

## SOME OBSERVATIONS ON AN IMMUNOLOGICAL TEST FOR PREGNANCY

by

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Until recently the only laboratory techniques for diagnosis of pregnancy were biological tests viz. Aschheim Zondek, Hogben, Hobson, Galli Mainini and Friedman test, etc. All these tests are unsatisfactory in many respects. They involve rearing of an animal colony in order to provide animals of the right type, right age and size, at the required time. Due to biological variations a great number of test animals may be required in each assay, to give reliable results. The assays are time consuming, laborious and expensive. Furthermore specificity of these tests is somewhat questionable. Budd (1963) described a chemical test; however, the same gives false positive reactions in patients with ovarian tumours, lactating mothers and missed abortions (Deshmukh *et al.* 1965), and diabetics (Patel and Sankari 1964) and our experience with the same has been disappointing. So development of a simple immunological test which does not require any cumbersome equipment, or highly specialised skill or technique and can be performed in a test tube, in a consultant's own clinic, is a welcome advance.

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Received for publication on 27-9-65.

An immunological test for detecting the chorionic gonadotrophic hormone in the urine of a pregnant woman, involving the principle of haemagglutination inhibition, was first described by Wide and Gemzel (1960), Swierczynska and Samochowiec (1960). Other techniques have also been described e.g. complement fixation technique to detect HCG in serum and urine by Brody and Carlstrom, and gel precipitation test to detect HCG in the urine by Mckean.

### *Present Study*

Princeton Laboratories of New York made available to us the reagents for immunological test called 'Preg test' to carry out the trial. The test is based on the principle of haemagglutination inhibition. The reagents consist of control serum (No. 1), HCG antiserum (No. 2) and cell suspension (No. 3). These are sheep's red blood cells with HCG coating. The reagents are supplied in small dropper bottles. They have to be constantly preserved in the refrigerator at 5°C, except for the time when the test is actually performed. The data presented below are analyses of 632 preg tests performed and these are reviewed along with the final diagnosis of the cases. The detailed procedure was described

earlier (Hingorani), and is summarized here. Two test tubes are set up in the transparent rack. One is the control tube and the other is reaction tube to determine the unknown.

*Procedure*

| Control                             | Unknown                          |
|-------------------------------------|----------------------------------|
| 6 drops of control serum (No. 1)    | 6 drops of HCG antiserum (No. 2) |
| 0.25 c.c. urine (1:3 dilution)      | 0.25 c.c. urine (1:3 dilution)   |
| One drop of cell suspension (No. 3) | One drop cell suspension (No. 3) |

The contents of both the tubes are mixed and tubes are allowed to remain undisturbed in the rack for 3 hours. Suspensions at the bottom of the tubes are observed and interpreted as shown in Figure 1.

*Results*

Results obtained with "Preg test" were checked with the provisional and the final diagnosis of the patients and only those patients where final diagnosis was obtained were included in the analysis. Results are shown in Table I.

TABLE I  
Results of HCG tests

|  | No. of tests |                 |
|--|--------------|-----------------|
| Correct positive .. ..                     | 420          | } Error<br>1.7% |
| Correct negative .. ..                     | 201          |                 |
| False positive .. ..                       | 3            |                 |
| False negative .. ..                       | 3            |                 |
| Non-conclusive<br>(No ring in the control) | 5            |                 |
| Total tests .. ..                          | 632          |                 |

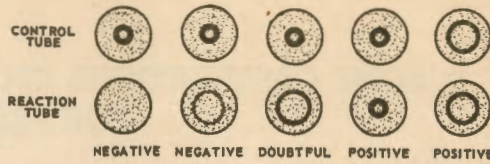


Fig. 1

*False positive*

Two false positives were obtained in the patients, over 40 years of age, and were possibly due to non-specific inhibition of agglutination or due to excessive pituitary leutinising hormone found in large amounts in this age group (Wide and Gemzell 1962, Butt *et al.* 1961). Barr (1963) had a similar experience.

One more false positive was in an infertile patient who had been given clomiphene (MRL-41) for induction of ovulation. Basal body temperature showed a biphasic curve thus indicating ovulation and elevation in the temperature persisted indicating pregnancy. However, on follow-up, patient was found not to be pregnant. In four other patients where MRL 41, had been administered, for induction of ovulation, no false positive results were obtained.

*False negative*

Three false negative results were obtained. Two were in the same patient. The test was performed 2 and 12 days after the first missed period. Later the test was repeated and found to be positive and pregnancy was confirmed. In another patient test done 10 days after the first missed period, was also falsely negative. However, only 1:3 dilutions were performed. In two other

patients, besides 1:3 dilutions, test was also performed in undiluted urine. Positive results were obtained with undiluted urines only, and therefore correct positive results were given in those cases instead of false negative. So now it has been our practice to perform tests on undiluted urine if 1:3 dilution gives negative results. It is possible that in very early pregnancy, chorionic gonadotrophins may be just sufficient to give a positive result in undiluted urine, though in five other patients, tests were positive as early as 2nd, 4th, 6th 8th and 10th day after the first missed menstrual period in 1:3 dilutions. The simplicity of the procedure makes it possible to repeat the test at frequent intervals.

In 5 cases there were no rings in the control and the reaction tube. So no conclusion could be drawn except that possibly there were non-specific antigens which interfered with the reaction. To remove these, it has been advised to use the 'Reagent powder' No. 4 ( $\frac{1}{2}$  gram capsule containing polydextran sephadex powder) and then perform the test on the filtered urine. In these cases the reagent powder did not remove the non-specific antigens. In one of the cases the urine was received in a face cream bottle and the contamination of urine with the face cream may have interfered with the results. So the instructions regarding the collection of the urine specimens in a clean container are worth emphasizing.

In ten cases doubtful reaction (wider ring in the reaction tube, see Fig. 1) were obtained with 1:3 diluted urines. In these cases, test was repeated with undiluted urine. Then

in two cases, a positive reaction and in six cases negative reactions were obtained and were interpreted as such. In the remaining two cases the result was doubtful with the undiluted urine also. This was reported as negative, as it is considered negative for diagnostic purposes (Southam *et al.*).

#### *Accuracy of Haemagglutination Inhibition Test*

The accuracy rate of haemagglutination test was tested by Wide and Gemzell (1960) against Friedman and Galli Mainini test, and was found by them of the same order. Southam *et al.* evaluated this test against rat hyperaemia test and concluded that diagnostic accuracy of haemagglutination inhibition test was found by them to be 98.5%, as against 84.5% by bio-assays. Barr in Edinburgh made a comparison between Hogben's test and an immunological test called "Prepuerin test". He concluded that Hogben's test was in agreement with clinical diagnosis in 99.2% of cases as against 97.4% obtained with immunological tests.

In Barr's series, in 10 cases, the immunological test was correctly positive, when amenorrhoea was less than 38 days, whereas Hogben's test was falsely negative in those cases. They commented that a positive result should be obtained, when there is detectable amount of chorionic gonadotrophin which was 3000 units in the case of biological test, as compared to 500 units in the case of immunological test. This makes immunological test more sensitive for early diagnosis of pregnancy.

Accuracy rate of the haemagglutination inhibition test as obtained in this study, is compared with that obtained by other authors is shown in Table II.

came negative on the 5th post-operative day.

#### *Choriocarcinoma*

There were four patients with

TABLE II  
*Accuracy rate of Haemogglutination Inhibition test*

| Authors               | No. of tests | % correct results                                 |
|-----------------------|--------------|---|
| Barr .. ..            | 1690         | 97.4%   |
| Southam et al .. ..   | 730          | 95% (with pregnancy)<br>98.5% (without pregnancy) |
| McCarthy et al .. ..  | 435          | 99.8%   |
| Fulthorpe et al .. .. | 350          | 98.2%   |
| Present series .. ..  | 632          | 98.3%   |

#### *Clinical application*

Apart from the diagnosis of pregnancy, the test was also performed in dilutions in the different weeks of pregnancy in 10 patients. The highest dilution that gave positive results in the first trimester in normal pregnancy was 1:500 in one case.

#### *Hydatidiform mole*

There were six cases of hydatidiform mole and the highest dilutions that gave positive results in these cases were 1:20 (2 cases), 1:50, 1:100; 1:200, 1:400 (one case each). In two cases of hydatidiform mole the test continued to remain positive in high dilutions, 21 and 23 days after the evacuation of the mole. On these patients hysterectomies were performed and diagnosis of chorioadenoma destruens was made. After operation in one case the test was initially positive 1:3 dilution and then with undiluted urine, and on the 10th day it became negative and remained negative. In the other case the test be-

choriocarcinoma and urines gave positive results, in 1:100 (2 cases) and 1:50 dilution (1 case) and 1:20 (1 case). One of these patients had a hydatidiform mole evacuated at another hospital three months earlier. Panhysterectomy was performed and the specimen confirmed the diagnosis of choriocarcinoma. Her urine on the sixth post-operative day gave a negative result even in the undiluted urine.

#### *Ectopic pregnancy*

In one case of abdominal pregnancy the test was positive in high dilutions, 1:50, and on repeated examinations the test became negative. At laparotomy the gross appearance, of macerated foetus at 4 months' gestation and the placenta, confirmed an earlier foetal death.

#### *Habitual abortion*

In three cases of habitual abortions, the test was performed soon after the period was missed. In these cases

vaginal examination is avoided as otherwise there is a risk of precipitating an abortion. So when this test confirmed the pregnancy as early as 2, 4, and 8 days after the first missed period, patients were put on hormone replacement therapy and in all the three cases pregnancies continued to term.

#### *Threatened and missed abortion*

This test has also been found to be particularly useful in the frequently encountered states of disrupted pregnancy. It is sometimes very trying to treat a case of so-called threatened abortion, particularly when the bleeding is minimal. The old standard treatments of rest in bed, hormones, vitamins etc. are being used for prolonged periods, in the hope of continuation of pregnancy. To the disappointment of the patient and the obstetrician, not infrequently these pregnancies end up in missed abortions. It is in these patients that the tests may be repeatedly performed if necessary; a negative test indicates an early evacuation and cuts short the trying, tiring and unfruitful confinement to bed in vain expectation.

#### *Intrauterine death*

In the two cases where the dates of intra-uterine death were known, the tests became negative 10 days after the foetal death.

#### *Early diagnosis*

It is useful to have a reliable and simple test for early diagnosis of pregnancy. Specially when clinical

examination is contraindicated, as in cases of habitual abortions, or is non-conclusive. The use of this test was also made for diagnosis of early pregnancy in patients with lactational amenorrhoea, who came to the family planning clinic for intra-uterine devices. Thus insertion of these devices in the presence of early pregnancy was avoided.

#### *Comments*

On account of simplicity of its performance, it is easy to run several tests and several dilutions at the same time. It is also easy to repeat the test at frequent intervals, so a small percentage of false negative in very early pregnancy could easily be rechecked by repeating the test or also performing the test on undiluted urines.

In the majority of cases, the results are obtained within 2 hours. It was observed that in winter the results could be read more correctly after 3 hours. As the reaction remains unaltered for several hours, the test could be set up in the evening and read the next morning if that was convenient. However, any form of shaking of tubes upsets the reaction, so results are best read after 2-3 hours.

Reagents are reasonably stable and when preserved at 5°C, do not deteriorate for one year. This care is particularly needed in hot climates as exposure to heat can cause deterioration of reagents and erratic results may then be obtained. Since only 3-5 tests can be performed with each reagent bottle, the chance of any prolonged exposure of reagent bottles to

high temperature with proper care is minimal.

The advantages of this immunological test may be summarised as under:

1. It is rapid.
2. It is simple.
3. It is easy to repeat.
4. Reagents are stable.
5. Animal colony is not required.
6. It has high sensitivity.
7. Several tests can be run at the same time.
8. Several dilutions can be done at the same time.
9. It has high accuracy.

With all these advantages one foresees wider utilization of the immunological tests for pregnancy.

#### *Summary and Conclusions*

Evaluation of an immunological test for pregnancy (Preg test) is made. The results were checked with the final diagnosis of the patients. Test was found to be rapid, simple, sensitive and highly accurate. Widening of its clinical applicability on account of simplicity in its performance is foreseen.

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