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Estimation of Human Chorionic Somatomammotropin (HCS) Levels during Normal Pregnancy Using a Rapid Radioimmunoassay

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ABSTRACT

The levels of HCS in plasma at different times during uncomplicated pregnancies were studied by a rapid radioimmunoassay. A steady rise was observed from the 22nd to the 37th week. After the 37th week, the HCS levels were fairly stable. The HCS levels were virtually unchanged in plasma samples stored at +4°C or −20°C for 1 to 7 days.

INTRODUCTION

Human chorionic somatomammotropin, HCS, was isolated from human placenta in 1961 by Ito & Higashi (1). In 1962, Josimovich & McLaren (2) verified the placental origin of the hormone. Several authors have measured HCS concentrations at different times during normal and abnormal pregnancies (3, 4, 5, 7). The hormone was earlier known by a variety of names such as human placental lactogen (HPL), purified placental protein (PPP), chorionic growth hormone-prolactin etc. In 1968, however, leading investigators proposed the name HCS (6). This name indicates the origin of the hormone as well as its known biological properties—somatotrophic and lactogenic activities. It is impossible to compare the absolute plasma HCS levels obtained by different investigators because of variations in the methods of assay and the standard preparations used. It is therefore necessary to establish normal ranges for each method.

PRESENT INVESTIGATION

The purpose of the present investigation was to determine the plasma HCS concentration in normal pregnant women by a rapid radioimmunoassay. Specificity and technical details have been thoroughly discussed by Letchworth et al. (8). As samples were withdrawn at different times of the day and had to be stored at +4°C for different periods of time before treatment and further storage at −20°C, the diurnal variations in HCS levels and the stability of HCS in the stored samples had to be investigated.

MATERIALS AND METHODS

Subjects

The circadian rythm was studied in seven women. The patients had been admitted to the Department of Obstetrics and Gynaecology of The University Hospital, Uppsala, for various reasons. Three had vaginal bleedings of unknown origin, one a premature labour which disappeared spontaneously and one an incompetent cervical os. The remaining two patients were observed because of obstetrical complications during previous pregnancies. One woman was investigated in the 29th as well as in the 34th week of pregnancy. All these patients subsequently delivered healthy infants. Samples were withdrawn every fourth hour over a 24 hour period during various gestational weeks of the last trimester of pregnancy.

The stability of HCS concentration was studied in samples collected from two subjects. Aliquots of the samples were stored as heparinized blood and plasma at room temperature, +4°C and −20°C. Assays were performed after 0, 1, 2, 3, 4 and 7 days of storage.

The HCS concentration during pregnancy was estimated in 384 plasma samples from women visiting the municipal antenatal clinic. Only women with regular menstrual periods and whose dates for the last menstrual period were known were included. All women were apparently healthy at the time of sampling. Subjects with urinary infection, hepatic disease, toxaemia of pregnancy, diabetes, Rh-immunization, anaemia or other complicating conditions were excluded.

The blood samples were withdrawn from a cubital vein into evacuated, heparinized glass tubes (Vacutainers,

Table I. *Reproducibility of assay. HCS concentrations in two reference plasma samples, A and B*

Batch $HCS^{125}I$	No. of standard curves	Mean value (HCS in μg/ml)		Coefficients of variation: Within assay		Between assay		Total	
		A	B	A	B	A	B	A	B
I	17	2.42	4.56	8.1	7.1	5.0	2.2	9.5	7.4
II	2	2.45	4.68	2.9	5.3	—	3.7	2.9	6.5
III	8	2.46	4.94	2.9	4.1	4.3	6.1	5.2	7.3

Becton & Dickinson, London, G.B.). The samples were stored not more than 2 days in a refrigerator at +4°C. The plasma was withdrawn after centrifugation and stored at −20°C prior to the assay. In most women, only one sample was withdrawn. In some cases two or more samples were obtained at different periods of pregnancy. Samples were collected every second week from the 22nd up to the 36th and then every week until delivery.

Analytical procedure

In general, the reagents were prepared and the HCS determined as described by Letchworth et al. (7).

For the assay, 3 ml polystyrene tubes (Cerbo, Trollhättan, Sweden) were used.

Dilutions of radioiodinated HCS and HCS antiserum were made in 0.05 M phosphate buffer pH 7.4 containing 0.3% human serum albumin (AB Kabi, Stockholm, Sweden), 0.9% NaCl and 0.025% thiomersal.

Highly purified HCS was obtained from Lederle Laboratories and used both as standard and for the preparation of $HCS^{125}I$. From a stock solution containing 16 μg/ml HCS in male plasma, final concentrations of 8, 4, 2 and 1 μg/ml were prepared by dilution with male plasma. These solutions were divided into portions, stored at −20°C and employed for the preparation of the standard curve.

The radioiodination of HCS was performed by the Chloramine-T method described by Greenwood et al. (9) giving a specific activity of approx. 30 μCi/μg. The labelled HCS was purified by gel filtration on Sephadex G-75. The radioiodine was purchased from Union Carbide, US.

Antiserum was purchased from Paines & Byrne, London, G.B. With the selected incubation time, a final dilution of 1 : 350 was found to give a steep curve over the range of standards employed.

The incubation volume (500 μl) consisted of (*a*) 200 μl buffer containing approx. 2 ng radioiodinated HCS, (*b*) 100 μl unknown plasma or male plasma containing known amounts of HCS, and (*c*) 200 μl HCS antiserum diluted in buffer (1 : 140).

All incubations were performed for 60 min. at room temperature. The antibody-bound and free HCS were separated by addition of 1 ml ethanol followed by centrifugation and aspiration of the supernatant with the aid of a Pasteur pipette.

The bound radioactivity was estimated in an automatic γ-counter (Wallac GTL 500). Normal counting time was 10 sec. All standards and unknowns were assayed in duplicate.

In all standard curves 2 controls (A and B) were included and determined in duplicate. During the test period, 27 standard curves and 3 different batches of $HCS^{125}I$ were employed. For each batch, the within and between assay variations were calculated. The results are compiled in Table I.

The total within batch variation is less than 10% for both controls. The differences in mean values of Control B indicate that a between batch variation may exist. It is, however, premature to make any statements about its magnitude and origin.

Table II. *Diurnal variations in HCS concentration in μg/ml*

The sampling was repeated every fourth hour. The starting point (hour 0) varied from patient to patient

Patient	Week of pregnancy	Time of sampling (hours): 0	+4	+8	+12	+16	+20	+24	Mean ± S.D.	Coefficient of variation
I. S.	33	5.8	4.9	4.3	4.2	4.0	4.1	3.8	4.44±0.69	15.5
M. W.	32	4.0	4.4	4.0	4.1	4.1	5.1	4.9	4.37±0.45	10.3
P. M.	38	4.2	4.5	4.7	4.3	4.3	4.2	4.2	4.34±0.19	4.4
U.-B. U.	39	6.5	6.5	6.2	5.9	6.7	6.4	6.3	6.36±0.26	4.1
S. H.	39	6.3	6.3	6.2	6.5	6.3	6.5	6.2	6.33±0.13	2.1
B. K.	39	4.4	4.6	4.6	4.6	4.7	4.1	4.1	4.44±0.25	5.6
M. H.	29	2.7	2.7	2.7	2.2	2.6	2.8	2.6	2.61±0.20	7.7
M. H.	34	3.9	4.7	4.2	4.1	4.1	3.9	4.1	4.14±0.27	6.5

Table III. *Stability of HCS concentration (μg/ml) in samples stored at room temperature, +4°C and −20°C*

	Subject	Storage time (days) 0	1	2	3	4	7	Mean ± S.D.	Coefficient of variation
Heparinized blood at +25°C	L.-B. O.	5.6	5.3	5.8	5.3	6.3	5.5	5.63 ± 0.38	6.8
Heparinized blood at +4°C	L.-B. O.	5.6	5.1	5.1	5.2	5.4	5.5	5.32 ± 0.21	4.0
	V. P.	4.4	4.2	4.1	3.4	4.2	4.3	4.10 ± 0.36	8.8
Plasma at +25°C	L.-B. O.	5.6	5.5	4.9	5.6	6.6	6.2	5.73 ± 0.59	10.3
Plasma at −20°C	V. P.	4.4	3.9	4.2	3.6	4.3	4.5	4.15 ± 0.34	8.2

RESULTS

The results from the study of the circadian rythm are shown in Table II. As may be seen from the table, no systematic variation was found during the 24 hour period covered. Table III demonstrates that HCS concentrations were quite stable under the conditions investigated. From the 22nd to the 37th week of pregnancy there was a steady rise in the HCS concentration (Fig. 1, Table IV). From the 37th to the 40th week the level remained fairly constant. After the 40th week a slight decrease was observed. In the last weeks of pregnancy the individual values showed a much greater spread.

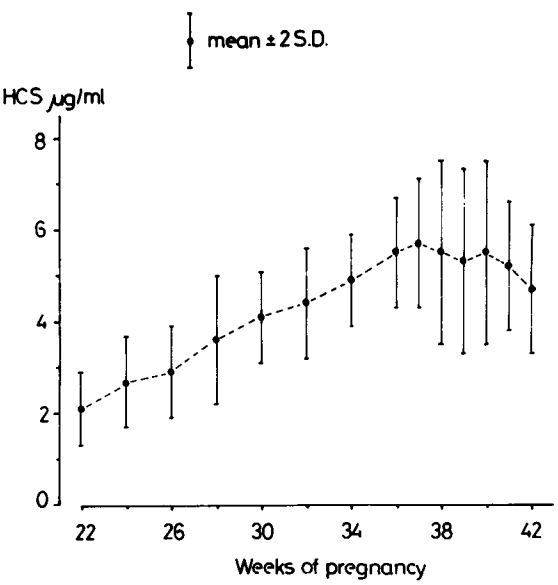

Fig. 1. HCS levels in 384 plasma samples from women with normal pregnancies.

DISCUSSION

The results in Tables II and III indicate that the techniques used in sampling and handling the samples were quite satisfactory and did not adversely affect the results. HCS seemed to be a very stable hormone and did not deteriorate on storage. This is of practical importance when plasma samples have to be sent to distant laboratories. As no systematic diurnal variation was found, samples may be drawn at any time.

The gradual increase in HCS levels with advancing pregnancy shown in this study confirms the findings of other investigators. The slight decrease in the last weeks of pregnancy may reflect the physiological involution of the placenta. HCS estimations with this simple and rapid method may be a valuable aid in the clinical management of high risk pregnancies. The assay is complete within two hours thereby permitting interventions with the minimum of delay if ne-

Table IV. *HCS levels in 384 plasma samples from women with normal pregnancies*

Weeks of pregnancy	No. of samples	Mean (μg/ml)	S.D.	2 S.D.
22	37	2.1	0.4	0.8
24	41	2.7	0.5	1.0
26	49	2.9	0.5	1.0
28	26	3.6	0.7	1.4
30	27	4.1	0.5	1.0
32	23	4.4	0.6	1.2
34	22	4.9	0.5	1.0
36	12	5.5	0.6	1.2
37	23	5.7	0.7	1.4
38	33	5.5	1.0	2.0
39	29	5.3	1.0	2.0
40	32	5.5	1.0	2.0
41	19	5.2	0.7	1.4
42	11	4.7	0.7	1.4

cessary. Previous reports indicate that a single value is not sufficient for assessing the placental function. Serial determinations may give more reliable information.

The normal range of HCS values during uncomplicated pregnancies reported here was established in order to assess values found in complicated pregnancies. An extensive study on pathological pregnancies is in progress. This rapid radioimmunoassay seems particularly suitable for this purpose.

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