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Peripheral melatonin concentration and its correlation with estradiol and progesterone levels during different months of pregnancy and after delivery in women

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SUMMARY

There is lack of basic information about peripheral melatonin level and its relationship with gestational hormones i.e., estrogens and progesterone, in human females. The present study addressed this issue and aimed to find the relationship between the levels of melatonin and estrogen and progesterone during different weeks of gestation, during parturition and during post-partum. The data suggest that there is a definite ratio of melatonin with both the steroids hormons before and during gestation as well as post-partum. An inverse relationship between the levels of estrogen and melatonin was observed in non-pregnant and post-partum women, while a direct relationship was observed during gestation. These two different types of hormonal relationships might be essential for maintenance of pregnancy. The inverse relationship of melatonin with progesterone during parturition may serve as a signal for parturition.

Key words: Melatonin, estradiol, progesterone, gestation, parturition, post-partum

Introduction

Melatonin, a pineal hormone, regulates the reproductive and physiological adaptations that occur in seasonally breeding mammals in response to changes in day length, but its role in reproduction of humans is still unclear (Reiter, 1998). Investigations, in both animals and humans, have provided evidences to the effect that the pineal gland plays an important role in the endocrine control of reproductive physiology (Matthews et al., 1993).

There is increasing evidences indicating that the pineal gland is affected by hormonal signals in the body. Some of the better-studied hormonal regulatory factors of pineal secretory activity are gonadal steroids and gonadotropins (Brzezinski et al., 1994). It is well established that melatonin can influence gonadotrophs, secreting LH and FSH (Morgan et al., 1991; Vanecek and Klein, 1995; Hattori et al., 1995). However, there is also evidence to suggest that melatonin acts at the level of the ovary to modify ovarian functions in mammals (Murayama et al., 1997). High levels of melatonin, which undergo seasonal variation, were detected in human pre-ovulatory follicular fluid (Brzezinski et al., 1987; Ronnberg et al., 1990; Yie et al., 1995a). In the human, melatonin-binding sites have been detected in granulosa-luteal cells (Yie et al., 1995b; Niles et al., 1999; Michelle et al., 2001) and, hence, melatonin can have a direct effect on ovarian steroidogenesis (Martin et al., 1982; Webley et al., 1886; Baratta et al., 1992; Brzezinski et al., 1992; Tamura et al., 1998; Balik et al., 2004). The effects of gonadotropins on follicular growth, ovulation and luteinization are associated with differences in FSH receptor (FSHR) and LH receptor (LHR) concentrations (Richards et al., 1995; Itoh et al., 1999). Further, GnRH and its receptor have also been detected in the ovary (Peng et al., 1994). Given that melatonin can alter steroidogenesis, it is possible that melatonin may be interacting with gonadotropins and GnRH to modulate the amplitude of the transductive signal (Silman, 1991; Morgan 1991). Further, melatonin is one of the hormones that control the timing and release of female reproductive hormones; thus, melatonin helps to determine when menstruation begins, the frequency and duration of menstruation cycles and when menopause sets in (Berga and Yen 1990; Santoro et al., 1996).

Pineal HIOMT activity, melatonin content, and serum and urine melatonin concentrations have been reported to fluctuate during the estrous and menstrual cycles (English et al., 1986; Berga et al., 1988; Helliwell and Williams 1992; Joshi et al., 1994). There is lack of

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information on the effect of melatonin during gestation in the human though it has been extensively studied in a rodent (Bishnupuri and Haldar, 2000). In view of lack of basic information about the relationship between melatonin and gestational hormones in women this study was undertaken to assess the serum melatonin level in women during different months of pregnancy and after delivery, and was correlated with estradiol and progesterone levels.

Material and Methods

The subjects

For the study on the relationship between hormones samples were collected from following four groups to which women were assigned on the basis of the period of their gestation.

Group 1: 20 non-pregnant women with ideal body weight (50-60 Kg)

Group 2: 20 pregnant women examined from week 5 to delivery

Group 3: 20 women after 24 to 28 hr of delivery

Group 4: 12 women examined up to 5 weeks after delivery.

Blood was collected from the women at 5-weeks interval (for melatonin at 10.00 pm under dim red light while for other hormones at 11am). Blood samples were collected from non-pregnant women one week after menstruation in a similar manner but sampling during menstruation was avoided. Informed consent was obtained from all subjects and guidelines from the ethical committee of the University (IMS) were followed. The obstetrics staff of the University collected the blood under the supervision of a Senior Gynecologist (Prof. L.K. Pandey, a Co-author). A further positive criterion was the good health of the females (without maternal metabolic disorders and fetal growth retardation). The age of the women was within the range of 22 to 32 years. The hospital allowed all women admitted (with labor pain and check up) with similar artificial light condition during hospitalization. The blood, after coagulation, was centrifuged at 1200 x g for 10 min. The sera were frozen and stored at –20 ^{0}C until the assay for melatonin, estradiol and progesterone.

Radioimmunoassay

Melatonin RIA was performed according to Rollag and Niswender (1976), using Guildhey anti-melatonin antibody (Guildhey, Surrey, UK). The recovery, accuracy and sensitivity for the melatonin RIA were 92%, 0.987 and 10 pg/mL, respectively. Intra- and inter-assay variations of melatonin were 9.0% and 15%. RIA of estradiol was performed using a commercial kit purchased from Leuco Diagnostics Inc. (Miss., USA). The recovery and sensitivity for estradiol were 102.2% and 1-45pg/mL, respectively. Intra- and inter-assay variations of estradiol were 9.2% and 4.3%, respectively. Progesterone assay was performed using a commercial RIA kit (Binax, Portland, Maine, USA). The sensitivity of the assay was 50 pg/tube and the intra- and inter-assay coefficients of variation were 6.5% and 8.7%, respectively.

Statistical analysis

Data were used to calculate the respective means and standard deviations. One-way ANOVA was also carried out.

Results

Levels of melatonin, estradiol and progesterone during different weeks of gestation, during parturition and at five weeks after delivery

The levels of estradiol and progesterone were low during the 5th week of pregnancy. The level of progesterone slowly increased from 10th week to 40th week while that of estradiol changed non-significantly up to 25th week, but a slight increase was observed after 30th week till 40th week. On the other hand, high levels of melatonin were recorded in pregnant women up to the 35th week. A sudden decrease in the level of melatonin was noted on the 40th week in all the women under observation around 39th to 40th week of pregnancy. We found a sudden decrease in progesterone, estradiol and melatonin levels immediately after the delivery. While the levels of melatonin and progesterone remained low thereafter, that of estradiol showed a slight increase (Fig. 1).

We compared the levels of different hormones between non-pregnant, pregnant (35th week), delivered (40th week) and post-partum (5th week) women. We found that estradiol level was high in non-pregnant women in comparison to the other groups and was insignificantly different in post-partum while the lowest level of estradiol was noticed during the early days of delivery (Fig. 2). Contrary to this, progesterone level was negligible in non-pregnant and post-partum females while it was significantly high in 35th week of pregnancy but decreased significantly during delivery, but the level was still higher than in non-pregnant women (Fig. 3). In non-pregnant women melatonin level was fairly low while it was significantly high in pregnant women and decreased significantly during delivery maintained low post-partum (Fig. 4).

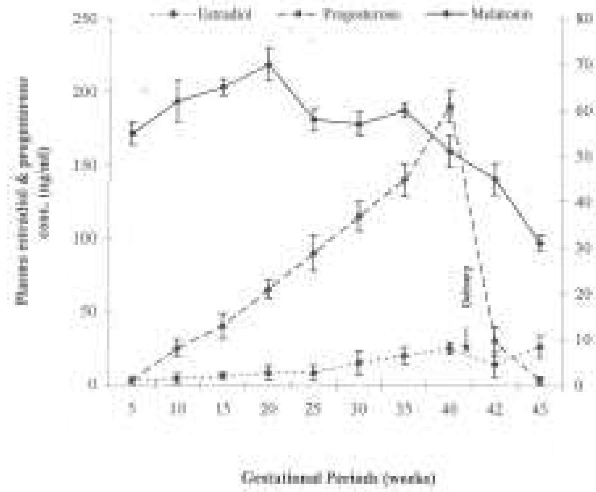

Fig. 1. Variation in the circulating levels of estradiol, progesterone and melatonin during different weeks of gestation, delivery and post-partum.

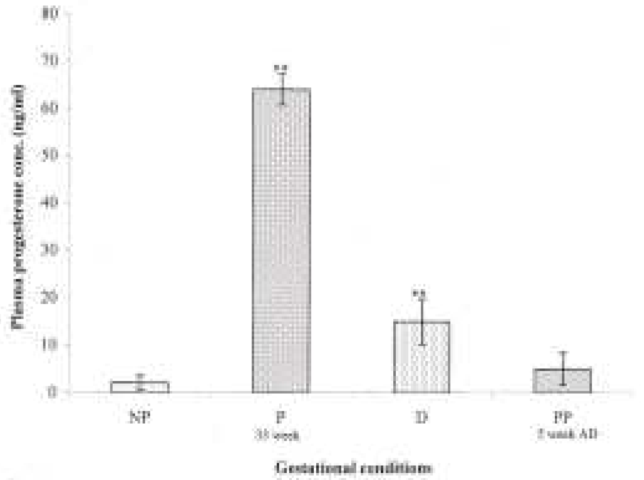

Fig. 3. Histogram showing the level of progesterone in non-pregnant (NP), pregnant (P; 35 week), delivered (D; within 24 hours of delivery) and post-partum (PP; 5 week) females.** $P< 0.001$, when compared with NP.

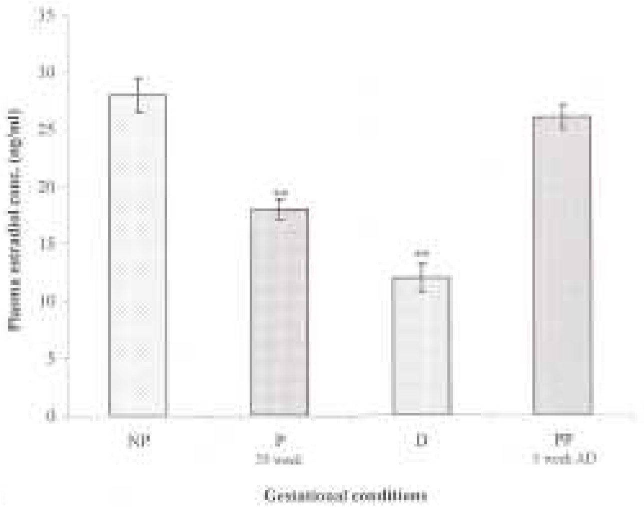

Fig. 2. Histogram showing the level of estradiol in non-pregnant (NP), pregnant (P; 35 week), delivered (D; within 24 hours of delivery) and post-partum (PP; 5 week) females.** $P< 0.001$, when compared with NP.

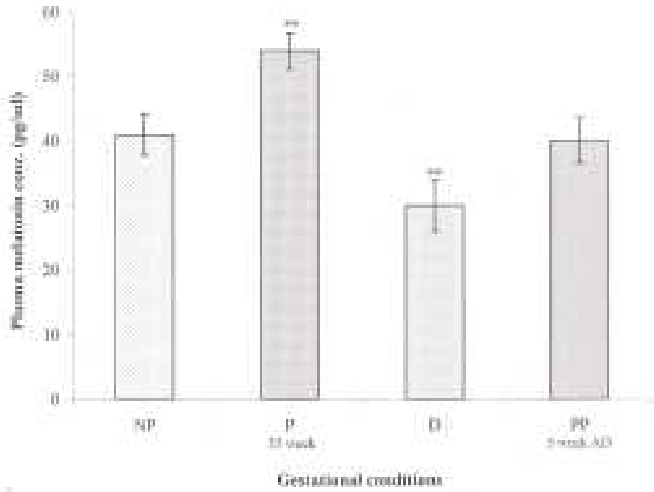

Fig. 4. Histogram showing the level of melatonin in nonpregnant (NP), pregnant (P; 35 week), delivered (D; within 24 hours of delivery) and post-partum (PP; 5 week) females.** $P< 0.001$, when compared with NP.

Discussion:

In the present study, we have shown for the first time a relationship between serum levels of estradiol and progesterone with melatonin from 5th week of pregnancy till delivery after 40th week and post-partum up to 5 week.

Our study shows that high level of melatonin and a moderately high level of estradiol are necessary for maintenance of pregnancy in women, as we had shown earlier in a seasonally breeding rodent *Funambulus pennanti* (Bishnupuri and Haldar, 1999, 2000). During this period there was a slow and steady increase of progesterone, which was highest during the 40th week which is a requisite for delivery. During the 40th week we found a sudden decline of melatonin, which might have "triggered – on" the parturition. Before delivery a direct relationship of estradiol and progesterone was noticed with that of melatonin, which became once again inversely proportional following delivery, as observed in normal non-pregnant women.

We further analyzed our data on the basis of hormonal pattern. Beginning with estradiol, it was highest in non-pregnant women but decreased significantly after 35th week of pregnancy up to delivery at 40th week and was insignificantly different from non-pregnant women after delivery.

Progesterone was extremely low in non-pregnant females (mostly un-detectable). It increased slightly in 35th week of pregnancy and was extremely high during delivery, i.e., the 40th week. Post-partum level of progesterone was insignificantly different from that of non-pregnant women. Interestingly, melatonin was low in non-pregnant women but increased significantly in pregnant women, became extremely low during delivery, and increased post-partum.

This hormonal pattern points clearly to the functional relationship between the three hormones at different times (early, middle and late) of gestation and supports our finding in mammals that high level of melatonin might be responsible for maintenance of pregnancy (Bishnupuri and Haldar, 2000; Nakamura et al., 2001). The inverse relationship between gonadal steroids and melatonin returned to normal after delivery, from 5th week onwards, while there was a direct relationship between these two hormones throughout pregnancy. In the initial stage of human pregnancy, there was low level of estrogen and progesterone and high level of melatonin in circulation. Those hormonal levels gradually changed after the first trimester with increasing progesterone so that in later periods of gestation, there was a high level of progesterone (Tulchinsky et al., 1972; Boroditsky et al., 1978; Kivela et al., 1990; Kivela 1991). Gonadal steroids and gonadotropins regulate pineal secretory activity; therefore, fluctuations in melatonin level of pregnant females are anticipated and it suggests a role for melatonin in gestation (Bishnupuri and Haldar, 1999, 2000). In a recent study, seasonal variations in human conception and birth rates in different geographical areas were analyzed (Roenneberg et al., 1990; Rozansky et al., 1992).

Although humans are not seasonal breeders, seasonal trends in their reproductive function have been ascribed, and the pineal gland appears to play an important role in the neuro-endocrine regulation of human reproductive physiology (Reiter et al., 1998). During short day length season, reduced activity of anterior pituitary and increased level of serum melatonin have been documented in population studies in temperate zone countries (Kauppila et al., 1987). Nocturnal plasma melatonin concentration on day 10 of the menstrual cycle has been found to be higher in winter than summer; conversely, nocturnal plasma LH levels are higher in summer than in winter (Kivela et al., 1988). In rats, the maternal pineal influences the gonadal and genital development and function of offspring, but this is yet to be confirmed in the human (Colmenero et al., 1991). The pineal, apparently, influences human reproductive function not only at the hypothalamus-pituitary level by inhibition of the hypothalamic pulsatile secretion of gonadotropins-releasing hormone, but also at the gonadal level, where melatonin receptors have been found (Niles et al., 1999).

The pineal appears to be involved in the neuroendocrine regulation of puberty and reproductive physiology (Colmenero et al., 2005); however, many aspects of the role of pineal in human reproduction remain obscure. The female reproduction, particularly the most important phase, i.e., gestation, has never been considered in this perspective. Further, it has not been clearly demonstrated in women whether melatonin exerts these effects by acting at the hypothalamic level or directly at the pituitary level. There is evidence to suggest that the inhibitory role of melatonin is exerted at the hypothalamic level, influencing the pulsatile secretion of GnRH (Silman, 1991). In patients with functional hypothalamic amenorrhoea, whose hormonal profile is decreased GnRH/ LH pulsatility, and a significant increase in the nocturnal peak amplitude and duration of melatonin has been documented (Silman et al., 1988; Brzezinski et al., 1988).

Studies have been carried out to investigate the mechanism by which melatonin inhibits the hypothalamic secretion of GnRH. Melatonin has been proposed to act by directly suppressing the hypothalamic pulsatile secretion of GnRH (Bittman et al., 1985). It has also been suggested that this inhibition is mediated by a change in dopaminergic and opioidergic activity (Rasmussen, 1993). However, the mechanism by which melatonin inhibits GnRH pulsatile release is not yet clear. In rats, melatonin has been observed to inhibit the pituitary response to LHRH (Martin et al., 1980; Vanecek and Klein, 1992; Hattori et al., 1995). The influence of pineal on reproductive function, however, is not limited to the hypothalamus–pituitary axis. Receptors are present in uterine tissue as well.

Melatonin also enhances the stimulatory effect of human chorionic gonadotrophin on progesterone production by human granulosa lutein cells (Brzezinski et al., 1992). These studies, therefore, suggest that melatonin may play a role in the modulation of luteal function and that an abnormal melatonin concentration might result in altered ovarian function. Till date hormonal correlation during gestation has never been assessed. Our data suggest that a definite peripheral ratio of estradiol and melatonin levels might be essential for maintenance of pregnancy, and level of progesterone and melatonin ratio for delivery.

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