Pre-treatment hematocrit (%)	32.20±4.55	31.03±3.68	0.28
Post-treatment hematocrit (%)	37.13±4.01	35.70±3.66	0.12
Pre-treatment endometrial thickness(mm)	9.50±4.81	9.73±4.63	0.84
Post-treatment endometrial thickness(mm)	6.40±2.54	6.00±3.25	0.25
Pre-treatment cycle length (days)	19.53±5.01	17.53±4.33	0.10
Post-treatment cycle length(days)	24.50±4.80	24.00±5.15	0.76
Pre-treatment duration of bleeding (days)	8.87±3.47	11.00±2.94	0.001
Post-treatment duration of bleeding (days)	6.63±2.74	7.47±2.00	0.01

Group A: 17β estradiol / norethisterone acetate (continuous – 28 tablets)
Group B: Estradiol valerate / norgestrel (cyclical – 21 tablets)

Table 2. Pre- and post-treatment ER and PR levels

	Receptor level	ER				PR			
		Pre		Post		Pre		Post	
		No,	%	No,	%	No,	%	No,	%
Group A	Low	9	(30%)	13	(43.3%)	9	(30%)	11	(36.7%)
	Medium	6	(20%)	15	(50%)	4	(13.3%)	7	(23.3%)
	High	15	(50%)	2	(6.7%)	17	(56.7%)	12	(40%)
Group B	Low	3	(10%)	8	(26.7%)	5	(16.6%)	9	(30%)
	Medium	2	(6.7%)	16	(53.3%)	2	(6.7%)	9	(30%)
	High	25	(83.3%)	6	(20%)	23	(76.7%)	12	(40%)

ER: Estrogen receptor PR: Progesterone receptor

Table 3. Changes in pre- and port-treatment ER and PR levels

		Group A	Group B	Total
		n (%)	n (%)	n (%)
Estrogen receptor	Unchanged	15 (50%)	10 (33.3%)	25 (41.7%)
	Decreased	14 (46.7%)	20 (66.7%)	34 (56.7%)
	Increased	1 (3.3%)		1 (1.6%)
	Total	30 (100%)	30 (100%)	60 (100%)
Progesterone receptor	Unchanged	19 (63.3%)	17 (56.7%)	36 (60%)
	Decreased	9 (30%)	13 (43.3%)	22 (36.7%)
	Increased	2 (6.7%)		2 (3.3%)
	Total	30 (100%)	30 (100%)	60 (100%)

Table 4. Changes in clinical parameters and menstrual patterns with treatment

	Group A	Group B	p
	Mean±SD	Mean±SD	
Increase in hemoglobin values (gr/dL)	1.77±1.07	1.83±1.15	0.80
Increase in hematocrit value (%)	4.93±2.77	4.67±3.55	0.61
Prolongation of cycle (days)	4.97±3.57	6.47±4.63	0.19
Decrease in duration of bleeding (days)	2.23±2.11	3.53±2.60	<0.01
Decrease in endometrial thickness (mm)	3.10±3.33	3.73±2.65	0.15

only one patient in Group A, remained within the same range in 15 and decreased in 14 ($p = 0.001$). In Group B, ER levels remained within the same range in 19 patients and decreased in 20 ($p = 0.0001$). No significant difference was determined between the changes in ER levels in groups A and B ($p = 0.153$) (Table 3).

PR levels increased in 2 patients in Group A, remained within the same range in 19 and decreased in 9 ($p = 0.065$). PR levels remained within the same range in 17 patients in Group B and decreased in 13 ($p = 0.0001$). No significant difference was determined in this change in PR levels between the two groups ($p = 0.380$) (Table 3).

Comparison of pre- and post-treatment clinical parameters in both groups revealed an increase in hemoglobin and hematocrit, prolonged duration of cycle, a decrease in endometrial thickness and a shortened bleeding time. These changes were significant ($p<0.05$). Intergroup comparison revealed no significant difference between the group in terms of changes in hemoglobin and hematocrit values, length of cycle and endometrial thickness. However, the decrease in bleeding time was significantly greater in the patients in Group B ($p<0.01$) (Table 4).

No response to medical treatment was achieved in three patients in both groups, and hysterectomy was performed.

4. Discussion

DUB is a very common problem that affects women's health in both medical and social terms. Two out of three hysterectomies are due to DUB. Much hysteroscopic endometrial destructive

surgery is also performed for this reason. Anemia due to excessive menstrual bleeding leads to a decrease in quality of life and increased health care costs. DUB is also the main cause of applications to gynecology clinics with treatment on an outpatient basis (11). DUB is the most widespread cause of iron deficiency in developed countries and of chronic anemia in developing ones (12).

Hormonal therapy being an effective therapeutic strategy in DUB suggests that hormone receptors are involved in the ethiopathogenesis.

The review of the literature, including studies of endometrial ER and PR in the use of DMPA (depot medroxyprogesterone acetate) (13), PR in Norplant use (14), endometrial sex steroid receptors in long-term subdermal levonorgestrel (15-17) and endometrial hormone receptors following levonorgestrel-releasing IUDs (18-19), revealed no previous studies involving changes in hormone receptor levels pre- and post-combined estrogen/progesterone therapy and the clinical response to that change.

Gorodeski et al. (20) measured wide ranges of total ER (TRE) and total PR (TPR) levels in the endometrium of women with DUB, and reported that TRP/TRE levels were significantly low in 18 out of 22 patients with DUB. Levels in the remaining 4 women were within control ranges.

Gleeson et al. (21) investigated cyclical changes in endometrial ER and PR in women with DUB or a normal menstrual cycle. They determined higher ER and PR in the late secretory phase in women with DUB compared to those with a normal menstrual cycle, while receptor levels were similar between the two groups at other stages of the menstrual cycle. A powerful correlation was determined between menstrual blood loss and late secretory endometrial ER levels. An increased local estrogenic effect was present in the premenstrual endometrium in DUB.

Chakraborty et al. (22) compared ER and PR in patients with DUB and with a normal menstrual cycle, and reported higher ER and PR levels in the DUB group.

Hurskainen et al. (23) examined the effect of the use of intrauterine levonorgestrel on chi-67 and sex steroid receptors in the endometrium of women with menorrhagia. They reported that after 6 months' use of an intrauterine system (LNG-IUS) containing levonorgestrel epithelial and stromal PR immune staining scores were significantly lower compared to proliferative endometrium before insertion of LNG-IUS. Comparison of biopsies taken during the use of LNG-IUS with biopsies from the secretory endometrium before insertion revealed a significant decrease in epithelial and stromal immunoreactivity (15,24). ER and PR immunostaining scores at 6 and 12-months' use of LNG-IUS were similar, and no significant difference was determined in women with or without menorrhagia at any time interval (23).

In a study performed with DMPA, Loockwood et al. (25) reported lower PRA and PRB release in the endometrium at times of bleeding with use of DMPA than that from the endometrium at times of no bleeding for all patients. No correlation was determined between number of days of bleeding and endometrial PRA and PRB release (25). Therefore, it seems that there is no direct association between breakage bleeding in use of DMPA and endometrial PRA and PTB release in the gland or stroma (13).

Most studies involving contraceptives containing progesterone alone have shown a decrease in endometrial PR compared to the endometrium of women with a normal cycle (15-18). Endometrial PR concentration in Norplant use was reported to be higher than that in women with a normal cycle in one study (14).

Our study involved women with DUB, and these patients were administered two different treatment regimens containing estrogen and progesterone. ER and PR levels in the endometrial glands were investigated in these patients. We observed a significant decrease in ER levels in the endometrial glands with both regimes, and particularly with estradiol valerate / norgestrel. There was also a significant decrease in PR levels in the endometrial glands in patients using estradiol valerate / norgestrel, but we observed no significant decrease in PR levels in patients using 17β estradiol / norethisterone acetate.

The increase in hemoglobin-hematocrit values, prolongation of cycle and decrease in length of bleeding and endometrial thickness were significant in both groups ($p<0.05$). At intergroup comparison, however, while the decrease in length of bleeding was marked in the estradiol valerate / norgestrel group ($p<0.01$), neither regimen was superior to the other in terms of other clinical parameters.

In conclusion, clinical responses achieved with hormonal preparates in the treatment of DUB are associated with decreases in ER and PR levels. A correlation can be established between receptor level determination at start of treatment and clinical response. The effect-clinical response relationship at receptor level can be established in more detail with future comparative studies involving different treatment modalities.

References

1. Davidson BR, Dipiero CM, Govoni KD, Littleton SS, Neal JL. Abnormal Uterine Bleeding During the Reproductive Years. J Midwifery Womens Health 2012; 57: 248-254.
2. Cecil AL. Evaluation of patients with abnormal uterine bleeding. Am J Obstet Gynecol 1996; 175: 784-787.
3. Charles SF. Dysfunctional uterin bleeding. Primary Care 1988; 15: 561-575.
4. Cindy MF. Management dysfunctional uterin bleeding. Drugs 1992; 44: 578-584.
5. Seth AS, Mark E, James LB. Modern treatments of menorrhagia attributable to dysfunctional uterine bleeding. Obstet and Gynecol Survey 1998; 54: 61-72.
6. Steven RB, Alan HD. Clinical manifestations and treatment of dysfunctional uterine bleeding. JAMA 1993; 269: 1823-1828.
7. James MS. Pathophysiology of abnormal uterine bleeding. Obstetrics and Gynecology Clinics of North America. Davis C (ed) 2000; 27: 219-235.
8. Lessey BA, Killam AP, Metzger DA, et al. Imunohistochemical anlysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J Clin Endocrinol Metab 1988; 67: 334-340.
9. Felix JC, Farahmand S. Endometrial glandular proliferation and estrogen receptor content during the normal menstrual cycle. Contraception 1997; 55: 19-22.
10. Bergeron C, Ferenzy A, Toft DO, Schneider W, Shyamala G. Immunocytochemical study of progesterone receptors in the human endometrium during the menstrual cycle. Lab Invest 1988; 59: 862-869.
11. Pitkin J. Dysfunctional uterine bleeding. BMJ 2007; 334: 1110-1111.
12. The initial management of menorrhagia; evidence based clinical guidlines. No. 1. Royal College of Obstetricans and Gynaecologist 1998.
13. Wisan S, Pichai C, Surasak T, et al. Endometrial progesterone receptors and bleeding disturbances in depot medroxyprogesterone acetate users. Hum Rep 2004; 19: 547-552.
14. Critchley HOD, Bailey DA, Au CL. Immunohistochemical sex steroid receptor distribution in endometrium from long-term subdermal levonorgestrel users and during the normal menstruel cycle. Hum Reprod 1993; 8: 1632-1639.
15. Critchley H, Wang H, Jones R. Morphological and functional features of endometrial decidualization following long-term intrauterine levonorgestrel delivery. Hum Reprod 1998; 13: 1218-1224.
16. Janne O, Ylostalo P. Endometrial eostrogenand progestin receptors in women bearing a progesterone-releasing intrauterine device. Contraception 1980; 22: 19-23.
17. Lau TM, Witjaksono J, Affandi B and Rogers PA. Expression of progesterone receptor mRNA in the endometrium during the normal menstruel cycle and in Norplant users. Hum Reprod 1996; 11: 2629-2634.
18. Zhu P, Liu X, Luo H, et al. The effect of a levonorgestrel-releasing intrauterine device on human endometrial oestrogen and progesterone receptors after one year of use. Hum Reprod 1999; 14: 970-975.
19. Weng M, Li L, Feng S, Xie M, Hong S. Effects of levonorgestrel-releasing intrauterin system on endometrial estrogen and progesterone receptors in patients with endometrial hyperplasia. Nan Fang Yi Ke Da Xue Xue Bao 2012; 32: 1350-1354.
20. Gorodeski IG, Geier A, Siegal A, et al. Decreased ratio of total progesterone to total estradiol receptor levels in endometria of women with adulth dysfunctional uterine bleeding. Gynecol Obstet Invest 1986; 22: 22-28.
21. Gleeson N, Jordan M, Sheppard B, Bonnar J. Cyclical variation in endometrial oestrogen and progesterone receptors in women with normal menstruation and dysfunctional uterine bleeding. Eur J Obstet Gynecol Reprod Biol 1993; 48: 207-214.
22. Chakraborty S, Khurana N, Sharma JB, Chaturvedi KU. Endometrial hormone receptors in women with dysfunctional uterine bleeding. Arch Gynecol Obstet 2005; 272: 17-22.
23. Hurskainen R, Salmi A, Paavonen J, Teperi J, Rutanen EM. Expression of sex steroid receptors and Ki-67 in the endometrioma of menorrhagic women:effects of intrauterine levonorgestrel. Mol Hum Reprod 2000; 6: 1013-1018.
24. Salmi A, Pakarinen P, Peltola AM. The effect of intrauterine levonorgestrel use on the expression of C-Jun, oestrogen receptors, progesterone receptors and Ki-67 in human endometrium. Mol Hum Reprod 1998; 4: 1110-1115.
25. Loockwood CJ, Runic R, Wan L, Krikun G, Demopolous Rand Schatz F. The role of tissue factor in regulating endometrial haemostasis: implications for progestin-only contraception. Hum Reprod 2000; 15: 144-151.