

## Radioimmunoassays Of Hormonal Changes Associated With Dysphoric Disorder In Premenstrual Women

Ragab H. EL-Yamani\* and Maha G. Soliman\*\*

\* Obstetric and Gynecology Dept. Faculty of Medicine, Al-Margab University, Great Socialist People's Libyan Jamahiriya and \*\* Pharmacology Dept (Medical microbiology and immunology unit) Notional Organization for Drug Control and Research

### Abstract

The neuroendocrinological changes that occur in patients with Premenstrual Dysphoric Disorder (PMDD) are poorly understood. The mechanism is likely to be a complex interaction between gonadal hormones and neurotransmitters. Melatonin is the predominant pineal neurohormone. The gonadal changes associated with melatonin are mediated via the hypothalamus. The purpose of this study was to evaluate melatonin concentrations in patients with PMDD. Also serum prolactin (PRL) and progesterone levels were estimated for any correlation with melatonin secretion. During the symptomatic late luteal phase, serum melatonin concentrations were determined by Radioimmunoassay (RIA) in 12 women with PMDD and 12 normal control women (NC). Also serum PRL and progesterone were estimated to all women by RIA. Melatonin concentration in patient with PMDD was significantly lower than in NC (mean 25.25 VS 79.25  $P \leq 0.05$ ). Serum PRL levels were significantly higher in PMDD subjects than in NC (mean 39.08 VS 20.17  $P \leq 0.05$ ). Serum progesterone levels were lower in PMDD than NC (mean 20.42 VS 33.58  $P \leq 0.05$ ). The results showed inverse correlation between melatonin and PRL levels ( $r = 0.931$ ,  $P \leq 0.001$ ) and direct correlation between melatonin and progesterone levels ( $r=0.894$ ,  $p \leq 0.001$ ). No correlation was found between PRL and progesterone level. Findings of the study suggest that lowered melatonin levels in women with PMDD may be due to involvement of the pineal gland in the pathogenesis of this disorder.

### Introduction

It is well known that clinical symptoms such as psychosis, epileptic seizures and sleep disturbances aggravate around the time of menstruation. In some healthy women, subjective sleep feelings or moods have been reported to change throughout the menstrual cycle, which suggests that sleep structure and sleep-wake rhythm may change during the menstrual cycle (Ito *et al.*, 1995).

Premenstrual Dysphoric Disorder (PMDD) is the new terminology used in the American Psychiatric Association's

Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). It more rigorously defines criteria for what historically has been referred to as premenstrual syndrome. Based on studies of its phenomenology and course, PMDD was categorized in DSM-IV as a depressive mood disorder (Parry, 1997).

Although the etiology of this condition has remained elusive despite considerable research, recent success in treatment studies would seem to

indicate progress. Efficacy has been shown both by drugs, which inhibit ovulation, and those, which directly interact with the central nervous system (CNS). The mechanism is therefore likely to be a complex interaction between gonadal hormones and neurotransmitters (melatonin and serotonin) with changes in the CNS initiated by fluctuating ovarian activity. The neuroendocrinological changes that occur in these patients are poorly understood (**Su et al., 1997**).

The pineal gland is an active neuroendocrine organ that responds to photic hormonal stimuli. Pineal activity can be viewed as the net balance between hormone and neuron mediated influences. It contains receptors for the active sex hormones and prolactin (**Reiter, 1991**). Melatonin is the predominant pineal neurohormone. It circulates in the blood like a classical hormone and affects distant target organs, especially the neuroendocrine centers of the CNS. In the absence of light at night an increase in melatonin biosynthesis in the pineal gland is stimulated by electrical signals originating from neurons in the suprachiasmatic nuclei. These neurons inputs from the lateral eyes (**Reiter, 1989**). Melatonin receptors have been identified in the human nucleus supra-chiasmaticus, which is the master circadian pacemaker (**Reppert et al., 1988**).

The gonadal changes associated with melatonin are mediated via the hypothalamus and suggest a general suppressive effect on GnRH pulsatile secretion and reproductive function (**Silman, 1991**).

The aim of this study was to replicate and extend previous work in which the authors observed lower melatonin secretion during the luteal phase in PMDD patients compared to

control subjects. Prolactin and progesterone levels were estimated for any correlation between them and melatonin concentration.

### Subjects And Methods:

The study included 12 patients who met DSM-IV criteria for PMDD and 12 apparently healthy control subjects (NC). All volunteers were between the ages of 25-35 years, non-smokers, resided in Cairo, with similar socioeconomic level and had not traveled in the 8 weeks prior to the study. All volunteers attended to El-Zahraa Hospital, Faculty of Medicine for Girls, Al-Azhar University. Measurements were made during symptomatic luteal menstrual cycle phases in PMDD patients. Samples were collected in the morning time (8-10 am) and within the same season (from January 1998 to March 1998). According to **Molina et al., (1996)** single morning blood sample (5 ml) was collected from each patient. Sera were obtained by centrifugation of the clotted blood samples, divided into 3 aliquots and stored at -20°C until assayed in the allowed time for melatonin, progesterone and prolactin (Yoshkor et al ., 2000; Och et al .,2000). Both progesterone and prolactin levels were determined by Radioimmunoassay (RIA) using Cota-A-Count kits supplied by (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum melatonin levels were determined also by RIA technique using the kit supplied by INRA, Nouzilly, France.

Statistical analysis and data presentation of the results were carried out by the use of the software **Microsoft Excel 5.0 (1994)**, **Microsoft Corporation, USA** and according to **Shwartz, (1980)** for IBM Computers.

**Results**

The results of the present investigation could be summarized as in table (1) :

Figure (1). Illustrates the significances changes of serum melatonin level between the control group and the PMDD groups . It was found that the serum melatonin levels during luteal phase, was significantly lower in patients with PMDD than that of the normal control group. On contrast, serum prolactin levels were significantly higher (  $P \leq 0.05$  ) in

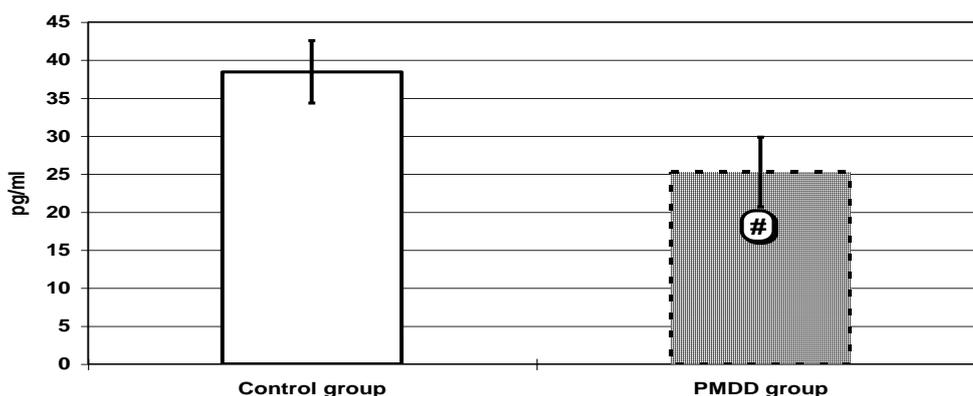
PMDD women than the normal control ( mean 39.08 VS 20.17 ) fig (2) .

Concerning the progesterone level during the luteal phase in patients fig (3) showed a significant lower concentration ( $p \leq 0.05$ ) than in normal subjects ( mean 20.42 VS 33.58.  $p \leq 0.05$  ).

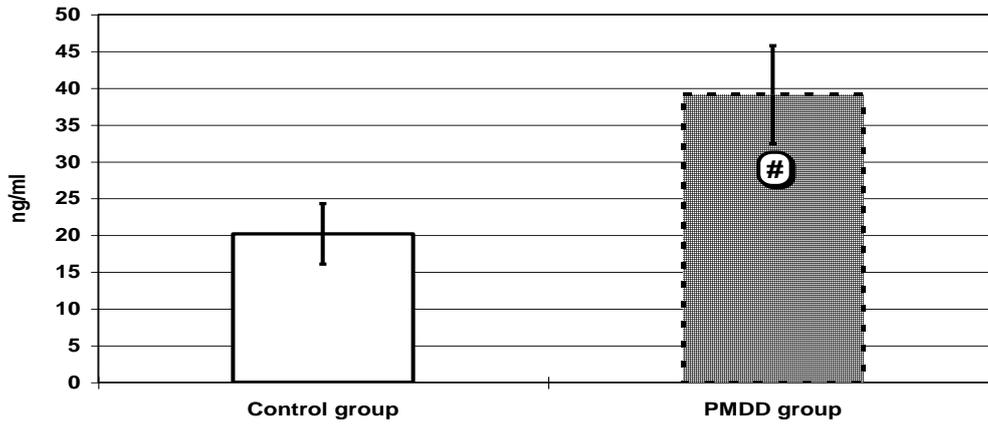
Fig (4) showed inverse correlation between melatonin and PRL levels (  $r = 0.931$  ,  $p \leq 0.01$  ) while fig (5) illustrated the direct correlation between melatonin and progesterone levels (  $r = 0.894$  ,  $p \leq 0.001$  ). On the other hand , no correlation was found between PRL and progesterone levels .

**Table (1): The descriptive statistics of the PMDD subjects and the NC.**

	Progesterone ng/ml		Prolactin ng/ml		Melatonin pg/ml	
	PMDD	NC	PMDD	NC	PMDD	NC
Mean	20.417	33.583	39.083	20.167	25.250	38.417
S.E	2.512	5.168	6.669	4.095	4.596	4.109
Median	18	30.5	32	17	21.5	37
S.D	8.702	17.901	23.102	14.186	15.921	14.235
Range	9-38	7-72	18-101	7-58	8-66	19-241
Sum	245	403	469	242	303	461
Count	12	12	12	12	12	12

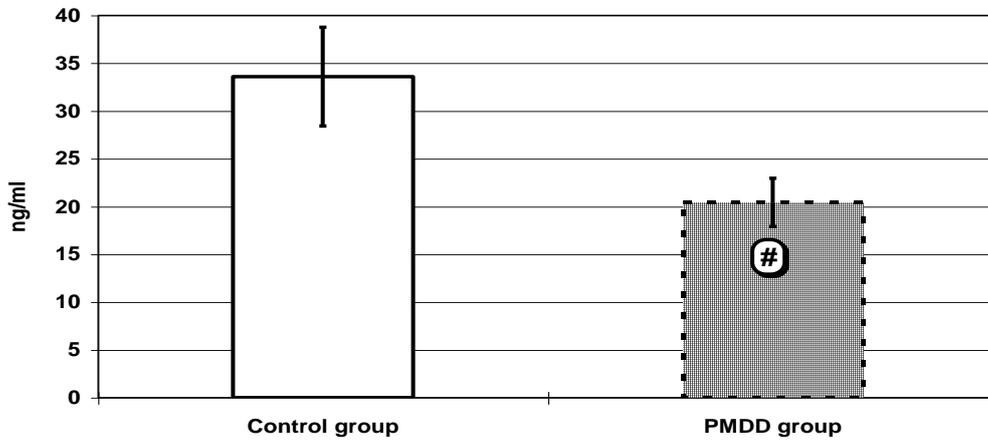


**Fig. (1) : The significance change of serum melatonin level between the control and the PMDD group (mean  $\pm$  S.E.).**



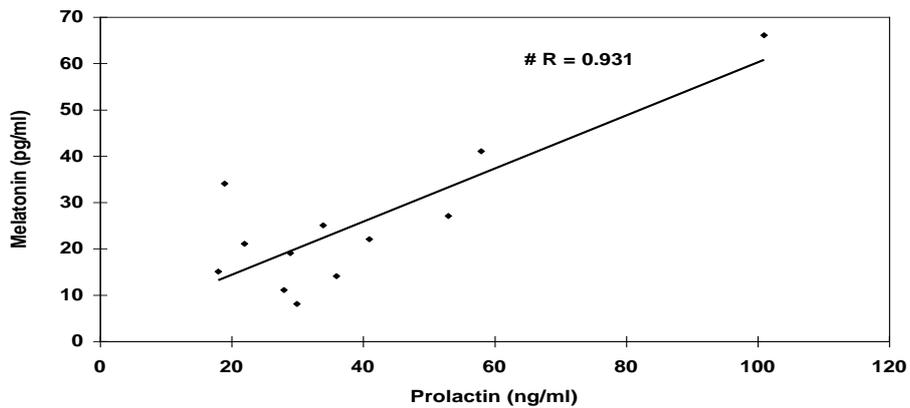
**Fig. (2) : The significance change of serum prolactin level between the control and the PMDD group (mean  $\pm$  S.E.).**

# Significant change from control at  $p \leq 0.05$



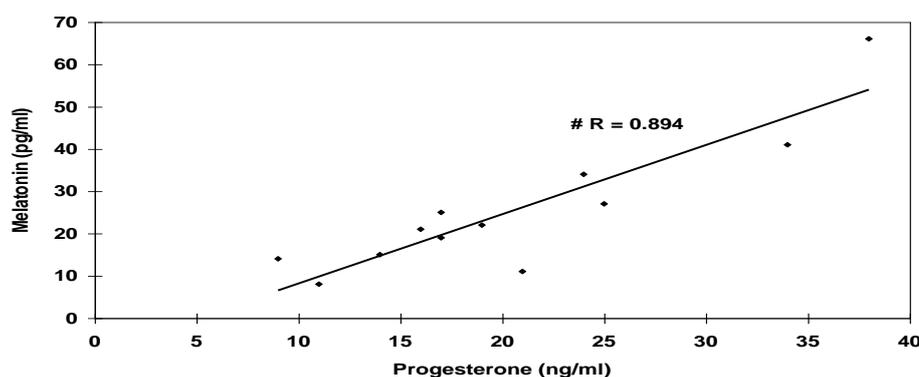
**Fig. (3) : The significance change of serum progesterone level between the control and the PMDD group (mean  $\pm$  S.E.)**

# Significant change from control at  $p \leq 0.05$



**Fig. (4) : The correlation between serum melatonin and serum Prolactin in the PMDD group**

# Significant correlation at  $p \leq 0.001$



**Fig. (5) : The correlation between serum melatonin and serum progesterone in the PMDD group**

# Significant correlation at  $p \leq 0.001$

## DISCUSSION

In the present study it was found that the serum melatonin levels, measured during the symptomatic luteal phases were significantly lower in patients with PMDD than NC (mean 25.25 VS 79.25  $P \leq 0.05$ ). These findings are in agreement with the findings of **Parry et al. (1997)** who reported that serum melatonin levels during the luteal phase were significantly lower in PMDD subjects than NC. They suggest that this observation do not likely represent a disturbance in pineal reactivity to suprachiasmatic nucleus efferent. Instead, the findings support a possible disturbance in PMDD in the clock itself or its coupling mechanisms. In another study by **Parry et al. (1997)**, they reported that in PMDD subjects during the luteal phase, melatonin onset time was delayed, duration was compressed, and area under the curve, amplitude, and mean levels were decreased than NC subjects.

**Murialdo et al. (1994)** reported that the normal increase in urinary metabolism excretion during the luteal phase was less pronounced in the PMDD patients. They suggest impaired pineal function in PMDD.

Parry's findings support low serotonin and melatonin metabolism in PMDD compared with NC, and an increasing number of rigorous studies report a reduction of symptoms with selective serotonin reuptake inhibitors (**Parry, 1997**).

The causal relationship, if any, between the decreased melatonin secretion and PMDD has not been established.

Preliminary data in rats and humans suggest that endogenous opioids augment melatonin secretion. Thus, it is possible that, withdrawal of endogenous opioids which has been found during the luteal phase (**Facchinelli et al., 1987**) induces suppression in melatonin secretion in PMDD subjects.

Data about women complaining of the PMDD are equally conflicting, some reports demonstrating significantly higher serum PRL concentrations both in the follicular and luteal phases, lower levels in the follicular phase, or no significant difference with control subjects (**Magos and Studol, 1984**).

In the present study it was noted that the serum PRL levels were higher in PMDD women than the NC (mean 39.08 VS 20.17  $P \leq 0.05$ ).

**Rubinow and Schmidt (1992)** reported excess prolactin secretion during the luteal phase in PMDD subjects than in NC. The present study showed significant correlation between melatonin and PRL levels ( $r = 0.931$ ,  $P \leq 0.001$ ). The results of **Brzezinski et al. (1994)** indicate that endogenous PRL do not affect the levels or circadian phase of melatonin. In vitro results showed that melatonin suppressed PRL secretion (**Bhगत et al., 1994**). In contrast to that **Terzolo et al. (1993)** reported that exogenous melatonin has a stimulatory effect on PRL release. Also **Okatani et al. (1994)** reported that oral melatonin administration induce a significant increase in serum prolactin concentration. They suggested that the stimulation is

not mediated by opioids. **Okatani and Sagara (1993)** suggested that melatonin can regulate nocturnal PRL secretion independent of sleep-related factors.

Progesterone and certain of its metabolites are anxiolytic (**Baker et al., 1995**). Some studies have reported lower progesterone concentration during the luteal phase in some patients with PMDD (**Munday et al., 1981**). Other studies have not confirmed this observation (**Taylor, 1979**).

**O'Brien et al. (1980)** actually found elevated plasma progesterone levels following ovulation in PMDD sufferers. The present study showed a significant lower progesterone concentration during the luteal phase in patients with PMDD than NC subjects (mean 20.42 VS 33.58  $P \leq 0.05$ ). No correlation was found between PRL and progesterone but a direct correlation was found between melatonin and progesterone ( $r=0.894$ ,  $p \leq 0.001$ )

**Brzezinski et al. (1994)** reported that endogenous progesterone do not affect melatonin levels. Also **Delfs et al. (1994)** reported that there is no consistent change in melatonin blood concentrations in response to the varying endogenous or exogenous concentrations of sex steroids.

Regarding the effect of melatonin on progesterone, **Sandyk et al. (1992)** reported that melatonin stimulates progesterone production. This is in contrast to in vitro animal studies which indicate a suppressive effect of melatonin on progesterone production (**Khan-Dawood and Dawood, 1993; Sirotkin, 1994**).

#### CONCLUSION:

Serum melatonin levels, during the late luteal phase, were significantly lower in PMDD women than NC suggesting that the pineal gland involved in the pathogenesis of the PMDD. There was an inverse correlation between melatonin and PRL levels and direct correlation between melatonin and progesterone levels. In future studies, attempts should be made to estimate and compare between serum melatonin levels, its metabolism and urinary excretion in patients with PMDD.

#### References

1. **Baker E.R., Best R.G. and Fredi R.L. (1995):** Efficacy of progesterone vaginal suppositories in alleviation of nervous symptoms in patients with premenstrual syndrome. *J. Assist. Reprod. Genet.*, 12: 205 - 209.
2. **Bhagat L., Duraiswami S. and Muralidhar K. (1994):** Mode of action of inhibin-like pineal antigonadotropin is different from melatonin during compensatory ovarian hypertrophy. *J. Pineal. Res.*, 16(4): 193 -197.
3. **Brzezinski A., Cohen M., Ever-Handi P., Mordel N., Schenker J.G. and Laufer N. (1994):** The pattern of serum melatonin levels during ovarian stimulation for in vitro fertilization. *Int. J. Fertil. Menopausal.* 39(2): 81-85.
4. **Delfs T.M., Baars S., Fock C., Schumacher M., Olcese J. and Zimmermann R.C. (1994):** "Sex steroids do not alter melatonin secretion in the human". *Hum. Reprod.* 9(1): 49-54.
5. **Facchinetti F., Martignoni E. and Petraglia F. (1987):** Premenstrual fall of plasma  $\beta$ -Erdorphin in patients with premenstrual syndrome. *Fertil. Steril.*, 47: 570 - 573.
6. **Ito M., Kohsaka N. and Honma K. (1995):** Changes in biological rhythm and sleep structure during the menstrual cycle in healthy women. *Seishin Shinkeigaku Zasshi/psychiatria et Neurologia Japonica*, 97 (3): 155-164.
7. **Khan-Dawood F.S. and Dawood M.Y. (1993):** Baboon corpus luteum: The effect of melatonin on in vitro progesterone production. *Fertil-Steril.*, 59 (4): 896-900.
8. **Magos A. and Studd J. (1984):** The premenstrual syndrome In progress in obstet and Gynecol, Stud. J. (ed) p. 334
9. **Molina C.A., Munoz H.A., Martin, G.J.A., Uberos F.J., Rodriguez C.T. and Acuna C.D. (1996):** 5-Methoxytryptophol and melatonin in children: differences due to age and sex. *J. Pineal. Res.*, 21(2): 73 - 78.
10. **Munday M., Brush M.G. and Taylor R.W. (1981):** Correlations between

- progesterone, estradiol and aldosterone levels in the premenstrual syndrome. *Clinical Endocrinology*, 14: 1-9.
11. **Murialdo G., Fonzi S., Costelli P., Solinas G.P., Parodi C., Marabini S., Fanciullacci M. and Polleri A. (1994):** Urinary melatonin excretion throughout the ovarian cycle in menstrually related migraine. *Cephalalgia* , 14(3): 205-209.
  12. **O'Brien P.M.S., Selby C. and Symonds E.M. (1980):** Progesterone, fluid, and electrolytes in premenstrual syndrome. *Br. Med. J.*, (280) 6224: 1161-1163
  13. **Och w., Smolka M., Mariakz. and Kozirowski ..(2000) :** Correlation of steroidal receptors and growth factors expression with peritumoral edema accompanying cerebral neoplasms. *Neurol Neurochir., Pol.* ; 34:91-101.
  14. **Okatani Y., Okada M. and Sagara Y. (1994):** Stimulation of prolactin secretion by melatonin is not mediated by opioids. *Horm-Res.*, 41(1): 38 -42.
  15. **Okatani Y. and Sagara Y. (1993):** Role of melatonin in nocturnal prolactin secretion in women with normoprolactinemia and mild hyperprolactinemia. *Am. J. Obstet. Gynecol.*, 168: 854 - 861.
  16. **Parry B.L. (1997):** Psychobiology of premenstrual dysphoric disorder *Seminars in Reproductive Endocrinology*, 15(1): 55 -68.
  17. **Parry B.L., Berga S.L., Mostofi N., Klauber M.R. and Resnick A. (1997):** Plasma melatonin circadian rhythms during the menstrual cycle and after light therapy in premenstrual dysphoric disorder and normal control subjects. *J. Biological Rhythms*, 12(1): 47 -64.
  18. **Parry B.L., Udell C., Elliott J.A., Berga S.L., Klauber M.R., Mostofi, N., Leveau B. and Gillin J.C. (1997):** Blunted phase-shift responses to morning bright light in premenstrual dysphoric disorder. *J. Biological Rhythms*, 12(5): 443 -456.
  19. **Reiter R.J. (1989):** The pineal gland. *DeGroot's Endocrinology*. Vol. 2<sup>nd</sup> ed DeGroot, L.J. (ed) p. 240.
  20. **Reiter R.J. (1991):** Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. *Endocr. Rev.*, 12: 151-180.
  21. **Reppert S.M., Weaver D.R., Rivkees S.A. and Stopa E.G.(1988):** Putative melatonin receptors in a human biological clock *Science*, 242: 78-81.
  22. **Rubinow D.R. and Schmidt P.J. (1992):** Premenstrual syndrome: a review of endocrine studies. *Endocrinologist*, 2: 47.
  23. **Sandyk R., Anastasiadis P.G., Anninos P.A. and Tsagas N. (1992):** Is the pineal gland involved in the pathogenesis of endometrial carcinoma. *Int.J. Neurosci.*, 62(1-2):89-96.
  24. **Shwartz D. (1980):** *Flammarion Medicine- Sciences Press*, 3<sup>rd</sup> edition-Paris..
  25. **Silman R. (1991):** Melatonin and the human gonadotrophin-releasing hormone pulse generator. *J. Endocrinol.*, 128: 7-11.
  26. **Sirotkin A.V. (1994):** Direct influence of melatonin on steroid, nonapeptide hormones, and cyclic nucleotide secretion by granulosa cells isolated from porcine ovaries. *J. Pineal. Res.*, 17(3): 112 -117.
  27. **Su T.P., Schmidt P.J. and Danaceau M.A. (1997):** Fluoxetine in the treatment of premenstrual dysphoria. *Neuropsychopharmacology*, 16: 346-356.
  28. **Taylor J.W. (1979);** Plasma progesterone, estradiol 17 $\beta$  and premenstrual symptoms. *Acta Psychiatrica Scandinavica*, 60: 76 -86.
  29. **Terzolo M., Revelli A., Guidetti D., Piovesan A., Cassoni P., Paccotti P., Angeli A. and Massobrio M. (1993):** Evening administration of melatonin enhances the pulsatile secretion of prolactin but not of LH and TSH in normally cycling women. *Clin. Endocrinol. Oxf.*, 39(2): 185 -191.
  30. **Yashkov Y.L., Vinnitsky L.I., Poroykova M.V. and Vorobyova N.T. (2000) :** Some hormonal changes before and after vertical banded gastropasty for severe obesity. *Obes. Surg.*, 10:48-53.

## القياسات المناعية الإشعاعية للتغيرات الهرمونية المصاحبة لمشاكل الأم الطمث في مرحلة ما قبل الدورة الشهرية

\*رجب حسنى اليماني - \*\*مها غازى سليمان

\*قسم النساء والولادة - كلية الطب - جامعة المرقب - الجماهيرية العربية الليبية العظمى  
\*\* الهيئة القومية للرقابة والبحوث الدوائية - قسم الفارماكولوجى - وحدة البكتريولوجى  
والمناعة

التغيرات العصبية الهرمونية التي تصاحب المرضى الذين يعانون الام ما قبل الدورة الشهرية غير معلومة ويعتقد ان ميكانيكية هذه التغيرات ناتجة عن التفاعل بين الوسائط العصبية وهرمونات الغدد التناسلية.

يعتبر الميلاتونين هو هرمون الغدة الصنوبرية الأكثر ظهوراً من بين الوسائط العصبية وان تغيرات الجهاز التناسلي المصاحبة للميلاتونين تحدث مروراً بجذع المخ. والغرض من هذه الدراسة هو تقييم تركيزات الميلاتونين فى مرضى آلام ما قبل الدورة الشهرية . أيضا تم قياس مستوى هرمون البروجسترون و هرمون البرولاكتين فى مصل الدم وتم ربط هذه القياسات بحدوث اى تغيرات فى مستوى الميلاتونين وذلك أثناء الفترة المؤلمة المتأخرة فى مرحلة ما بعد التبويض .وقد تم قياس الميلاتونين فى مصل الدم باستخدام القياسات المناعية الإشعاعية وذلك فى عدد اثنتي عشر امرأة يعانين من حالة آلام ما قبل الدورة الشهرية، كذلك تمت القياسات فى عدد اثنتي عشر امرأة ذات حاله طبيعية وذلك كمجموعه حاكمه وقد تم قياس مستوى البروجسترون و البرولاكتين فى السيرام باستخدام طريقة القياسات المناعية الاشعاعية .وقد وجد ان مستوى الميلاتونين فى النساء المريضات كان اقل بصورة واضحة عن المجموعه الحاكمه . ووجد أيضا ان مستوى هرمون البرولاكتين كان مرتفعاً بصورة واضحة فى النساء المريضات عن المجموعه الحاكمه .اما مستوى هرمون البروجسترون فقد وجد انه منخفض بصورة واضحة فى المجموعه المريضة عن المجموعه الحاكمه.

وقد اوضحت الدراسة وجود علاقة عكسية بين مستوى الميلاتونين وهرمون البرولاكتين ووجود علاقة طرديه بين مستوى الميلاتونين ومستوى هرمون البروجسترون ولكن لم نجد اى علاقة ارتباطية بين مستوى هرمون البروجسترون وهرمون البرولاكتين .  
وقد اوضحت نتائج الدراسة ان انخفاض مستوى الميلاتونين فى النساء المريضات بآلام ما قبل الدورة الشهرية قد ينتج عن تأثير الغدة الصنوبريه بصورة مباشرة فى الأعضاء التناسلية.