SERUM HUMAN PLACENTAL LACTOGEN (HPL) LEVELS IN NORMAL PREGNANCY

by

G. K. RASTOGI,* F.R.C.P. (E.), M.R.C.P. (Lond.),
M. K. SINHA,** M.Sc.,
S. JAYARAMAN,*** B.V.Sc.
and
P. K. DEVI,**** M.S., F.R.C.S.

It is now well established that human placenta secretes a protein hormone Human Placental Lactogen (HPL), also referred to as placental Somatomammotropin. It shows immunological cross-reactivity with pituitary growth hormone and possesses both mammotropic and somatotropic effects (Jasiomovich and Maclaren, 1962, Kaplan and Grumbach 1968). This hormone is produced by syncytiotrophoblast layer of the placental epithelium (Sciarra et al, 1963). It is present in measurable amounts in early pregnancy and circulating levels in maternal serum progressively increase with advancing pregnancy (Grumbach et al, 1958, Beck et al, 1965, Samaan et al, 1966).

The introduction of the techniques of radioimmunoassays (Yalow and Berson, 1960) and improved method for labelling of peptide hormones with radioiodine to achieve high specific activity (Greenwood et al, 1963) have facilitated the measurement of a variety of polypeptide and glycoprotein hormones in the biological fluids with high degree of accuracy and specificity.

The present paper describes a double antibody technique of radioimmunoassay of serum HPL and also summarises our preliminary results in normal pregnant women at different periods of gestation.

Material and Methods

Morning venous blood samples were collected from healthy male and female subjects, healthy pregnant women at different duration of pregnancy and the first week after parturition. The sera were separated and kept deep frozen at −20°C until assayed.

The reagents used in the assay, purified HPL for both standards and iodination and anti-HPL rabbit serum (CT 3399) were kindly supplied by the National Pituitary Agency, NIAMDD, Bethesda, Md; USA. Human placental lactogen was iodinated with ¹²⁵I (Radiochemical centre, Amersham (UK) by the chloramine-T method of Greenwood et al, (1963). The labelled hormone from the reaction mix-
ture was separated by gel chromatography on Sephadex G-50 (1 x 15 cm column) equilibrated and eluted with barbitone buffer (0.05 M, pH 8.2). The second antibody (anti-rabbit gamma globulin goat serum, 2nd AB) was raised in our laboratory. A double antibody technique described previously for other hormone assays from this laboratory was used for HPL also (Rastogi et al, 1973 and Rastogi et al, 1973).

Protocol of the assay

The entire assay was carried out in 10 x 75 mm conning glass test tubes. The reagents were added in the following order.

1. Buffer (0.05 M barbitone, containing 0.3% bovine serum albumin and 0.01% merthiolate, pH 8.2) to make final volume 1 ml.
2. 100 ul of standard HPL preparation (range 0.5—100 ng/ml) or serum samples diluted to 1/10 to 1/100 in buffer (early pregnancy 1/10, mid-pregnancy 1/50 and late pregnancy 1/100).
3. 100 ul of diluted hormone free human serum to standard dilution tubes to equalize the protein content with that of unknown sample tubes.
4. 100 ul of anti-HPL rabbit serum (1st AB, 1:12,000).
5. ¹²⁵I-HPL (approximately 10,000 counts/100 Sec. (100-250 ug HPL) in 100 ul.
6. After 72 hours of incubation at 4°C, 100 ul of non-immune rabbit serum (1:100) in 0.1 M EDTA pH 7.8 and 100 ul of 2nd AB (1:8).

Twenty four hours later the incubation mixture was centrifuged at 4°C for 15 minutes at 2000 rpm. The supernatant was decanted and the precipitate representing bound ¹²⁵I-HPL was counted for 100 seconds in a well type manual gamma spectrometer at the photopeak of ¹²⁵I. The standard curve was drawn by plotting the bound ¹²⁵I-HPL (% of total) on the linear scale against the standard dilutions on the log scale along the horizontal axis. From the % bound ¹²⁵I-HPL in unknown samples the HPL content of the dilute serum in ng/ml could be read directly and by multiplying it by the dilution factor the serum HPL levels were obtained as ug/ml serum. The non-specific radioactivity trapped in the precipitate was assessed from the control tubes containing no 1st AB and it was always less than 5%. The purity of the ¹²⁵I-HPL was assessed in each assay by setting up tubes with excess 1st AB. All unknown samples were assayed in duplicate and the standard HPL dilutions in triplicate.

The cross-reaction of the anti-HPL rabbit serum with varying concentrations of LH, FSH, TSH, HCG and HGH was also checked. Routinely in all the assays, aliquots from three pooled sera having different concentrations of HPL were assayed for intra- and inter-assay variations.

Results

Figure 1 shows the standard curve for HPL. The sensitivity of the assay was 0.1 ng per tube. TSH, LH, FSH, & HCG upto concentrations of 100 mU/ml, 100 mU/ml, 100 mU/ml and 1 IU/ml respectively were not able to significantly displace ¹²⁵I-HPL from HPL antibody (1st AB). Human growth hormone showed significant cross-reaction only when added in very high concentration, 10 to 20 ng HGH manifesting the displacement effect of 0.2 ng HPL (1:50-100 ratio). The inter- and intra-assay coefficient of variations were less than 10%. Both in healthy men and women no HPL
TABLE I
Comparative Values of Serum HPL Levels at Term and Standard used by Different workers

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>HPL level at or near term (ug/ml)</th>
<th>S.D.</th>
<th>No. of samples studied</th>
<th>Standard HPL preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaplan &amp; Grumbach (1965)</td>
<td>5.6</td>
<td>±2.1</td>
<td>23</td>
<td>Kaplan CGP-10-5</td>
</tr>
<tr>
<td>Beck et al (1965)</td>
<td>25.0</td>
<td>-</td>
<td>-</td>
<td>Beck PPP</td>
</tr>
<tr>
<td>Samaan et al (1966)</td>
<td>3.3</td>
<td>±1.0</td>
<td>71</td>
<td>Friesen HPP</td>
</tr>
<tr>
<td>Spellacy et al (1966)</td>
<td>10.8</td>
<td>-</td>
<td>50</td>
<td>Lederle 438-7C-125</td>
</tr>
<tr>
<td>Sciarra et al (1968)</td>
<td>3.98</td>
<td>±1.64</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saxena et al (1968)</td>
<td>19.8</td>
<td>±6.0</td>
<td>-</td>
<td>Lederle PP (H)-1</td>
</tr>
<tr>
<td>Samaan et al (1969)</td>
<td>6.5</td>
<td>-</td>
<td>10</td>
<td>Friesen no 67 a</td>
</tr>
<tr>
<td>Saxena et al (1969)</td>
<td>6.8</td>
<td>±2.1</td>
<td>15</td>
<td>Lederle PPP (H)-1</td>
</tr>
<tr>
<td>Singer et al (1970)</td>
<td>8.5</td>
<td>±2.7</td>
<td>12</td>
<td>Friesen PPP (H)</td>
</tr>
<tr>
<td>Jasimovich (1970)</td>
<td>8.4</td>
<td>±5.0</td>
<td>-</td>
<td>Lederle PP (H)</td>
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<tr>
<td>Letchworth et al (1971)</td>
<td>5.6</td>
<td>±1.2</td>
<td>47</td>
<td>Lederle PPP-496 C11</td>
</tr>
<tr>
<td>Present study (1974)</td>
<td>4.06</td>
<td>±0.86</td>
<td>8</td>
<td>NPA, NIAMDD</td>
</tr>
</tbody>
</table>

Figure 1
Showing the dose response standard curve for HPL radioimmunoassay and cross-reaction by HGH, LH, FSH, and TSH.

like immunoreactivity was detected. In the first post-partum week, either the serum HPL levels were undetectable or were negligible (less than 10 ng/ml).

Figure 2 shows the serum HPL levels during different periods of pregnancy.

From fifth week onwards human placental lactogen could be detected in the serum of pregnant women and the level of HPL increased progressively with increase in
the duration of pregnancy and the maximum serum HPL values (4.06 ± 0.33 SEM, ug/ml) were observed at term (40 weeks).

Discussion

A double antibody technique for the radioimmunoassay (RIA) of HPL has been described. This RIA is specific for HPL as there was practically no cross reaction with luteinizing hormone, follicle stimulating hormone, human chorionic gonadotropin and thyroid stimulating hormone. However, only human growth hormone at the concentration of 100 and 200 ng/ml significantly cross-reacted with HPL. Since sera were assayed in 1/10 or greater dilution, the HGH levels would have to be >1 ug/ml to be detectable in HPL assays. Such high levels of HGH are not known even in HGH producing tumours.

As expected the serum HPL levels in healthy male and non-pregnant female subjects were undetectable and early in the post-partum period also only very low concentration of HPL, still circulating after delivery, was detectable.

Consistent detection of HPL in serum as early as 5 weeks of gestation suggests that the sensitivity of the present assay of HPL is similar to that reported by others workers (Beck, et al., 1965 and Kaplan and Grumbach, 1965). In this study the serum HPL levels are similar to those reported by Samaan et al, 1966 and Sciarra et al, 1968 but when compared to results of other groups our values are slightly lower, (Kaplan and Grumbach, 1965; Beck et al, 1965; Spellacy et al, 1966; Saxena et al, 1968; Samaan et al, 1969; Saxena et al, 1968; Singer et al, 1970; Jasimovich et al, 1970 and Letchworth et al, 1971). As is shown in the table, discrepancy in reported HPL values by different workers is perhaps due to the use of different standards. The value of HPL estimation as a test of placental function and comparison with other conventional tests in normal pregnancy, threatened abortion, and complete abortion is under evaluation. This report provides base line data in north Indian women and would be of value to other laboratories in the country setting up similar assays.

Summary

A double antibody radioimmunoassay of human placental lactogen (HPL) has been described. Serum HPL levels were estimated in 148 women with normal pregnancy at different weeks of gestation. HPL was detectable in the serum as early as 5 weeks of gestation and its serum levels increased progressively from 0.55 ug/ml ± 0.3 (SEM) at 5-8 weeks to 4.06 ± 0.8 at 37-404 weeks. No HPL could be detected in the serum of healthy non-pregnant women, healthy men or in the first week post-partum.

References


