PRENATAL SEX DETERMINATION BY DEMONSTRATION OF Y BODY USING FLUORESCENT STAIN

by

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Sensational discovery in cytogenetics is the use of chemical, used in the treatment of malaria, the quinacrine dihydrochloride, which has remarkable and yet unexplained propensity for staining the stem end of Y chromosome. Cervenka *et al.*, (1971) and Alain Rook *et al.*, (1971) were the first to use this new technique using fluorescent stain to identify the Y sex chromosome rather than the heterochromatic X chromosome in the interphase nuclei in amniotic fluid cells. This method was used successfully to determine sex from buccal smear (Pearson *et al.*, 1970) and to identify the Y chromosome in human spermatozoa. This method is superior to Barr body analysis mainly because of its simplicity and prompt results and in no specimen was there any difficulty in immediate identification of Y body (Cervenka *et al.*, 1971). As this method has not been evaluated in our country, a study was undertaken to assess the acceptability and reliability of this method.

Material and Methods

Liquor amnii in a total of 170 antenatal patients attending the Postgraduate Institute of Medical Education and Research, Chandigarh, India was studied for prenatal sex determination by detection of Y body using quinacrine dihydrochloride stain modified by Cervenka *et al.*, (1971). These patients were divided into 2 main groups.

Group I—120 patients above 20 weeks of gestation.

Group II—50 patients in whom the pregnancy was between 14-20 weeks.

Liquor amnii for study was obtained by the following methods.

Group I—20-40 weeks of gestation—

Abdominal route: 1. Transabdominal amniocentesis—42 cases. 2. At caesarean section—17 cases.

Vaginal route: 1. At artificial rupture of membranes—38 cases, 2. transvaginal puncture—23 cases.

In group II (between 14 to 20 weeks gestation). Abdominal route: 1. Transabdominal amniocentesis—26 cases,

2. at hysterotomy—16 cases.

Vaginal route:—from intact sacs in cases of complete abortion—8 cases.

Prenatal sex prediction was confirmed at birth or at abortion by the apparent sex of the baby. Buccal smears of adult males and females were used as controls and to check staining procedures with each batch of slides examined.

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Technique

About 5-10 cc of collected amniotic fluid was centrifuged for 5 mts. at 2000-2500 revolutions/per minute. Thin smears were prepared from the sediment and staining of liquor amnii was done by method of Cervenka *et al.*, (1971). Smears were fixed in absolute alcohol for 15 minutes and stained with 0.5% quinacrine dihydrochloride solution for 25 mts. Later washed with distilled water, dried and mounted in top and seen under fluorescent microscope.

Observations and Discussion

The sex of the foetus was considered as male, when a Y chromosome fluoresces brightly as a single spot of about 0.25 um. diameter, seen in amniotic cells (Fig. 1). In male liquor amnii, such fluorescing spots were seen clearly in approximately 40-60% of cells screened. The sex was labelled as female when such fluorescing body were not seen under fluorescent microscope.

Group I

fluorescent stain in 49 cases, while in one case (16 weeks gestation) no cells could be seen under flourescent microscope due to technical failure.

The percentage of accuracy found in group I is in full agreement with Cervenka *et al.*, as there was no difficulty in screening Y body positive cells in male amniotic fluid nearing term pregnancy.

In Cervenka *et al.*, (1971) series most of the cases were nearing term, only 2 cases were taken by transabdominal amniocentesis between 16-20 weeks. In Rook *et al.*, series of 20 cases (1971), employing the technique used in the present study, most of the cases were below 6 months, as study was carried out in patients whose pregnancy was being terminated. Rook *et al.*, (1971), stressed "although the smears with Y body were from males, in few cases it could not be detected by the method used" Possibilities they put (1) many of the cells were damaged or not viable—and this certainly seemed to account for unsatisfactory smears for sex chromosome determination, (2) a small Y chromosome may fail to fluoresce. Boragaonkar and Hollander, (1970) found 6 males with small Y chromosome which did not fluoresce in their study.

TABLE I

Correlation Between Predicted Prenatal Sex (Quinacrine dihydrochloride stain under fluorescent microscope) and Apparent Sex at Birth

Total 120	Predicted sex by Quinacrine stain		Apparent sex at birth		No. of incorrect prediction	%age of correct prediction
Female	Female	54	Female	54	0	100
			Male	0		
Male	Male	66	Female	0	0	100
			Male	66		

It can be seen from the above Table that there was a complete correlation between predicted sex by fluorescent stain and apparent sex at birth.

There was thus complete correlation of sex at birth and predicted sex by

In the present study the results obtain-

Group II

TABLE II

Correlation Between Predicted Prenatal Sex (by Quinacrine dihydrochloride stain under fluorescent microscope) and Apparent Sex at Birth

Total (50)	Predicted sex by Quinacrine stain		Apparent sex at birth		No. of incorrect prediction	%age of correct sex prediction
Female	Female	18	Female	18	0	100
			Male	0		
Male	Male	31	Male	31	0	100
1 case	no cells seen		Female		1	Overall 98% accuracy

TABLE III

Comparison of Results of Antenatal Sex Prediction from Amniotic Fluid by Various Workers Using Fluorescent Stain for Demonstrating Y body

S. No.	Author	Year	Number	Percentage of accuracy
1.	Cervenka et al	1971	15	100
2.	Rook et al	1971	20	85
3.	Present series	1972	50 (20 wks)	98
			120 (20 wks)	100

TABLE IV

Percentage of Y Body Positive Cells in Amniotic Fluid

S. No.	Author	Year	Number	Percentage of Y body
1.	Cervenka et al	1971	16	68–87
2.	Rook et al	1971	20	3–9
3.	Present series	1972	170	40–60

ed are in between the findings by above two workers.

It is our impression that smears stored for a week or more showed less reliable staining and we recommend the screening of fresh preparation, this point has already been stressed in literature by (Pearson *et al.*, 1970 and Cervenka *et al.*, 1971).

Screening of particular case took less than 5 minutes. The usefulness of this rapid method of diagnosing the sex chromosome constitution of an individual is well attested by volume of information which has been gathered by staining interphase nuclei for female sex chromatin.

The present technique is (i) complimentary to the previous ones used for sex determination, (ii) with regard numerical abnormalities of Y chromosome, (iii) may also prove of value in investigation of structural chromosomal aberrations involving Y chromosome.

Amniocentesis

Amniocentesis was considered to be a safe procedure. The main complications encountered were, abdominal discomfort in 6 patients (3 in each group), blood stained tap in two cases and in one case no liquor was obtained at the first attempt which when repeated after 30 hours was successful.

Summary

Liquor amnii in total of 170 antenatal cases was studied for prenatal sex determination by demonstration of Y body using Quinacrine dihydrochloride stain. Prenatal sex prediction was confirmed at birth or at abortion by the apparent sex of the baby.

The overall accuracy in group 1—100%, Group II—98% (in one case—improper technique of staining hindered with prediction of sex).

Y body fluoresces brightly when stained with acridine derivatives. 40 to 60% of Y body positive cells were observed in males, and no positive cells in females. Drawback of this procedure is that it requires a costly instrument (fluorescent microscope) which cannot be made available in ordinary hospitals. The cost of reagents etc. is negligible.

References

1. Alain Rook, Lilliam, Y. Hsu, Melvingertner Kurt Hirschhorm: Nature. 230: 53, 1971.
3. Borgaonker, D. S. and Hollander, D. H.: Nature. 230: 52, 1971.
2. Cervenka, J., Gorlin, R. J. and Bendel, R. P.: Obst. & Gynec. 37: 912, 1971.
6. Pearson, P. L., Babrow, M., Vosa, C. G.: Nature, 226: 78, 1970.
5. Daftary, V. G., Ram, J., Parikh M. N. and Masani, K. M.: J. of Obst. & Gynec. of India, 11: 99, 1960.

See Fig. on Art Paper III