A COMPARISON OF SERUM HUMAN PLACENTAL LACTOGEN AND URINARY OESTROGEN EXCRETION IN THE MOTHER AS PLACENTAL FUNCTION TESTS

by

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Summary

Three hundred patients with complications of pregnancy had paired maternal serum levels of human placental lactogen (HPL) and 24 hours total urinary oestrogen levels performed serially. Serum HPL determinations were found to be of little use in predicting fetal distress, the Apgar score at birth, or light-for-dates infants. There was no correlation between HPL levels and the severity of hypertensive vascular disease complicating pregnancy, fetal weight, placental weight or 24 hour urinary total oestrogen levels. Twentyfour hour urinary total oestrogen levels were found to be more reliable than serum HPL levels in predicting the fetal outcome.

The recognition of fetal jeopardy is a daily concern of the obstetrician and a simple, reliable laboratory test for placental function is urgently needed. The function of the placenta has long excited interest and, by the 18th century the respiratory function was recognised. Since then a great many other functions have been ascribed to this organ and the list will doubtless be still further extended in the future. In assessments of placental function three factors should be considered. Firstly, the placenta has many functions and any single test may not assess all these. Secondly, the placenta and the fetus are functionally interdependent and some tests are as likely to measure processes in the fetus as in the placenta. Thirdly, most tests have of necessity to be made on the mother and may detect changes in her rather than in the placenta or fetus. The concern of placental function tests in clinical practice is not with placental function as such but with its effect on the fetus. A 24 hour maternal urinary total oestrogen or oestriol excretion has been the most widely employed index of feto-placental integrity. However, inaccurate urine collection, steroid therapy and glycosuria might produce inconsistent results. The considerable diurnal and day to day variations in the level of oestrogen are other adverse factors in utilising this particular tests as a prognostic index of feto-placental unit. Hence a simple, quick but reliable method to assess fetal welfare has been sought.

Human placental lactogen was isolated from human placenta in 1961. It is secreted in progressively increasing quantities during pregnancy by the syncytiotropho-

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blast (Sciarra *et al*, 1963). It can be measured in the serum of the pregnant woman from 6 weeks until term. It is found to be increasing gradually and reaches its highest value between 36 and 38 weeks, after which a slight decrease in the level is noted (Geazzani *et al*, 1971; Joshimovich *et al*, 1970; Samaan *et al*, 1969; Saxena *et al*, 1968; Teoh *et al*, 1971). Teoh *et al* (1973) did not find any circadian rhythm. They also reported that the levels of HPL were not affected by stress or metabolic changes. Ylkorkala *et al* (1973) stated that the maternal posture had no effect on HPL levels in maternal serum. The short half life of 20 minutes and other qualities already described suggested that assays of HPL in maternal serum might prove to be a reliable index of fetoplacental integrity.

There has been no unanimity regarding the usefulness of serum HPL as an indicator of fetal prognosis. Hence a study was undertaken to evaluate the reliability of assays of HPL in maternal serum as a placental function test. The results were compared with that of serial urinary oestrogen assays in relation to the fetal outcome.

Material and Methods

Twenty-four hour total maternal oestrogen excretion in the urine was assayed as described by Campbell and Gardner (1971), which involves a simple fluorimetric method. The method is capable of analysing upto 150 samples per day. The results correlated well with an established manual assay.

HPL was assayed using the method of radio-immunoassay as described by (Letchworth *et al*, 1971).

The mean 24 hour total urinary oestrogen and HPL values and their 95 per cent confidence limits for each week of gestation from 26 to 41 weeks were determined from 950 measurements performed on 50 normal patients with uncomplicated pregnancy, of known maturity, and who delivered an infant weighing above the tenth centile standardised for maternal parity, duration of gestation and sex of the infant (Thomson *et al*, 1968).

Three hundred patients, who were considered to be at risk from placental insufficiency, were monitored by serial serum HPL and 24 hour maternal urinary total oestrogens. Assays were performed once or twice a week for out-patients and 2 or 3 times a week for in-patients. Table I shows the clinical diagnosis in 300 patients thought to be at risk from placental insufficiency.

TABLE I

Initial Clinical Diagnosis in 300 Patients Thought to be at Risk From Placental Insufficiency

Pre-eclampsia	60
"Hypertension"	30
"Light-for-dates"	52
Poor weight gain	52
Previous bad obstetric history	22
Antepartum haemorrhage	22
Previous light-for-dates baby	16
Postmaturity	16
Elderly primigravida	12
Premature labour	10
Urinary tract infection	6
Proteinuria	2
Total	300

Total urinary oestrogen levels and HPL levels were considered abnormal if they were below the lower limit of normal range, or if two or more successive determinations prior to delivery showed a reduction in the levels by 30 per cent or more. The results were divided into groups according to whether the serial maternal urinary oestrogen and serum HPL levels had been normal or abnormal. These groups were then analysed accord-

ing to the clinical outcome of the pregnancy in terms of whether (i) there had been objective signs of fetal distress during pregnancy; (ii) the Apgar score of the infant at one minute had been five or less; (iii) the birth weight of the infant was above or below the tenth centile (Thomson *et al*, 1968).

Results

During the last 16 weeks of normal pregnancy, serial HPL levels showed a slow but steady rise upto 37 weeks, thereafter a slight decrease in the levels were noted (Fig. 1).

Fig. 1 shows the mean HPL values and their 95 per cent confidence limits for each week of gestation from 26 to 41 weeks.

Table II shows the mean and standard deviation of HPL levels from 26 to 41 weeks of gestation.

Fig. 2 shows the mean urinary oestrogen levels and their 95 per cent confidence limits for each week of gestation from 26 to 41 weeks.

Table III shows the mean and the standard deviations of the total urinary oestrogen levels from 26 to 41 weeks of gestation.

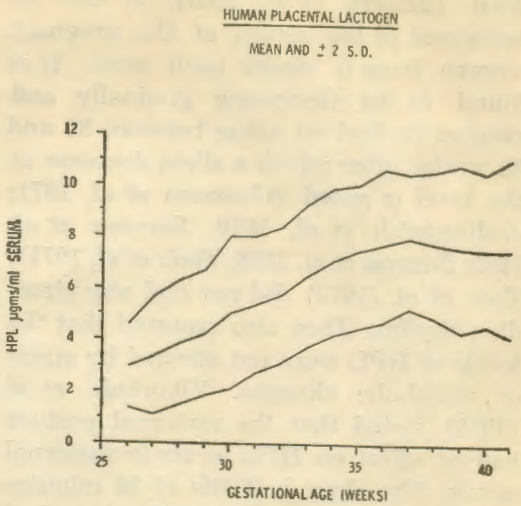

Fig. 1
Mean serum HPL levels and plus or minus two standard deviations in normal pregnancy.

TABLE II
Mean HPL Levels and Standard Deviation for Each Week of Gestation From 26 to 41 Weeks

Gestation in weeks	Mean HPL levels ug/ml	Standard deviation	Number of samples
26	3	0.08	40
27	3.4	1.14	55
28	3.9	1.20	60
29	4.3	1.20	65
30	5.08	1.48	75
31	5.40	1.30	55
32	5.78	1.30	75
33	6.54	1.36	60
34	7.08	1.42	55
35	7.5	1.50	60
36	7.68	1.48	50
37	7.88	1.30	65
38	7.75	1.42	70
39	7.62	1.60	55
40	7.55	1.48	60
41	7.60	1.72	50

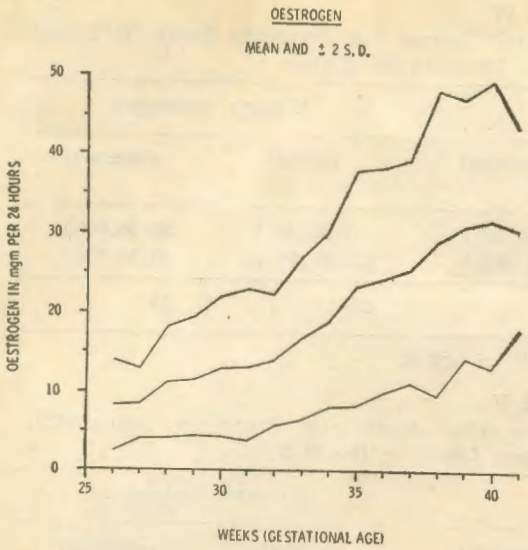

Fig. 2

Mean 24 hour urinary oestrogen and plus or minus two standard deviations in normal pregnancy.

16 (48.4%) were preceded by abnormal HPL levels while 22 (66.6%) were preceded by abnormal levels of urinary oestrogen excretion. The difference remained the same whether the labour was spontaneous or induced. Two hundred and sixty seven cases were not complicated by fetal distress but of these 14 (5.25%) had been preceded by abnormal levels of HPL and 28 (10.5%) by abnormal levels of urinary oestrogen excretion. Though the incidence of false negative results (51.6%) in the HPL series was high, the false positive results appeared to be insignificant (5.25%).

Table V relates the assay in the mother to the Apgar score of the infant at one minute. There were 30 babies born with an Apgar score of 5 or less at one minute after birth, of which 9 (30%)

TABLE III

Mean Oestrogen Levels and Standard Deviation for Each Week of Gestation Between 26 to 41 Weeks

Gestation in weeks	Mean oestrogen levels in ug/ml	Standard deviation	Number of samples
26	8.3	2.93	40
27	8.6	2.29	55
28	11.25	3.52	60
29	11.72	3.88	65
30	12.92	4.57	75
31	13.33	4.71	55
32	14.18	4.07	75
33	16.88	5.11	60
34	19.20	5.52	55
35	23.34	7.48	60
36	23.79	6.94	50
37	25.22	6.97	65
38	28.92	9.02	70
39	31.00	8.09	55
40	31.30	9.03	60
41	30.50	6.27	50

Table IV relates the assay results in the mother to the presence or absence of fetal distress in labour. There were 33 cases of fetal distress in labour, of which were preceded by abnormal serum HPL levels, while 16 (53%) were preceded by abnormal levels of urinary oestrogen. Of 270 babies with an Apgar score of 6 or

TABLE IV

The Relationship Between the Incidence of Fetal Distress and Preceding Serum HPL and 24 Hour Urinary Oestrogen Levels in the Mother

Clinical signs of fetal distress	Number of cases	Serum HPL		Urinary oestrogen	
		normal	abnormal	normal	abnormal
Present	33	17(51.6%)	16(48.4%)	11(33.3%)	22(66.6%)
Absent	267	253(94.75%)	14(5.25%)	239(89.5%)	28(10.5%)
Total	300	270	30	250	50

P = >0.05. P = <0.05.

TABLE V

Relationship Between Apgar Score at One Minute After Birth and Preceding Serum HPL and 24 Hour Urinary Oestrogen Levels in the Mother

Apgar score at one minute	Number of cases	Serum HPL		Urinary oestrogen	
		normal	abnormal	normal	abnormal
0– 5	30	21 (70%)	9 (30%)	14 (47%)	16 (53%)
6–10	270	249 (92%)	21 (8%)	236 (88%)	34 (12%)
Total	300	270	30	250	50

P = > 0.05. P = <0.05.

over, 21 (5%) were preceded by abnormal serum HPL levels and 34 (12%) by abnormal levels of urinary oestrogen.

Table VI relates the assay results in the mother to the birth weight of the baby. There were 48 babies whose birth weight was less than the tenth centile (Thomson *et al*, 1968). Of these only 14 (30%) were preceded by abnormal serum HPL levels and 33 (69%) were preceded by abnormal levels of oestrogen. Two hundred and fiftytwo had a birth weight above the tenth centile, but of these 16 (6.3%) were preceded by abnormal serum HPL levels and 17 (6.7%) by abnormal levels of urinary oestrogen.

TABLE VI

Relationship Between Birth Weight of Infan and Preceding Serum HPL and 24 Hour Urinary Oestrogen Levels in the Mother

Birth weight	Number of cases	Serum HPL		Urinary oestrogen	
		normal	abnormal	normal	abnormal
Below tenth centile	48	34 (70%)	14 (30%)	15 (31%)	33 (69%)
Above tenth centile	252	236 (93.7%)	16 (6.3%)	235 (93.3%)	17 (6.7%)
Total	300	270	30	250	50

P = > 0.05. P = < 0.05.

Fig. 3 shows that there is poor correlation between birth weight and the serum HPL levels assayed within a week of delivery.

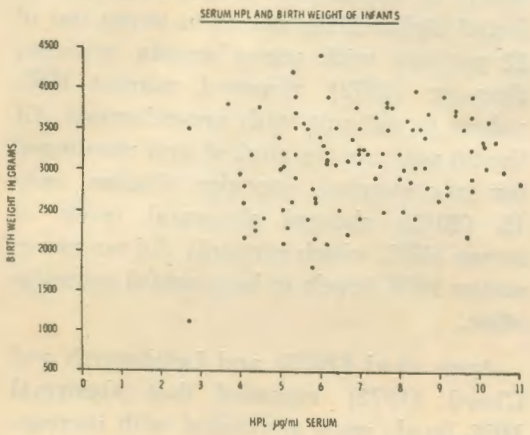

Fig. 3
Serum HPL levels within one week of delivery and fetal weight.

Fig. 4 shows that there is little correlation between placental weight and the serum HPL levels assayed within a week of delivery.

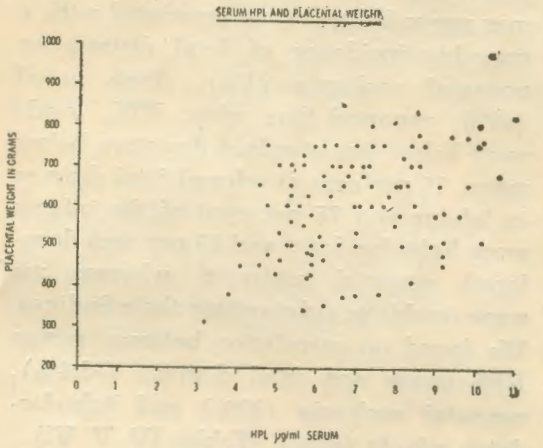

Fig. 4
Serum HPL levels within one week of delivery and placental weight.

Fig. 5 shows that there is no correlation between serum HPL levels and urinary oestrogen levels assayed within a week of delivery.

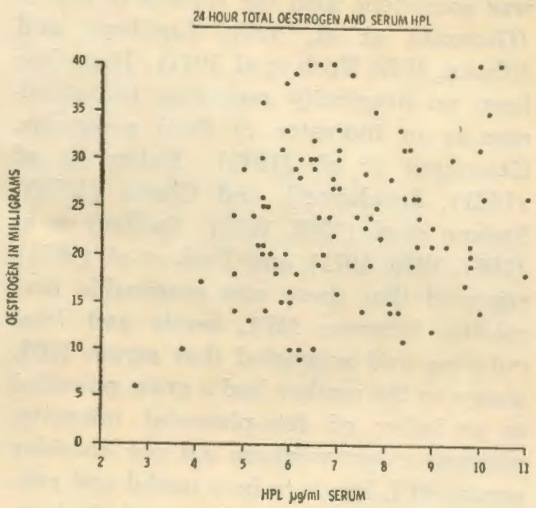

Fig. 5
Serum HPL levels and total urinary oestrogen levels within one week of delivery.

Of the 90 patients with hypertensive vascular disease complicating pregnancy 75 (80%) had normal levels of serum HPL and only 15 (20%) had abnormal serum HPL levels. In this group there were 2 still-births, one at 31 and the other at 36 weeks of gestation. Both were preceded by abnormal serum HPL levels and total urinary oestrogen levels. There was no correlation between HPL levels and severity of the hypertensive disease in pregnancy.

Discussion

Several workers have suggested that serial serum HPL levels may be useful in the assessment of placental function and fetal prognosis. Grumbach *et al* (1964) and Sciarra *et al* (1963) indentified the placenta as the sole organ to produce HPL and directed much attention to its role as an indicator of placental function. Our study on serum HPL values showed that the levels of HPL increased steadily

upto 37 weeks and thereafter gradually decreased until delivery; this was consistent with the reports of others (Geazzani *et al*, 1971; Lindberg and Nilsson, 1973; Teoh *et al*, 1971). There has been no unanimity regarding its usefulness as an indicator of fetal prognosis. Genazzani *et al* (1971), Kellar *et al* (1971), Letchworth and Chard (1972), Saxena *et al* (1968, 1969), Spellacy *et al* (1967, 1970, 1971) and Teoh *et al* (1971) reported that there was reasonable correlation between HPL levels and fetal outcome and suggested that serum HPL assays in the mother had a great potential as an index of feto-placental integrity, whereas other workers did not consider serum HPL levels to be a useful and reliable index of placental function (Josimovich *et al*, 1970; Samaan *et al*, 1969; Sciarra *et al*, 1968; Singer *et al*, 1970; Spellacy *et al*, 1966; Spencer *et al*, 1971).

Our results suggest that serum HPL determinations are of no value in predicting the fetal outcome. Spellacy *et al* (1970, 1971) and Teoh *et al* (1971) stated that HPL levels below 4 microgrammes per millilitre after 30 weeks were suggestive of a fetus at risk, more so when pregnancy was complicated by hypertensive vascular disease. They reported that the incidence of false positive results were high though the incidence of false negative results were very low, whereas our study showed a higher incidence of false negative results with very low incidence of false positive results which made the value of HPL assays very unreliable as a prognostic index of feto-placental unit. There is no unanimity regarding its usefulness in patients with hypertensive vascular disease complicating pregnancy. Lindberg and Nilsson (1973) Spellacy *et al* (1971) and Teoh *et al* (1971) noted low levels of HPL in mild pre-eclampsia, lower still in severe pre-eclampsia. Whereas Letchworth and Chard (1972) found lower levels in mild pre-eclampsia compared with levels in severe pre-eclampsia. Singer *et al* (1970) found higher HPL values in seven out of 12 patients with pre-eclampsia, whereas Spencer (1972) reported normal HPL values in patients with pre-eclampsia. Of the 90 patients we studied and monitored for hypertensive vascular disease, only 15 (20%) showed abnormal levels of serum HPL, which certainly did not prove serum HPL levels to be a useful investigation.

Anne *et al* (1975) and Letchworth and Chard (1972) reported that abnormal HPL levels were associated with increased incidence of perinatal asphyxia. Letchworth and Chard (1972) reported that the incidence of fetal distress in labour or neonatal asphyxia was 71 per cent when there were 3 or more HPL levels of less than 4 microgrammes per millilitre between 35th and 40th week of pregnancy. Levels above 5 microgrammes per millilitre were associated with a very low incidence of fetal distress or neonatal asphyxia (4%). Anne *et al* (1975) reported that when HPL levels were below two standard deviation below mean, 75 per cent developed fetal distress in labour and 75 per cent of the infants were light-for-dates and 75 per cent developed neonatal asphyxia, whereas we were unable to substantiate their findings. We found no correlation between serum HPL levels and fetal distress (48.4%), neonatal asphyxia (30%) and light-for-dates infants (30%) (Tables IV, V, VI).

There were reports from other workers who did not consider assays of HPL levels to be a useful index of feto-placental function (Josimovich, 1970; Samaan *et al*, 1969; Sciarra *et al*, 1969; Singer *et*

al, 1970; Spellacy *et al*, 1966; Spencer *et al*, 1971). Samaan *et al*, (1969, 1971) reported that the HPL levels did not disappear from the maternal serum after fetal death had occurred. They considered that neither a fetus nor an intact feto-placental circulation was necessary for the production of HPL.

Anne *et al* (1975), Lindberg and Nilsson (1973) and Teoh *et al* (1971) found correlation between fetal weight and HPL levels in maternal serum, whereas we were unable to substantiate their findings (Fig. 3). Our findings confirmed the reports of other workers such as Singer *et al* (1970), Spellacy *et al* (1966) and Spencer (1971). We also did not find any correlation between HPL levels and placental weight (Fig. 4O, whereas Saxena *et al* (1968, 1969), Sciarra *et al* (1968) and Selenkow *et al* (1968) showed good correlation between serum HPL levels and placental weight. Our study did not show any correlation between serum HPL levels and 24 hour total urinary oestrogen levels (Fig. V).

The main clinical criterion for evaluating the clinical usefulness of tests of feto-placental function is the ability to predict those pregnancies which will end in producing a light-for-dates infant or fetal distress in labour or neonatal asphyxia. Any test which produces a significant incidence of false negative results where the test suggests feto-placental function is satisfactory but the fetal outcome is poor, and false positive results where the test shows that the feto-placental function is unsatisfactory but the fetal outcome is satisfactory, discredits its use in the management of patients at risk of placental insufficiency. The end point of chronic placental insufficiency is chronic fetal distress leading to fetal death. Any claim for good placental function test must be backed by the demonstration of the method's ability to predict impending fetal death before it actually occurs. There were 4 perinatal deaths in this group of 300 patients who were considered to be at risk from placental insufficiency. There were 2 stillbirths and 2 neonatal deaths. The levels of HPL were abnormal prior to the 2 stillbirths, whereas they were normal in the group of 2 neonatal deaths. The levels of oestrogen were low prior to all these 4 perinatal deaths.

The hormone is assayed using a fairly sophisticated radio-immuno-assay, and while it has its enthusiastic proponents, it has not found general acceptance. In conclusion, although the level of HPL in maternal serum may give some indication of placental function, the high incidence of false negative results limit the clinical value of this investigation. There is no evidence in this report to show that assays of serum HPL levels give sufficient indication of placental dysfunction to justify altering clinical management.

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