Agricultural Journal 10 (1): 7-11, 2015
ISSN: 1816-9155

Late Luteal Hyperprolactinemia (LL-HPRL) is not a Disease..!!!

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Abstract: Prolactin (PRL) is an exceptional hormone of pituitary gland with important implications for normal reproduction as well as for sexual behavior. It increases in the first trimester of pregnancy (physiological hyperprolactinemia). The objective of this study is to evaluate the usefulness of LL-HPRL as an indicator for early ongoing pregnancy in patients undergoing treatment for infertility. This is a retrospective study on one hundred and eleven women who had beenundergoing treatment for infertilityand their husbands were known to be reproductively fertile. Women with knownother causesof infertility, besides anovulation and luteal phase deficiency were excluded from the study population. They were divided into two groups matched for age and Body-Mass Index (BMI); control group A: 76 pregnant women on no anti-LF-HPRL therapy and study group B: 35 pregnant women on anti-LF-HPRL therapy. All women in both groups had been treated for hyperprolactinemia until their PRL levels were normal. Later, after one menstrual cycle, both groups had been enrolled in a Controlled Ovarian hyperstimulation (COS) Program. The COS aimed to induce 2-3 mature follicles/women/cycle. Duringthe study period (34 months), the ovulation, pregnancy, abortion and live-birth rates were evaluated. The withdrawal rate of patients was also recorded. No significant differences were observed between the two groups of women regarding baseline hormonal levels (FSH, LH, LH/FSH, testosterone, estradiol and PRL ($p<0.03$). However, the progesterone levels at day 21 of the cycle was significantly different between study and control groups (4.52±4.91 and 5.36±4.73, respectively, $p<0.02$). The serum PRL at 28th day of ovulation induction cycle was significantly different between the study and control groups (28.32±11.89 and 7.53±5.69, respectively, $p<0.001$). The ovulation, ongoing pregnancy, life-birth rates were significantly higher in the study group in comparison to control group ($p<0.001$). Early abortion and cancelation rates were significantly higher in control than in study group ($p<0.001$). High serum prolactin levels in the Late Follicular menstrual Phase (LF-HPRL) in infertile women treated by ovulation induction after having completed therapy for hyperprolactinemia is an early indicator for diagnosis of ongoing pregnancy, consequently treatment of LF-HPRL is not recommended.

Key words: Late-luteal hyperprolactinemia, pregnancy, FSH, COS, Yemen

INTRODUCTION

Prolactin (PRL) is an exceptional hormone of pituitary gland with an important role in normal reproduction as well as sexual behavior. Physiologically, PRL is a peptide hormone essentially secreted by the anterior pituitary and to a lesser extent by other extrapituitary tissues (Ben-Jonathan *et al*., 1996). The endometrium is one of the first extrapituitary sites that have been reported to synthesize and secrete PRL (Brosens *et al*., 1999). It is synthesized by decidualized endometrial cells in the late secretory phase in a non-conception cycle and also throughout pregnancy. Wu *et al.* (1995) and Ben-Jonathan *et al.* (2008) had scientifically, documented that PRL synthesis increases if pregnancy occurs and the production is maintained until the final stage of pregnancy (Wu *et al*., 1995). So, it plays an important role in implantation, decidualization and subsequent placentation of the human endometrium. Furthermore, lack of endometrial PRL during the implantation window seems to be implicated in reproductive failure (Garzia *et al*., 2004).

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Successful pregnancy is dependent on growth and modulation of the endometrium in preparation for implantation of the blastocyst. This complex process involves secretory transformation of glandular epithelial cells followed by decidualization of stromal cells (Jabbour and Critchley, 2001). The process of decidualization is poorly understood but a key stimulus is progesterone acting on estrogen-primed endometrial stromal cells leading to dramatic transcriptional reprogramming (Gellersen and Brosens, 2003). Among the numerous genes that are upregulated during decidualization, PRL is one of the most impressively induced decidua-derived PRL is one of the most abundant secretory products in the amniotic fluid (Bao *et al*., 2007; Gellersen *et al*., 2007; Jabbour and Critchley, 2001; Labied *et al*., 2006).

To verify the role of late luteal hyperprolactinemia as a lab finding in early pregnancy, we prospectively analyzed a group of infertile women who had reproductive failures due to anovulation. All of these patients had already completed treatment of hyperprolactinemia and undergoing controlled ovulation induction protocol plus programming intercourse. Accordingly, this retrospective study aims: to highlight the importance of physiological features of late follicular hyperprolactinemia and to use it as a new medical terminology for early diagnosis of ongoing pregnancy after the treatment of anovulation by mono or bi-follicular Controlled Ovarian Stimulation (COS) plus programming intercourse.

MATERIALS AND METHODS

- Study design: retrospective case control descriptive clinical study
- Study population: infertile patients attending allow IVF center for infertility treatment
- Study place: Allow IVF Center Sana'a Yemen
- Study period: Feb., 2009 to Dec., 2011

The present study involved one hundred and eleven (n = 111) women undergoing treatment for infertility. They were divided into two groups: control group included 76 pregnant women who were not treated for LF-HPRL and study group included those treated by Bromocriptinemesilate 2.5 mg daily for LL-HPRL. The husbands of both groups were reproductively fertile. The inclusion criteria of women involved in this study were anovulation and Luteal Phase Deficiency (LPD). All women with an additional cause of infertility had been excluded. Previously, all women had been treated for pathological hyperprolactinemia until the serum prolactin was within the normal range. Both groups had been involved in the same program of COS to get at least 2-3 mature graafian follicles, (we called this protocolmono or bi-follicular COS) (Table 1).

The study was approved by the Review Board of the Allow IVF Center Ethical Committee meeting 3S/2014. The purpose of the protocol was carefully explained to all the women their written consent was obtained before beginning the study. Six injections of human postmenopausal gonadotropin (HMG, total 450 IU per cycle) were administrated for each woman according to the scheme above and human chorionic gonadotropin injection (hCG, 5000-10000 U single dose, intramuscular) was administrated on the day when the graafian follicles reach 17-22 mm in diameter (Table 1).

Each patient underwent serial Trans-vaginal Ultrasonography (TV-USG) measurements by the same experienced physician using an ultrasonic scanner (Aplio, Toshiba Medical Systems, Rome, Italy) equipped with a 7.5 and 5.0 MHz vaginal probe. Scans were performed every 2-3 days beginning on the 6th day after starting injection of hCG and after the onset of menses. When the follicular dimensions (arithmetic mean of the two main diameters of the follicle) achieved at least 17 mm, the TV-USG was performed daily. When the follicle dimensions yielded at least a mean diameter of 19-20 mm, each woman was injected with human Chorionic Gonadotropin (hCG, 5000-10000 U), intramuscularly for induction of ovulation was asked to have intercourse twice daily with 10-12 h rest period in between, however, for precise scheduling of intercourse we recommended them to do intercourse exactly after 24 and 36 h from the hCG injection.

Prolactin measurement: Serum prolactin was measured by specific radioimmunoassay before ovulation induction at day 2 to 3 of the cycle and at day 28 of ovulation induction cycle.

During the study, the ovulation, pregnancy, abortion live-birth rates were evaluated in each woman.

Table 1: Ovulation induction protocol (mono or bi-follicular COS)

Days of cycle	HMG injections (75 IU mL^{-1})	Ultra-sound	hCG 5000-10000 U
2	2	Yes	No
3	2	No	≈Done
4	Nil	No	-
5	1	No	-
6	1	Yes	-
7	Nil	No	-
8	-	No	-
9	-	Yes	-
10	-	-	-
11	-	-	-
12	-	Yes	-
13 daily	-	Yes	-
14	-	Yes	-

The ovulation was retrospectively defined with the observation of a decrease in follicular dimensions andliquid in the cul-de-sac confirmed by plasma prolactin assay >10 ng mL^{-1} (SI 32 nmol L^{-1}). Anovulatory women received a further dose of 100 mg natural progester 1 IM, in the absence of spontaneous with drawal bleeding after 40 days from the last progesterone-induced uterinebleeding. Ovulation rate was calculated as the percentage of ovulatory cycles per total number of menstrual cycles. The pregnancy rate was defined as the percentage of pregnancies per total number of cycles. A rising β-hCG and the sonographic evidence of intrauterine gestational sac were considered criteria to define a pregnancy. The abortion rate was defined asa percentage of miscarriages during the first 12 weeks of gestation per total pregnancies. The live-birth rate was obtained after a 9 months extension of the follow-up period and was defined as the percentage of women with a live baby per total number of women who became pregnant.

Statistical tests were used depending on the nature of the data. In addition to descriptive statistics to determine the mean, standard deviation and Standard Error of Mean (SEM), paired t-test, Chi-square and Kruskal-Wallis one-way analysis of variance were used for analysis of data. Correlation coefficients were calculated according to Pearson. Data were analyzed using 'Statistical Package for the Social Sciences' SPSS 20.5; Inc. Chicago, IL). A $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The data of the present study show no significant differences between both groups of women A and B regarding age, BMI, base-line hormone levels number of graafian follicles after ovulation induction ($p<0.47$) (Table 2). The data pointed that the age factor was significantly higher in group B in comparison to group A ($p<0.031$) as well as the number of ovulation induction cycles ($p<0.002$, Table 2). No significant differences ($p<0.471$) were noted between the two groups regarding the number of yielded follicles after ovulation induction protocol.

The concentration of serum prolactin was significantly higherat days 27-28 of the cycles in group A than in group B ($p<0.001$, Table 2 and Fig. 1).

Moreover, there was no significant difference between the ovulation rates in both groups ($p<0.056$). The pregnancy and life birth rates were however significantly higher ($p<0.002$) in group A in comparison to group B while the abortion rate was higher in group B as compared to A (Table 3).

Table 2: Data of patients involved in this study

Data of patients	Group A	Group B	p-values
Age (years)	24.63±4.49	29.8±5.74	0.031
Body Mass Index (BMI) (kg/m^2)	25.64±3.57	26.6±3.62	0.143
Duration of infertility (years)	11.35±4.73	9.68±5.49	0.191
Baseline FSH (mIU mL^{-1})	7.37±3.28	8.04±2.9	0.131
Baseline LH (mIU mL^{-1})	5.05±3.18	7.78±3.72	0.074
LH/FSH	0.78±0.48	1.18±0.83	0.163
Progesterone at the day 21 of cycle (pg mL^{-1})	4.52±4.91	5.36±4.73	0.022
Baseline testosterone (ng mL^{-1})	0.59±0.24	0.60±0.25	0.081
Baseline estradiol (pg mL^{-1})	50.44±19.79	69.01±18.62	0.063
Estradiol at the day 12 of cycle (pg mL^{-1})	796.74±336.45	572.62±283.68	0.153
No of graffianfollicle	2.29±1.16	2.18±0.48	0.471
No of ovulation induction cycles	1.48±0.73	2.89±0.61	0.002

NS: Not Significance; nil: no results

Table 3: Pregnancy, life birth and abortion rates among control and study groups

Parameters	Group A	Group B	p-values
Ovulation rate/Patient	73.68% (56/76)	71.43% (25/35)	0.0560
Pregnancy rate/Cycle	23.26% (40/172)	12.87% (8/101)	0.0020
Life birth rate /Patient	31.58% (24/76)	22.86% (8/35)	0.0001
Pregnancy failure rate/Patient	42.11% (32/76)	48.57% (17/35)	0.0400

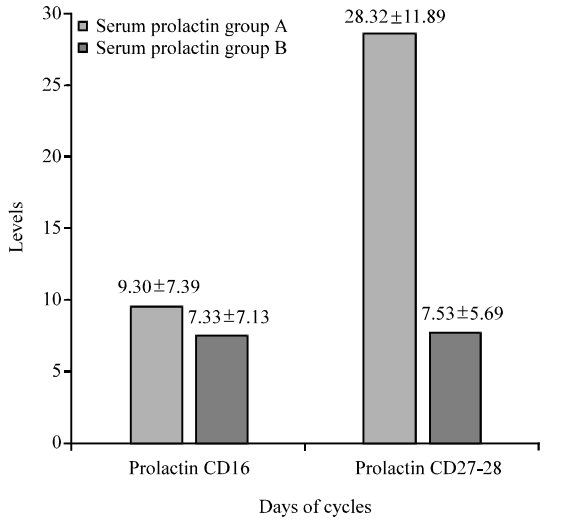

Fig. 1: Serum prolactin levels in the day 16 and 28 of ovulation induction cycles of patients involved in this study

Table 4 shows the ovulation, pregnancy and life-birth rates for each cycle of treatment in both control and treated groups. The cumulative ovulation rate over the first 3 months period was not statistically ($p = 0.74$) different between the two groups whereas it was significantly higher in group B as compared to group A (8/11 (72.72%) vs. 5/8 (62.5%), respectively; $p = 0.02$) during the later period of treatment of ≥4 months. The overall pregnancy rate for the whole period of treatment was significantly different between the two

Table 4: Ovulation, pregnancy and life-birth rates in control and study groups during treatment period

	Ovulation rate (No. of ovulatory cycles/No. of cycles (%))			Pregnancy rate (No. of pregnancies/No. of cycles (%))			Life-birth rate (No. of life-birth cases/No. of cycles (%))		
Cycles	Group A	Group B	p-values	Group A	Group B	p-values	Group A	Group B	p-values
≤2	40/49 (81.63%)	10/13 (76.92%)	0.74	17/49 (34.69%)	7/11 (63.63%)	0.010	16/49 (32.65%)	4/13 (30.78%)	0.71
3	11/19 (57.89%)	7/11 (63.63%)	0.08	9/11 (47.37%)	4/11 (36.36%)	0.040	6/19 (31.58%)	2/11 (18.18%)	0.01
≥4	5/8 (62.5%)	8/11 (72.72%)	0.02	4/8 (50%)	3/11 (27.27%)	0.002	2/8 (25%)	2/11 (18.18%)	0.03

groups but none of the women in either group had multiple pregnancies. The life-birth rate was not significantly different between the two groups in the early period of treatment (≤2 months), however, it became significantly higher in group A at a later period (≥3 months) ($p = 0.01$, Table 4).

Our policy at Allow IVF Center is not to recommend administering prolactin-lowering drugs at the end of the menstrual cycle of infertile anovulatory women due to the high probability of pregnancy. For that reason, we are retrospectively analyzing our data to find out if serum levels of PRL in the late luteal phase area consequence of conception in those infertile anovulatory women who have been treated for hyperprolactinemia.

The data shows that patients' age may play a significant role in the noted differences in cumulative life-birth rates between the two groups. Recently, Arce and Smitz (2013) reported a correlation between age and life-birth rate as well as ovulation rate. In this, the live-birth rate was lower in group B (22.86%) than in group A (31.58%). This finding is supported with those reported by Amer *et al.* (2002), Nahuis *et al.* (2011) and Goudarzi *et al.* (2014).

The higher concentration of the prolactin in group A at days 27-28 of cycle is significantly associated with ongoing pregnancies. It was documented that prolactin is a special reproductive hormone that correlates with implantation (Garzia *et al.*, 2004) as well as reproduction and sexual behavior (Ben-Jonathan *et al.*, 2008). Earlier, in 1979, the potential role of prolactin during implantation was reported showing that the prolactin is the major hormone secreted during decidualization of human endometrial stroma *in vivo* (Maslar and Riddick, 1979). On the other side, the high level of prolactin is essential for maintenance of normal production of progesterone during early pregnancy (Perks *et al.*, 2003). Bussen *et al.* (1999) showed a marked decrease in the prolactin level in patients with spontaneous abortion when compared to that of women who progressed to term (Bussen *et al.*, 1999).

The temporal pattern of expression of prolactin receptors throughout the menstrual cycle indicates that prolactin plays a differentiation rather than a mitogenic role on the glandular epithelial cells (Jabbour and Critchley, 2001). During the luteal phase, the abundant expression of prolactin and its endometrial receptor suggests that prolactin may be regulating the endometrial function in early conception and trophoplastic implantation. A role for prolactin in implantation has been outlined in the prolactin and prolactin receptor knockout mice (Horseman *et al.*, 1997).

In the group B treated with anti-prolactin drugs starting from the luteal phase of ongoing stimulated cycles, the placental development was impaired leading to deterioration of the maternal-fetal circulation through the anti-angiogenic effects of anti-prolactin treatment causing early abortion and fetal loss. Impairment of placental development or placental insufficiency has been observed in pregnancies complicated by pre-eclampsia and intrauterine growth retardation. In the pregnant uterus, blood vessel development in the placenta may be promoted by decidual prolactin via its classical receptors: both the long and intermediate forms of the receptor are highly expressed in placental tissue. The bottom line is that prolactin is essential for development of decidualized endometrium during the secretory phase of the menstrual cycle and early pregnancy (Lynch *et al.*, 2009).

For that reason we are not recommending starting treatment of hyperprolactinemic women in the second phase of the menstrual cycle in those who are undergoing ovulation induction protocols for treatment of infertility. In mice, bromocriptine treatment during early gestation impedes postpartum maternal care affects maternal postpartum behavior (Price and Bridges, 2014).

CONCLUSION

Late luteal hyperprolactinemia in women undergoing ovulation induction protocols for treatment of anovulatory factor of infertility is not a disease and is an early indicator of an ongoing pregnancy, it is therefore absolutely not recommended to be treated. Treatment of late follicular hyperprolactinemia leads to high abortion rate in anovulatory women treated by gonadotropin injections.

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