COMPARISON OF TWO METHODS FOR THE DETERMINATION OF LH (HI-GONAVIS AND LH RADIO-IMMUNO ASSAY) FOR PREDICTING OVULATION

by

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Ever since Wide and Gemzell (1960) were successful in immunologically determining chorionic gonadotropin in the urine by making use of haemagglutination inhibition reaction, and Brody and Carlstrom (1962) reported the use of complement fixation reaction, a variety of kits has been developed for immunological determination of gonadotropin in the urine. Since then, these kits have been utilised for diagnosing and controlling choriocarcinoma and for pregnancy testing. However, these kits lack sensitivity in identifying gonadotropin levels in normal menstrual cycles and in patients with disorders of ovulation. In the meantime, radioimmunoassay has been introduced for determining urine levels of gonadotropins and this method has improved the sensitivity. This technique, however, involved sophisticated methodology and is not available for routine examination. In 1969 Schuurs developed Luteonosticon which uses the same haemagglutination inhibition reaction, and this kit has made semiquantitative assay of low urine levels of gonadotropins possible. Recently, Mochida Pharmaceutical Company has marketed Hi-Gonavis, a kit for determining chorionic gonadotropin (HCG) and the luteinizing hormone (LH) in the urine, that is very simple in procedure and which has a high sensitivity.

Accurate prediction of approaching ovulation would be highly useful in the diagnosis and management of infertility. The clinical parameters such as cervical mucus, vaginal cytology, and the variation of the basal body temperature are not sufficiently informative for a reliable prediction, and the customary radioimmunological determinations for establishing the LH peak are impracticable because of the cost and the long time for the test.

The aim of the present study was to investigate the correlation between the LH values found in the urine using Hi-Gonavis kit and those measured in the plasma using radioimmunoassay, and also to test whether the mid-cycle LH peak could be determined with sufficient reliability and promptness to be of pratical use for the prediction of ovulation in the treatment of infertility.

Material and Methods

Twenty-five new women attending the special fertility clinic took part in the

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study; the mean age was 25.8 years (range 20-30), all the patients had regular menstrual cycles (28 ± 2), and all had full clinical and biochemical studies to exclude thyroid, adrenal, pituitary, and hypothalamic dysfunction and other systemic illness. Ovulation was confirmed in all patients using plasma progesterone levels on day 20 of two cycles prior to entry into the study. Patients gave their informed consent to the study.

A second group of 10 patients who had irregular but ovulatory cycles (33-42) and were attending the special fertility clinic was also studied for two cycles using the Hi-Gonavis test to improve fertility. These patients had difficulty in conceiving due to difficulty in timing the possible ovulation time and basal body temperature was not helpful.

A third group of 10 patients who had irregular and anovulatory cycles (35-49) and were treated with clomiphene therapy to induce ovulation prior to artificial insemination were included in the study.

Each patient brought two samples of urine, one collected between 11 p.m. and 12 a.m. and the second sample was collected between 7 and 8 a.m. from day 10 to 20 for two successive menstrual cycles. Blood was drawn between 7 and 8 a.m. on arrival of patient with the two urine samples, during the same period, for 2 cycles.

Patients in the second and third groups were investigated from day 10 for 2 to 3 weeks until the test showed the possible peak level, and for a period of 5 days after the peak level of LH in the serum and urine.

The Hi-Gonavis test is based on in vitro haemagglutination reaction using anti-HCG antibody sensitized erythrocytes. The erythrocytes sensitized with anti-HCG rabbit gamma globulin were agglutinated in a tube when LH or HCG was present and the ring formation at the bottom of the tube did not take place. Thus, the result is in contrast to the usual haemagglutination inhibition test for the detection of pregnancy.

Composition of the reagents:

1. Anti-HCG antibody-sensitized erythrocytes:

   The lyophilized sheep erythrocytes sensitized with the anti-HCG rabbit gamma globulin.
2. Buffer (PBS) for preparing a sensitized erythrocyte suspension: a pH 6.4 buffer containing in each ml.:

   —13.6 mg of potassium dihydrogen phosphate (anhydrous)

   —7.1 mg of sodium hydrogen phosphate (anhydrous)

   —4.5 mg of sodium chloride

   to be used for suspending the lyophilized erythrocytes.
3. Bovine serum albumin (BSA) for diluting test samples of urine: The lyophilized bovine serum albumin, to be used for diluting test urine samples. It is dissolved in 2.5 ml PBS at the time of use (each ampoule contains 2.5 mg of BSA).

Principle

When a urine sample containing HCG or LH is added to an ampoule which contains the lyophilized anti-HCG antibody sensitized erythrocytes, and the contents are mixed, haemagglutination reaction occurs as a result of antigen-antibody reaction between the antibody on the erythrocyte surface and the HCG/LH contained in the urine sample. Consequently, no formation of a precipitation ring occurs, but the erythrocytes precipitate in a smooth mat form at the bottom of the

ampoule. This smooth mat precipitation is read as the positive reaction.

If neither HCG or LH is contained in the test, or the hormone concentration is insufficient in the test urine sample, the erythrocytes precipitate to form a precipitation ring. This formation of a precipitation ring is read as the negative reaction. Circles ranging to the whole circumference without interruption, even though they are thin and have a large diameter, are evaluated as ring formation present. The sensitivity of Hi-Gonavis somewhat varies from batch to batch, but gives a positive haemagglutination in the presence of 5 to 12.5 I.U. of HCG per litre of urine (one I.U. of HCG is equivalent to 2.5 I.U. of LH). The lower detection limit of the test is considered to be 12.5 I.U./L. for LH and 5 I.U./L. for HCG (Ishizuka and Arii, 1974. Mizuno *et al* 1973).

Procedure

The Hi-Gonavis test was done in three dilutions: 1:4; 1:6; 1:8.

1. Reconstitute BSA by adding 2.5 mls of PBS.
2. Pipette 0.3 mls, 0.5 mls, 0.7 mls of BSA solution into test tubes number 1, 2 and 3 respectively.
3. Attach a filter tube to the pipette and suck up 0.1 ml of urine to be tested and discard filter and transfer the filtered urine into test tube 1 or 2 or 3 as per dilution required.
4. Set up one ampoule containing anti-HCG sensitized erythrocytes in front of the test tube containing urine dilution to be tested.
5. Reconstitute anti-HCG serum sensitized erythrocytes by adding 0.4 mls of PBS and shake the ampoule until a homogenous suspension is formed.
6. Add 0.1 ml of the diluted urine into the ampoule of reconstituted anti-HCG sheep erythrocytes and mix thoroughly by shaking. Allow to stand in a rack undisturbed for 2 hours on a stable surface at room temperature.
7. Read the results after two hours by observing the pattern on the bottom of the ampoule.

The Hi-Gonavis test is done in 1:4 dilution initially, and if positive it is repeated in 1:6 dilution and finally in 1:8 dilution if the 1:6 is positive. The results are obtained by multiplying final positive dilution by sensitivity, which determines the level of hormone content in the urine.

Serum LH was assayed using LH RIA kit (The Radiochemical Centre. Amersham, Bucks., England).

Results

Figure 1 shows the LH level measured in the urine with the corresponding serum LH values.

Since the Hi-Gonavis test is only semi-quantitative, a relatively broader range of scatter of the plasma values is expected for the individual titre stages. These ranges have been indicated in Fig. 1 for the various titre stages by shading.

Since the first group of patients investigated consisted exclusively of women with ovulatory cycles, the lowest serum level was 3 I.U./LH/L. The mean serum LH level in the patient in whom no LH excretion could be detected in the urine by the Hi-Gonavis test (LH 12.5 I.U./L. urine) was 5.6 I.U./L. The serum level of 5.9 to 7.9 corresponded with 12.5 I.U./LH/L. in the urine. The plasma levels of this group clearly over-lapped onto the next titre stage 25 I.U./LH/L. urine). Fifty I.U. of LH/L. in the urine corresponded to 12 to 18.2 I.U./LH/L. in the serum. Serum LH values of 12.3 to 33.4 I.U./L. corresponded with 75 I.U./LH/L. in the urine. Serum LH level of 20 to

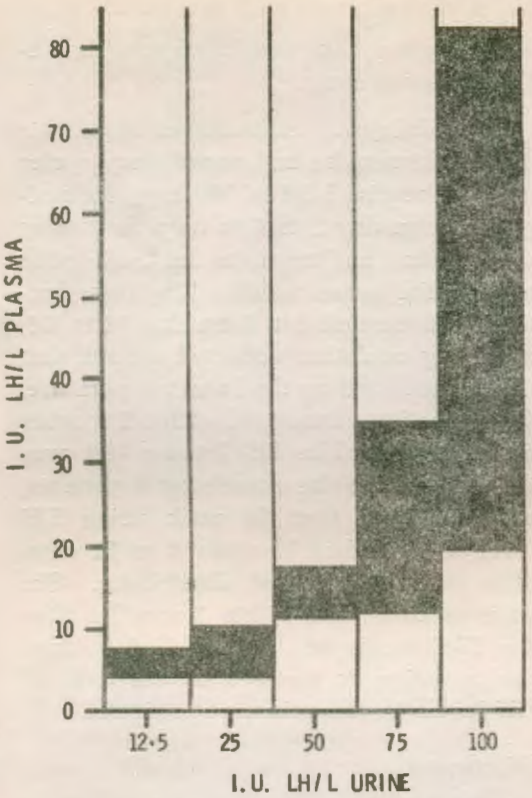

Figure 1 shows comparison of LH values in the urine (Hi-Gonavis) with the corresponding LH values in the plasma (RIA). The shaded areas represent the range of the individual titre stages of Hi-Gonavis (calculated from the mean plasma values).

82.5 I.U./L. corresponded with 100 I.U/L. urine and more, which was characteristic of the mid-cycle LH peak.

Figure 2 shows the serum LH levels measured every 24 hours from day 10 to 20 and urine LH levels measured every 12 hours during the same period.

The day with the highest serum LH concentration was called day 0 or the day of ovulation; days previous to this were designated by minus signs and the days thereafter without any sign. The mean values and the standard deviations of the individual days were then calculated from the radio-immunologically measured values. The values of the semiquantitative method (Hi-Gonavis) were not dis-

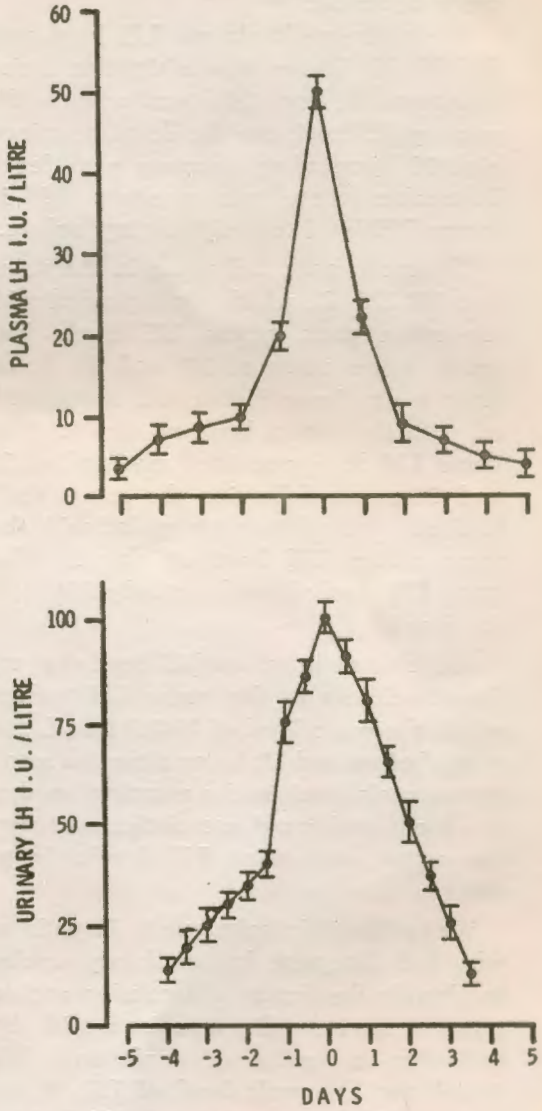

Figure 2 shows the mean and 2 standard deviations of the LH levels measured in the plasma and the median and quartiles of LH in the urine, determined with Hi-Gonavis. The day of the highest level of LH in the serum has been taken as the day of ovulation (day O); the days before have been marked with minus signs and the days after with no sign.

tributed continously, so that in those cases the median was calculated as the central value together with the upper and lower quartile spread, i.e., the 50 per cent range of scatter.

The mean peak plasma LH level was 50 I.U./L. There was a significant rise in serum LH level 24 hours prior to the mean peak level and the level remained high 24 hours after the mean peak level. Thereafter, there was a sharp fall in serum LH level to pre-ovulatory values.

The mean peak LH value in the urine was 100 I.U./L., which corresponded with the mean peak serum LH level. The mean urine value at 12 and 24 hours prior to day 0 was higher and corresponded with the serum level on day 1. The urine LH level remained elevated at 12 and 24 hours following the mean peak level and the values corresonded with the mean serum LH level on day 1. The urine LH level showed a steady fall after the day 1.

The change in serum LH level was reflected closely in the urine LH values which showed a level of 75-100 I.U./L., 12 hours before and 12 hours after the mean serum peak level in the morning on day 0. We found good correlation between the serum and urine LH levels ($r = 0.91$).

We applied this technique in 10 patients who had irregular but ovulatory cycles to identify the timing of ovulation and to improve the infertility associated with the difficulty in timing of ovulation. We found that the peak level of LH in the urine varied between 75 and 100 I.U./L. and this occurred approximately 14 to 15 days prior to the onset of the following menstruation and the coitus was timed accurately to achieve a conception rate of 70 per cent in 3 months. Applying Hi-Gonavis test, we found that we could improve fertility without therapy to regulate the cycle, thus avoiding hyperstimulation in some cases.

The third group consisting of 10 patients who had irregular and anovulatory cycles (35-49) received 50 to 100 mg of clomiphene from day 2 to 6 to help the timing of ovulation and regulate the cycle prior to artificial insemination. The insemination was carried out from day 14 to 16, assuming ovulation occurred around that time as assessed by the basal temperature. Only 2 patients conceived within 2 courses of treatment. The Hi-Gonavis test was then applied to the remaining 8 patients, and it showed that the peak urine LH level was reached between 8 to 11 days after the last tablet of clomiphene. Six patients conceived within 2 months after the Hi-Gonavis test was applied to time the ovulation as assessed on the basis of LH levels in the urine.

Discussion

Hormone determination methods have acquired increasing significance in recent years amongst the clinical methods of establishing optimum time for conception. The determination of LH has gained particular significance, since LH is released from the hypophysis to a high level in mid-cycle and brings about ovulation from the mature follicle of the ovary. This takes place within 40 hours when the highest LH concentration in the blood has already been exceeded (Boyers *et al*, 1977; Friedrich, 1975; Gitsh and Spona, 1973; Thomas *et al*, 1973; Yussman and Taylor, 1970). Yussmann *et al* (1970) confirmed that the ovulatory surge of serum LH occurred 24 hours prior to ovulation and suggested that the prediction of imminent ovulation by means of a rapid LH assay would be practicable. LH degrades very rapidly because of a short

half life such as 20 minutes (Henzl and Segre, 1970). Nevertheless, the mid-cycle LH increase can be measured somewhat earlier in the blood than in the urine.

The radioimmunoassay is the most sensitive of all HCG and LH assay methods currently available, and is of high reliability; however, its procedures are considerably complicated, requiring special facilities and techniques which are expensive, and it also takes a considerably longer time to get the results. Hence, the use of serum LH levels using radioimmunoassay technique in the management of infertility is not practicable.

Our study showed a good correlation between serum and urine LH values ($r = 0.91$). It confirmed that the Hi-Gonavis test could be used to identify the ovulation peak of LH in the urine with reasonable accuracy to improve infertility associated with irregular or anovulatory cycles requiring therapy or for those who were receiving artificial insemination. Mizuno *et al* (1973), Brandau (1978), and Völker (1978) were able to show that Hi-Gonavis and radio-immuno assay exhibit a good correlation for LH in urine samples, and that the test gave a high level of reproducibility. Tamda (1976) stated that there was good correlation between serum LH and urine LH -(Hi Conavis), but the time difference between the first LH increase in the blood and the first LH increase in the urine did not become apparent when only one LH assay was done daily.

We found values of 50 or more I.U/LH/L. urine in women whose serum LH was 12 I.U./L. or more were usually found within 36 hours before the mid-cycle peak of LH. From a correlation of the times of the pre-ovulatory LH increase and the LH urine elimination, it can be deduced that when 50 I.U./LH/L. urine is first detected in the cycle, ovulation can be expected within the next 12 to 36 hours. The Hi-Gonavis test showed a significant rise in urine LH level 12 hours before the peak serum level on day 0, followed by peak level in the morning on day 0 coinciding with the serum peak level. The urine LH level remained elevated at 12 and 24 hours following the peak level of serum LH or day 0. We found 12-hourly LH determination in the urine was more useful in identifying the imminent peak serum LH level. The correlation of the plasma LH levels and the urine LH levels measured with the Hi-Gonavis test confirmed that with this test, the ovulation peak of LH in the urine can be established exactly, and ovulation can be predicted sufficiently early for management of infertile patients.

We applied this test in 10 patients with irregular but ovulatory cycles to identify the timing of ovulation to improve infertility. We found the Hi-Gonavis test was useful for this group and that medication to regulate the cycle could be avoided. We also studied a second group of 10 patients with anovulatory irregular cycles receiving clomiphene therapy prior to artificial insemination. Timing the ovulation using temperature charts was successful only in 2 patients. When Hi-Gonavis was used to time ovulation accurately, there was a significant increase in conception rate. Six out of eight conceived in two months, using the Hi-Gonavis test to time the ovulation.

Thus, it is as a rule possible to rely on it without taking other parameters into consideration. This is advantageous for laboratories with no possibility of examining the patient from the clinical point of

view. Further advantages of this test are the short determination time of 2 hours and that the tests are less expensive and can be repeated at 4 and 6-hourly intervals if necessary. Because of its ease of operation and short determination together with the relatively easy interpretation of the results, the Hi-Gonavis test can be used quite satisfactorily in any laboratory and even by non-gynaecologists.

We have now applied the Hi-Gonavis test in 2 urine samples at intervals of 12 hours from day 10 onwards to identify the timing of ovulation in patients who have irregular ovulatory cycles and in those who receive artificial insemination, and the results are encouraging.

The test with Hi-Gonavis which makes use of high sensitivity haemagglutination reaction permits the assay of urinary LH/HCG with such a high sensitivity that it is virtually comparable to that of radio-immuno assay. It is simpler, less time-consuming, and relatively less expensive as compared with similar methods introduced so far. Hi-Gonavis is, therefore, of high practical value in the infertility clinic.

Summary

The immunological LH/HCG test Hi-Gonavis was used to measure the LH-excretion in urine of 25 patients with regular ovulatory cycles (28 ± 2), of 10 patients with irregular ovulatory cycles and of 10 patients with irregular anovulatory cycles treated with clomiphene during 2 to 3 cycles. The level of LH in plasma was determined simultaneously by radio-immuno assay. 12.5 I.U./LH/L. urine, the limit of sensitivity of Hi-Gonavis, corresponds on average to plasma LH level of 7.5 I.U./L. Fifty I.U. of LH per litre in the urine corresponded with 12 I.U./LH/L. in the serum 48 hours before the mid-cycle peak. Serum LH level of 20 I.U./L. corresponded with 75 I.U./LH/L. in the urine 24 hours prior to ovulation. From the correlation of the pre-ovulatory increase of LH in the plasma and the LH excretion in the urine, it can be concluded that the detection of 75 I.U./LH/L. urine indicates the ovulation within the next 24 hours.

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