DIAGNOSTIC ACCURACY OF A RADIORECEPTOR ASSAY FOR HCG IN EARLY PREGNANCY

by

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In recent times when there is an increased awareness of the use of contraceptives and early termination of pregnancy is frequently practised as a fertility regulation procedure, it becomes imperative to make a precise, specific and rapid diagnosis of pregnancy at the earliest stage.

Reports have indicated that due to lack of such a sensitive test for pregnancy, menstrual regulation or miniabortions are performed in a significant number of non-pregnant women. (Fortney *et al*, 1977). The procedure though considered safe, may be associated with a certain degree of morbidity. Besides, in developing countries with limited facilties it would inflict an unnecessary strain on available operative services. Thus, there is an urgent need for a simple pregnancy test which is sensitive and reliable around the time of the missed period.

The specific binding affinity of HCG and LH hormones to plasma membrane receptors from bovine corpora lutea has been successfully used in the development of very sensitive *in vitro* receptor assay (RRA) for early pregnancy diagnosis (Saxena *et al*, 1974; Landsmen, and Saxena, 1974). The test is based on the principle of competitive protein binding similar to that used for Radiommunoassays (RIA) except that here specific tissue receptors are used instead of a specific antibody. While these tests have a high sensitivity similar to *RIA*, they do not have the disadvantage of non-specific immune responses. In most cases the receptor will bind the biologically active form of the hormone molecule contributing to the biospecificity of this test. Rosal *et al* (1975) have reported that the RRA for HCG can detect HCG-like material in amounts as little as 5 miu or 0.4 ng/ml ± 15% as early as a week after conception with a fair degree of accuracy.

Material and Methods

The test was carried out in 685 female subjects for a variety of reasons as indicated in Table I. Blood samples (2-3 ml) were collected by venepuncture. Sera were separated by centrifugation and frozen at –20°C if not used immediately.

Cases designated as Medical termination of Pregnancy (MTP) and Menstrual regulation (MR) constituted the largest group 43.8% and were selected from the family planning clinics. Blood from these cases was collected before the operation

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TABLE I
Scope of HCG RRA in Clinical Conditions

Clinical conditions	No. of patients	%
Pregnancy diagnosis	151	22.04
MTP & MR	300	43.80
Pts. on various contraceptives	64	9.34
Infertility Rx cycles	164	23.94
Ectopic	6	00.88
Total	685	100.00

and the currettings were subjected to histopathology for confirmation of the test results. In other conditions, other confirmatory tests for pregnancy like clinical follow-up, bioassay on urine using rat ovarian hyperemia (ROH) test, HCG RIA, plasma progesterone and in some cases repeat receptor tests were carried out.

Results

Table II shows the distribution of sample material by days past missed period.

TABLE II
Distribution of Cases by Days Past Missed Period

Days Past Missed Period	No. of cases	%
Before missed period	95	13.87
1- 7 Days Past missed period	110	16.06
8-15 Days Past missed period	241	35.18
>15 Days Past missed period	239	34.89
Total	685	100.00

It is understandable that 2/3rd of the samples were from subjects who had gone over their period by more than 7 days. In 16.06% cases, the subjects had just missed their period by only a few days. These were either regular users of contraceptives or patients undergoing treatment for infertility. The remaining

TABLE III
Accuracy of HCG RRA

Days past missed period	Total	+Ve Results				−Ve results			
		Total confirmed +ve		False +ve		Total confirmed −ve		False −ve	
		No.	%	No.	%	No.	%	No.	%
Before missed period	95	18	83.33	3	16.67	77	85.71	11	14.29
1- 7 Days past missed period	110	51	100.00	0	—	59	93.22	4	6.78
8-15 Days past missed period	241	183	99.45	1	0.55	58	98.28	1	1.72
> 15 Days past missed period	239	196	97.45	5	2.55	43	97.67	1	2.33
Total	685	448	97.99	9	2.01	237	92.83	17	7.17

13.87% whose blood samples were tested during the late luteal phase were infertile subjects undergoing treatment in the department and closely monitored. In these cases blood was routinely drawn around D_8-D_{14} following the basal temperature shift.

Table III shows the positive and negative results obtained with the receptor test and their degree of accuracy based on clinical follow up and other supporting data. It indicates that when the test was positive its degree of accuracy was about 98%. In negative tests the degree of accuracy was 92.8%. There were in all 26 false tests, 14 of which were in samples collected before the missed period.

It is also evident that the efficacy of the test increased significantly in the week following the missed period.

Table IV describes the supporting data used for confirmation of this test. In some subjects more than one parameter was used. Essentially, confirmation was based either on subsequent clinical follow-up for pregnancy or histopathology of the currettings obtained in operated cases. The bioassay on 137 urine samples by R.O.H. was also quite reliable after the 6th week of pregnancy. In a few cases high titres of plasma progesterone > 14 ng/ml, HCG RIA and repeat RRA on subsequent samples were carried out to confirm the data.

Table V: In infertility cases undergoing treatment, where basal temperature charts (BBT) were regularly maintained or midcyclic LH levels were available, one could ascertain the presence of HCG in the early implantation stage of the embryo by this test. Samples collected

TABLE IV
Supporting Data for HCG RRA

Supporting data	Total	Confirmed RRA results	False RRA results
Clinical follow up	318	296	22
Histopathology	305	302	3
Bioassay urine	137	125	12
Plasma progesterone	5	4	1
HCG RRA	4	4	—
Repeat HCG RRA	9	2	7

In some patients more than one parameter was used.

TABLE V
HCG RRA Before Missed Period in Relation to Post Ovulation Day

Post ovulation day	Total tests	Confirmed tests		False tests	
		No.	%	No.	%
< 7 Days	9	9	100.0	—	—
8-12 Days	54	44	81.48	10	18.52
13-18 Days	19	16	84.21	3	15.79
OV. day indefinite	13	12	92.31	1	7.69
Total	95	81	85.26	14	14.74

before 7th post-ovulatory day were all negative and confirmed to be so later on. Beyond 8th post-ovulatory day, the accuracy of the test in positive cases was over 80% which is indeed remarkable as per Fig. 1.

FIG. 1

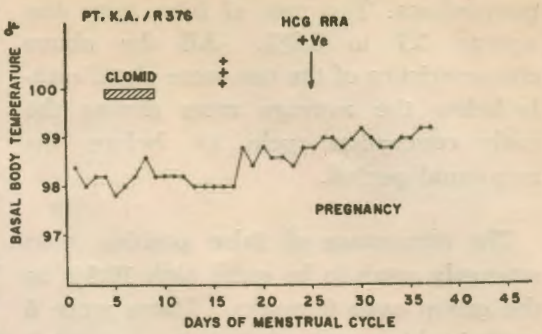

Table VI describes the overall statistical characteristics of the test at various times of gestation.

The efficiency of the test is about 96.2% which goes up significantly with weeks of gestation. The predictive value of positive tests is about 98% and also shows an improvement with duration of pregnancy, except in the group over 6 weeks. The overall predictive value of negative tests was 92.8% and tends to

TABLE VI

Characterization of HCG—RRA in Pregnancy Diagnosis

	Overall	Gestation period in days			
		<30	31–37	38–45	>45
1. Efficiency: all confirmed tests / total tests x 100	96.2%	85.3%	96.4%	99.2%	97.5%
2. Predictive value of +ve test: true +ve tests / all +ve tests x 100	98.0%	83.3%	100.0%	99.4%	97.4%
3. Predictive value of —ve test: true —ve tests / all —ve tests x 100	92.8%	85.7%	93.2%	98.3%	97.7%
4. False +ve rate: false +ve tests / all non-preg. pts. x 100	3.9%	4.3%	1.9%	1.7%	10.6%
5. False —ve rate: false —ve tests / all pregnant pts. x 100	3.7%	42.5%	7.3%	0.5%	0.5%

improve with the length of period of ammenorrhoea. The rate of false tests was around 3.7 to 3.9%. All the above characteristics of the test were significantly below the average rates during the early conception cycle, i.e. before the menstrual period.

The percentage of false positive tests strangely seem to be quite high 10.6% in the group over 6 weeks. There were 5 cases in this group where sera gave positive results eventhough the women were not pregnant. These cases probably had high LH levels or some other substances cross reacting with HCG in this system (Roy *et al*, 1977).

Discussion

The results just described indicate the high precision and efficiency of the receptor assay for early pregnancy diagnosis. This information can be very vital to the clinican especially in cases of early abortions, bleeding disorders, ectopics, infertility treated cycles, cases for MR or MTP and in patients using contraceptives especially the long-acting ones.

On all such cases the HCG titres may be low and not detectable by routine laboratory tests. Yet early diagnosis and management may make a great difference to the patient's prognosis. The currently available immunological instant tests give fairly accurate results by 6th-7th week of gestation, but not earlier.

The highly sensitive HCG test by RIA can detect pregnancy by D_9-D_{12} following ovulation (Kosasa, 1973) but the test itself takes 2-3 days to perform and becomes fairly expensive and time consuming.

The RRA in comparison has the advantage of high efficiency and speed. It can be performed in about 2 hours. Its greatest disadvantage is the use of isotopic label which has a limited half life and requires a Gamma Counter in the testing laboratory.

If the isotope can be substituted by an easily available and detectable marker, it will make a great difference in the large scale applicability of this test.

Summary

(1) Results of HCG radioreceptor assay on 685 serum samples in different clinical conditions are presented.

(2) The test has the advantage of high reliability and speed. Can be completed in 2 hours.

(3) The test system could detect pregnancy even before the missed period. Its efficiency was 85.26% around this period.

(4) The efficiency increased very much around the 5th-6th weeks of gestation.

(5) Its disadvantage is that it requires an isotopic label and thus cannot be used in the field.

(6) It has great scope in ectopics, threatened abortion, sequelae of abortions, menstrual regulation, infertility and menstrual disorders.

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References

1. Fortney, J. A., Miller, E. R. and Kessel, E.: Studies in Family Planning, **8**: 257, 1977.
2. Kosasa, T., Levesque, L., Goldstein, D. P. and Taymor, M. L.: J. Clin. Endocrinol Metab. **36**: 622, 1973.
3. Landesman, R. and Saxena, B. B.: Fertil. Steril., **25**: 1022, 1974.
4. Rosal, T. P., Saxena, B. B. and Landesman, R.: Fertil. Steril. **26**: 1105, 1975.
5. Roy, S., Klein, T. A., Scott, J. Z., Kletzky, O. A. and Mishell, D. R., Jr.: Obstet. Gynec. **50**: 401, 1977.
6. Saxena, B. B., Hasan, S. H., Haour, F. and Gollwitzer, M. S.: Science, **184**: 793, 1974.