

SERUM PROLACTIN LEVELS AT DIFFERENT STAGES OF MENSTRUAL CYCLE AND DURING A 24-HOUR PERIOD IN MALAY WOMEN

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SUMMARY

A radioimmunoassay technique has been used to investigate the variation in the serum human prolactin (hPRL) levels at different stages of menstrual cycle and during a 24-hours period in Malay women. The results showed that the serum hPRL concentrations during menstrual (24.5 ± 4.3 ng/ml), preovulatory (36.6 ± 7.4 ng/ml), ovulatory (29 ± 5.3 ng/ml) and postovulatory (26.6 ± 5.2 ng/ml) periods were not significantly different among each other. The hPRL level was highest during night-sleeping, at 0200 hr and was significantly different from the values obtained at 1400, 2000, 0800 and 1400 (last sampling) hours ($P < 0.01$).

INTRODUCTION

Several reports in the literature on the concentration of human prolactin (hPRL) in blood during menstrual cycle have been controversial. A number of workers have reported a rise in circulating levels of hPRL at mid-cycle with higher levels persisting during luteal phase

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(L'Hermite *et al.* 1972; Robyn *et al.* 1973). On the contrary, Hwang *et al.* (1971), Friesen *et al.* (1972) and McNeilly and Chard (1974) have failed to show any consistent changes in hPRL level during the menstrual cycle. McNeilly (1975) has shown that both hPRL and luteinizing hormone increased at mid-cycle, however, hPRL levels showed marked irregular variation. The present study was carried out in order to add some information in an attempt to explain this controversy. Furthermore, there is no report as yet available regarding the prolactin levels at different stages of menstrual cycle in Malay women.

Workers are in agreement with regard to the variation of 24-hour prolactin levels in the blood of normal healthy women. Nokin *et al.* (1972) and Sassin *et al.* (1972) have found that the hPRL levels in women were highest during sleeping. Further, Sassin *et al.* (1973) have shown that the night-time rise in hPRL secretion was due to sleep, and not to an intrinsic 24-hour circadian rhythm. In the present study, we have measured the serum hPRL levels consecutively at six-hourly intervals (1400, 2000, 0200, 0800 and 1400 hrs) in Malay women.

MATERIALS AND METHODS

hPRL levels at different stages of menstrual cycle

Twenty one apparently normal healthy, unmarried women (20-21 yrs old), with regular menstrual cycle and no galactorrhea and amenorrhea have participated in the study. Subjects were not taking any contraceptive pill at least six months prior to the study. Out of these, 18 were second year medical students, and the other three were from the general public. In an attempt to reduce stress, the subjects were well-informed regarding the objective of the experiment and the procedures employed. Relevant particulars about the subjects were

taken, including the last three normal menstrual periods, length and duration of cycle and stages in the cycle during blood sampling. Subjects were also asked to monitor their daily basal body temperatures (ie immediately after waking up from night sleep) by means of a fertility thermometer and basal temperature chart (BTC) throughout the study. Efforts were made so that the study on each subject could be completed throughout one cycle. By means of a syringe, 2.0 ml of blood were drawn through a venous puncture between 1400-1430 hours on the second day of each stage of menstrual cycle, except during ovulation. For the ovulatory period, blood samples were taken on the day when the basal body temperature increased, which normally occurred 14 days prior to next menstruation. Should the temperature fail to rise, the blood samples were still taken, and the ovulatory periods were predicted based on the last three normal menstrual periods. Subjects were also told to note any illness during the study to ensure that no blood samples were taken during this period.

hPRL level during a 24-hour period

Twelve apparently normal healthy unmarried women (20-26 years old), ten of whom were factory workers, have participated in the study. As in the first study, the subjects were also well-informed regarding the objectives of the study and the procedures employed. Subjects were also not taking any contraceptive pill at least six months prior to the study. Two mls of blood were withdrawn through a venous puncture at six-hourly intervals consecutively at 1400, 2000 0200, 0800 and 1400 hours. To minimise the effect of stress, blood samples were withdrawn from right and left arm, alternatively.

Specimen collection and preparation

Immediately after withdrawal, the blood was transferred into a specimen bottle, and left to clot. The serum was separated by means of centrifugation at 1000-3000 r.p.m. for 10 min, and transferred into two polythen tubes (duplicate). This serum was frozen in a deep freezer of -17°C to 20°C in temperature. As in the case of most proteinaceous material, alternate freezing and thawing were avoided. At the time the tests were to be performed, the serum and all reagents were brought to room temperature.

Assay procedure

The assays for hPRL were carried out with the use of a double-antibody radioimmunoassay (RIA) technique. The reagents and the procedures employed were supplied by Abbot Laboratories, Diagnostic Division, North Chicago, IL 60064, Canada. All samples were

measured in duplicate. The intra- and interassay coefficient of variation were 5.8% and 13.6% respectively. Estimates for intra- and interassay variation were obtained by repeated estimation of the hPRL concentration from a pooled source of human serum. Cross reactivity with thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, growth hormone and adrenocorticotrophic hormone did not occur because of the specificity of the antiserum used in the hPRL assay when measured at the 50% of inhibition point.

Statistical analysis of results

The significance of the difference between means was assessed by means of Duncan's new multiple range test (Duncan, 1955).

RESULTS

hPRL level at different stages during menstrual cycle

It was the aim of this study to measure the serum hPRL at different stages during one complete menstrual cycle. However, due to the technical difficulties involved, only six out of twenty one subjects have fulfilled this criterion. The commonest reason for such a situation was the illness and the unavailability of the subjects at the time of sampling. Should this happen, the blood sample for that stage, in particular, ovulatory period, will be taken in the next cycle. However, based on the six subjects from whom the blood samples were withdrawn throughout one cycle, it was found that the mean hPRL levels during menstrual, preovulatory, ovulatory and postovulatory periods were not significantly different from the respective values obtained from the twenty one subjects ($P > 0.1$). Thus the serum hPRL values obtained from twenty one subjects were taken and here presented (Table I, Fig. 1).

From the data, it can be seen that the mean serum hPRL concentrations \pm standard error of the means (SEM) during menstrual, preovulatory, ovulatory and postovulatory periods were 24.5 ± 4.3 , 36.6 ± 7.4 , 29.8 ± 5.3 and 26.6 ± 5.2 ng/ml respectively, and were not significantly different from each other ($P > 0.05$).

hPRL level during a 24-hour period

The results for this study were summarised in Table I and Fig. 2. The data shows that the level of hPRL at 0200 hr (82.1 ± 10.8 ng/ml) was significantly greater than those of 1400 hr (first sampling) (29.2 ± 7.2 ng/ml), 2000 hr (27.9 ± 4.8 ng/ml), 0800 hr (26.3 ± 8.7 ng/ml) and 1400 hr (last sampling) (19.7 ± 3.2 ng/ml) ($P < 0.01$ in all cases).

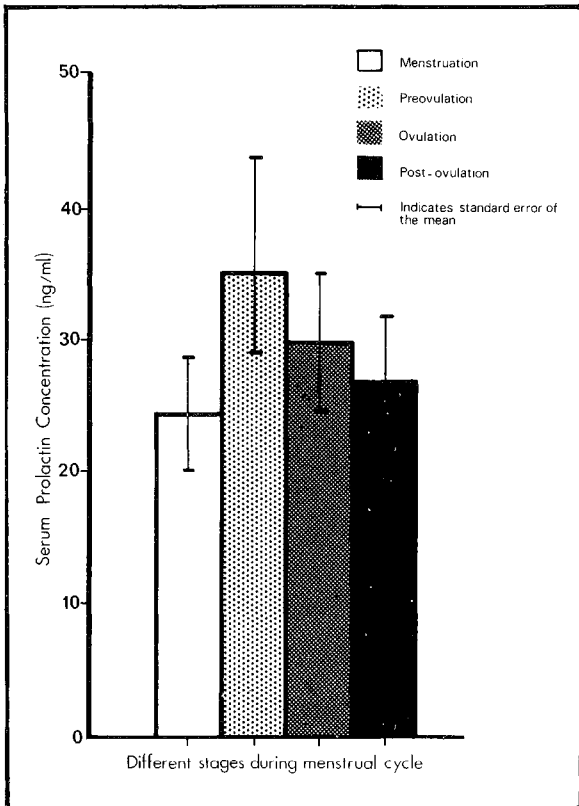


Fig. 1 Serum Prolactin concentration at different stages during menstrual cycle.

DISCUSSION

One common problem arising in the study of hPRL is the considerable variations involved not only between individuals but also within individuals at different times of sampling (McNeilly, 1975). Similarly, our studies have shown that this was the case, based on the large values of standard deviation, and such a problem could have been due to the fact the hPRL is very susceptible to stress (Noel *et al.*, 1971). In this study, efforts were made to minimize the effect of stress on the subjects. Precision estimates for intraassay (5.8%) and interassay (13.6%) variation compared quite favourably with the estimates reported in humans by Barberia *et al.* (1975), 10.5% and 13.5% respectively.

The results of this study indicated that the hPRL concentration during menstrual cycle did not show any specific pattern. These were in agreement with those reported by Hwang *et al.* (1971), Friesen *et al.* (1972), McNeilly and Chard (1974) and McNeilly (1975). These findings, therefore, raise a question regarding the role of estrogen in increasing the serum PRL level, particularly just before ovulation (Ehara *et al.* 1973). This argument

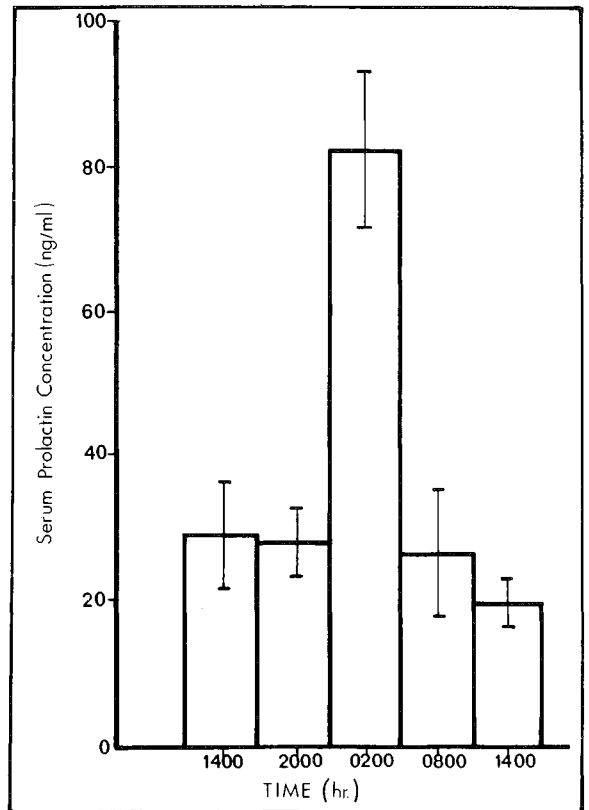


Fig. 2 Serum Prolactin concentration during a 24-hour period. Bars indicate standard error of the mean.

was based on the studies in cows that estrogen may raise serum PRL level (Schams and Karg, 1972). Although in the present study the concentration of serum estrogen was not measured, other workers have shown that prolactin levels bear no relationship to the concentration of estradiol — 17 β or progesterone measured in the same sample (McNeilly and Chard, 1974). Exogenous treatment of estrogen was however found to cause a marked increase in hPRL release (Meites, 1966). This increase was brought about in two fashions: reduction of hypothalamic prolactin inhibitory factor (PIF) which has been documented in rats (Nicoll *et al.* 1970) and augmentation of the release of hPRL from the pituitary by a direct action on the pituitary lactotrophs (Abu — Fadil *et al.* 1976). Thus, the question whether this increase in hPRL concentration could have been due to the administration of estrogen at the dosage level greater than the physiological dose, remains to be investigated.

The present study has also shown that hPRL level reached its peak value at 0200 hours. This study was in agreement with those of Nokin *et al.* (1972) and Sassin *et al.* (1972) who found a night surge of hPRL secretion.

Nokin *et al.* (1972) found that the peak values for serum hPRL were between 0100 and 0500 hours. In one of our studies (unpublished data), blood samples from three women volunteers were taken at six-hourly intervals consecutively during a 24-hour period (1700, 2300, 0500, 1100 and 1700 hours). We have indeed found that at 0500 hr the concentration of serum hPRL was at the highest level. We, therefore, inferred that the peaks of serum hPRL during sleeping at night were between 0200 and 0500 hours. A study by Sassin *et al.* (1973) has shown that there was an immediate and complete shift of prolactin release with the shift of sleep. Thus, the nocturnal rise of prolactin depended upon the occurrence of sleep, and not on an inherent rhythmic release related to time of the day as is the case for ACTH — cortisol system (Krieger *et al.* 1971).

The mechanisms by which hPRL concentration increased during sleep are unknown. A question also remains to be answered whether prolactin release is in fact stress-related as is the case for ACTH (Selye, 1950). This might be justified from the point of view that stress increases hPRL secretion (Noel *et al.*, 1971). However, this might not be an acceptable argument to explain the mechanism for the nocturnal rise of hPRL secretion since during night-sleeping, the subject is free from stress. Thus, several nervous mechanisms might be involved for the occurrence of such phenomenon. It is hoped that in our future studies involving pregnant women and under pathological conditions, the disappearance of nocturnal rise in serum hPRL may bring some light to the issues in question.

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