
NOTE

Evaluation of Serum FSH and LH in Various Infertile Women Treated with Clomiphene Citrate

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Synopsis

Serum FSH and LH levels were measured serially by radioimmunoassay in fifteen menstrual disordered or infertile subjects for two menstrual cycles, one control cycle and another cycle treated with clomiphene citrate. Lack of normal cyclic patterns that usually appear in ovulatory individuals of serum FSH and LH, however, within the range of normal levels in most subjects, as well as the day-to-day fluctuation of gonadotropin levels were observed in amenorrheic or anovulatory patients. By regarding the early rise of gonadotropins after clomiphene intake as a favorable response, those with fluctuating gonadotropin levels during untreated cycle were found to respond considerably to clomiphene citrate. Thus, a predictability is suggested. In the clomiphene-induced ovulatory cycles the serum FSH and LH exhibited either similar to those usually seen in normal ovulatory cycle or without a sequence of normal pattern but midcycle surge. The discordancy of gonadotropins stimulated by clomiphene which suggested the cause of temporal failure with this medicine was pointed out.

As one of the successful medical inducers of ovulation into the management of human infertility, the effect of clomiphene citrate has been studied by several investigators (Greenblatt et al., 1962; Roy et al., 1963; Roland, 1970; Yu et al., 1970), including its influences upon pituitary gonadotropins (Dignam et al., 1969; Jacobson et al., 1968 a; Jacobson et al., 1968 b; Seki et al., 1969). However, among those subjects with ovulatory disorders or infertile women it remains difficult to predict their response to clomiphene citrate. Therefore, it was the purpose of this study (1) to attempt to differentiate the particular patterns of gonadotropins of infertile subjects and those normal women; (2) to attempt to determine whether the variations of serum gonadotropins of those infertile subjects could have a tendency which may suggest the predictability of their response to clomiphene; (3) to attempt to determine the mode of response of gonadotropins to treatment with clomiphene. For this, serum levels of FSH and LH were measured serially for a prolonged period of time for one control and another clomiphene treated cycles in a variety of infertile subjects by radioimmunoassay.

Materials and Methods

Serum FSH and LH were determined by double-antibody radioimmunoassay. Purified human pituitary FSH (3,500 IU FSH and 100 IU LH per mg) obtained from Calbiochem and highly purified human pituitary LH, LER 960 (923 IU LH and 1.9 IU FSH per mg), kindly supplied by National Institute of Arthritis and Metabolic Diseases (NIAMD), NIH, were iodinated with \(^{125}\)I by the method of Greenwood et al. (1963).

Antisera to FSH and LH prepared in rabbits with pituitary FSH or LH, obtained from Calbiochem were diluted to 1: 500 and 1: 10,000 respectively for assays.

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LER 907, kindly supplied by the NIAMD, was used as standard as ng/ml in both FSH and LH assays. The details of the methodology are described elsewhere (Seki, 1972 a).

From 15 subjects of menstrual disordered or infertility, with breakdown of their pathologic conditions to 4 second grade amenorrhea; 2 first grade amenorrhea; 2 oligomenorrhea; 3 anovulatory cycle; 2 corpus luteum insufficiency; one endometrial failure; and one so called functional sterility, sera were obtained for determination of FSH and LH through one control and another clomiphene treated cycles. All samples for particular subjects were frozen at $-20^\circ$C until tested simultaneously in one radioimmunoassay as well as in a replicate assay. Final estimate of the concentration of serum FSH and LH was based on the mean of the two independent determinations.

For evaluation of ovulation basal body temperatures (BBT) urinary pregnanediol values and endometrial figures obtained on early day of menstruation were studied. Urinary pregnanediol was measured with modified Klopper's method, which gives 0.5 to 1.2 and 1.4 to 3.0 mg per 24 hr urine for normal follicular and luteal phases respectively. The endometrium were dated according to the criteria of Noyes et al. (1950).

Clomiphene was given to all subjects in a dose of 100 mg/day for 5 days (Seki, 1971) starting from the 5th day of menstruation or bleeding by progesterone (Proluton 50 mg) injection.

Results

Some representative patterns of serum FSH and LH in subjects with menstrual disorders or infertility are presented:

1). Second grade amenorrhea:

Subject 1 (S. M.) Primary amenorrhea. This 26-year-old subject experienced only once scanty vaginal bleeding at the age of 14. She did have normal secondary sex development, though culdoscopy examination encountered atrophic ovaries. The serum FSH and LH levels were fluctuating from day to day in the level of higher than normal. Clomiphene administration resulted in less fluctuating and slightly diminished, but still higher than normal gonadotropin levels, and a significant rise in serum FSH levels on the late days of the cycle (Fig. 1).

Subject 2 (N. S.) Secondary amenorrhea. This 29-year-old housewife, nulligravida, experienced her menarche at the age of 14 years. Her cycles were irregular with interval of more than 6 months unless estrogen and progesterone were administered. She received these injections which caused withdrawal bleeding after seven months amenorrhea just before she was studied. Both serum FSH and LH levels were normal or lower than normal, especially the serum LH levels which were undetectable in several occasions. Though there was BBT elevation and spontaneous menstruation occurred during the control.

![Fig. 1. Serum levels of FSH and LH, the response to clomiphene (lower), and BBT (upper) in primary amenorrheic woman, subject 1 (S. M.). FSH and LH are in LER 907 ng/ml serum, pregnanediol (P) is in mg/24 hr urine and numbers with endometrial biopsies (E) indicate the dating. Menstruation is depicted by a square.](image-url)
cycle, the pregnanediol values and endometrial picture showed anovulation. Administration of clomiphene stimulated an ovulatory type of response in serum FSH and LH. Marked elevation of serum LH levels, by the influence of HCG, in the late days of studied period and positive Pregnosticon test indicated that she was pregnant (Fig. 2).

2). First grade amenorrhea:

Subject 3 (Y. M.) Secondary amenorrhea. This 36-year-old nulligravida gave a history of amenorrhea for 6 months and constantly high BBT. This subject was studied during a prolonged period of amenorrhea. Levels of FSH were within or lower than the range of normal and rather constant from day to day. Serum LH levels were within the normal range and also limited in day to day variation. Administration of clomiphene did not give any significant change to both serum FSH and LH levels (Fig. 3).

3). Oligomenorrhea:

Subject 4 (A. T.). This 27-year-old nulligravida had her menstrual cycle characteristic of 3 months interval since her menarche at the age of 14 years. The amount of flow was said to be increased and increased size of the uterus was detected by pelvic examination. Serum FSH and LH levels were constant from day to day within the low normal range.
though serum FSH levels were occasionally undetectable. After clomiphene administration no definite change of both gonadotropin levels but an elevation on the latter days of the cycle was noticed. However, no ovulation was encountered. The endometrial biopsy revealed a picture of adenoacanthoma with neoplastic columnar epithelia which showed partly squamous metaplasia (Fig. 4).

4). **Anovulatory cycle:**

Subject 5 (T. K.). This 22-year-old unmarried subject experienced her menarche at the age of 13 years. She had a history of irregular vaginal bleeding since she was in high school. The levels of serum FSH and LH were within the range of normal but acyclic with considerable degree of fluctuation, especially the serum FSH. After administration of clomiphene, a moderate elevation of both serum FSH and LH followed by a definite ovulatory peak at the midcycle were observed. The biphasic BBT and the value of pregnanediol were suggestive of ovulation (Fig. 5).

5). **Corpus luteum insufficiency:**

Subject 6 (Y. K.). This 25-year-old subject had a history of artificial abortion at the age of 22 years. Her BBT showed patterns of anovulatory cycle. This subject was studied
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6. Functional sterility (Oligomenorrhea with ovulation):

Subject 7 (N. K.). This 28-year-old married nulligravida experienced her menarche at the age of 13 years. Her menstruation had always been irregular with 2 to 4 months interval. She was studied during a spontaneous ovulatory cycle. The levels of serum FSH and LH were quite normal though occasional undetectable serum LH levels obtained during the luteal phase in both cycles. A definite early clomiphene peak of both serum FSH and LH was noticed during clomiphene administration. However, it did not influence her status (Fig. 7).

during a cycle of spontaneous ovulation with corpus luteum insufficiency pattern of BBT. Both serum FSH and LH levels were normal, though they were not like those of typical normal ovulatory cycle. The pregnanediol values during the latter days of the cycle were lower than usual ovulatory subjects but endometrium showed a postovulatory secretory figure. Clomiphene stimulated a normal cyclic pattern of serum LH levels with an early elevation, as well as serum FSH. Increased post-ovulatory pregnanediol excretion to a normal luteal phase value was also noticed (Fig. 6).
Discussion

In this series of infertile or menstrual disordered women two thirds, that is, ten of the subjects, showed serum FSH and/or LH levels of within the range of normal. On the other hand, three subjects displayed lower than normal and two subjects exhibited higher than normal levels of serum FSH and LH. Thus, only a prolonged serial determination of serum samples, but not a single randomly selected serum specimen, can be useful for appraisal of the abnormal subjects by the lack of normal pattern of either or both FSH and LH. In some few occasions, strange and yet, ovulation was found to occur during the period of serum collection in cases of prolonged period of anovulation. In two subjects of this series the feature of normal cyclic gonadotropin secretion with ovulation was observed during the cycles studied, though their previous one year BBT showed monophasic anovulatory patterns.

Another peculiar evidence found in those of menstrual disorders is the dynamic day-to-day variation of their serum FSH and LH or both, which is scarcely seen in normal ovulatory cycle. This makes one speculating about the improper and inharmonious shaky pituitary-ovarian relationship, that is, inadequate stimulation by the pituitary or unfavorable feedback by the ovary in these subjects.

The results of this study support the opinion that the hypothalamic-pituitary portion being the primary action site of clomiphene (Igarashi et al., 1967; Kato et al., 1968; Roy et al., 1964; Schally et al., 1970; Seki et al., 1972 b) as in those who responded to clomiphene an early elevation of gonadotropin(s) appeared during or immediately after administration of clomiphene.

A satisfactory criteria for predicting responsiveness to clomiphene have yet to be established. In this study the full scale ovulatory-type of response was obtained only in those subjects who showed the early rise of gonadotropins by clomiphene administration, though, the early clomiphene peak did not insure ovulation. Moreover, the early elevation of gonadotropin(s) was more frequently observed in patients with their serum FSH and/or LH levels fluctuating from day to day during the control cycles. Thus, the prediction of ovulation can be made by serial determination of serum gonadotropins with (1) fluctuating gonadotropic levels during the control cycle and (2) an early rise of gonadotropins by administration of clomiphene. The degree of variation of serum gonadotropins from day to day (rhythmicity) in amenorrheic women is considered to be a function of the stimulatory activity of the hypothalamus, as well as its reserve of activity. The early clomiphene peak of FSH is considered to be more functionally significant than that of LH in the role of induction of ovulation, for the former resembles the early follicular FSH rise whereas the latter has no counterpart in a normal ovulatory cycle.

The mode of response of serum FSH and LH to clomiphene can be characterized by an early elevation during or two to three days after administration of clomiphene in those whose hypothalamo-pituitary-ovarian axis is healthy enough to respond. Then, it is followed by (a) a characteristic normal ovulatory pattern of FSH and LH secretion; or (b) an “ovulatory surge” but without normal ovulatory cyclic pattern. In this study, the ovulatory surge appeared during 4 to 8 days mostly 6 days after cessation of clomiphene.

Among those subjects in which induction of ovulation was failed but with the evidence of early clomiphene rise of FSH and LH, the discordance of FSH and LH was noticed. The characteristic features of serum gonadotropins in these subjects were (1) a more prominent LH elevation than FSH elevation during or immediately after clomiphene intake; and (2) a discrepancy (time lag) of FSH and LH midcycle peak. These facts, particularly the latter, are thought to be very important and
suggest a new category in the mode of failure of induction of ovulation by clomiphene. Those patients showed, though unfavorable during the cycle, the ability to respond to clomiphene and might have the chance to ovulate by continuous trial with this medicine, whenever the mode of secretion of gonadotropins became coordinate. This can well explain the conception that an initial failure to respond to clomiphene can not be considered as ultimate failure and Kistner (1965) advised six consecutive cycles of therapy before clomiphene is deemed a failure.

Some subjects with inadequate FSH midcycle peak but apparent LH midcycle surge were ovulated by clomiphene therapy. This makes one doubtful if FSH midcycle peak is necessary in the role of induction of ovulation. Yet, there still remains the possibility that the FSH peak was not picked up in these subjects.

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