ANTENATAL SEX DETERMINATION

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Introduction

Barr and Bertram first of all in 1949 noticed a chromatin body, which they called as nucleolar satellite, lying adjacent to the larger nucleolus in neurones of the female cat in 30.43% of the cells.

Antenatal sex determination is important in diagnosing the type of hermaphroditism (Moore *et al* 1953) the developmental problems associated with gonadal agenesis (Polani *et al* 1954, Wilkins *et al* 1954) and the gonadopituitary and adrenal genital dysfunction in gynaecologic patients. It has a clinical application for legal abortions in cases of sex linked hereditory diseases like haemophilia, Hunter's, syndrome and muscular dystrophy.

It is neither important nor necessary to satisfy patient's inquisitiveness about the sex of her baby but it is definitely important for those cases where pregnancy has to be terminated for one of the above indications.

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Material and Method

A total of 110 cases were selected from indoor wards of U.I.S.E. Maternity hospital attached to G.S.V.M. Medical College, Kanpur. Study was carried out in 2nd and 3rd trimesters of pregnancy only as the number of cells in amniotic fluid in the first trimester of pregnancy was not sufficient to produce any conclusive results. Similar observation has been made by Agarwal and Devi in 1976, according to them 8% cases below 20 weeks of pregnancy did not contain sufficient number of cells according to the selection of criteria of cells. The route by which the amniotic fluid was collected varied according to gestation period. The patients were divided into 2 groups.

Group A included 40 patients (36.3%) with a gestation period of 14 to 20 weeks.

Abdominal route I Transabdominal aminocentesis.

36 cases (95.0%) 25 cases (62.5%).

II Directly from the sac at the time of Hysterotomy. 13 cases (32.5%).

Vaginal route—Both the patients were admitted as cases of threatened abortion who inspite of all care changed into inevitable abortion and liquor was collected from the bulging sac. 2 cases (5.0%).

Group B included

70 cases (63.6%) belonging to 20-40 weeks of gestation.

Abdominal route 30 cases (42.8%)

(i) Transabdominal amniocentesis in 20 cases (28.6%).

(ii) At the time of caesarean section 10 cases (14.3%).

Vaginal route 40 cases (57.1%)

(i) By ARM 20 cases (28.6%).

(ii) During spontaneous rupture of membranes. 20 cases (28.6%).

About 5-10 c.c. of amniotic fluid was collected after the patient had evacuated her bladder. Meticulous care was taken to avoid all infections. A long needle with stylet was used for collection of fluid by transabdominal approach in order to avoid admixture with maternal cells, whereas a catheter was introduced into the sac where vaginal approach was used. The fluid thus collected was then centrifuged at a rate of 2000 revolutions/mt for five minutes. The vernix and supernatent fluid was removed. Most cells found in the amniotic fluid sediment were non-nucleated, or the nucleus appeared disintegrated.

These cells are probably desquamated from the foetal skin. The cells that are well preserved and in a satisfactory condition for study of sex chromatin is easily identified as a dark donal, planoconvex, rounded or triangular mass, 0.5 to 1.5 μ in diameter lying in intimate contact with inner surface of nuclear membrane. Sediment was smeared on the microscopical slides coated with egg albumin and fixed in equal proportion of ether and alcohol for atleast ½ hr. The slides were then stained with 1% cresyl violet by the method described by Moore and Barr (1955). Various other chromatin stains are available e.g. Fuelgen stain, Guard's stain, Acetoorcein, haematoxylene, Carbol fuschin, thromin, gallocyanin stain. In Fuelgen and Guard's stain, the process is rather long and difficult. The sex was determined by the inspection of atleast 100 cells which had clear nuclear detail. If one or more typical female configuration of the chromatin mass were seen in 15-60% of the amniotic fluid cells nuclei, the foetus was predicted to be female. Male foetus was predicted when the sex chromatin limits ranged between 0.10%. Chromatin negative nucleus has a finely granular nucleoplasm and well defined nuclear membrane with no sex chromatin. All determinations of sex from the cells of amniotic fluid were performed and noted down carefully and compared with the phenotype either at the time of M.T.P. abortion or delivery. Two slides minimum were prepared in each case and studied under oil immersion.

Observations and Discussion

Table I, shows the prenatal sex determination from the amniotic fluid cells and also the sex of the foetus as found at the time of M.T.P. abortion or delivery in Group A, which comprised of 40 patients. Their gestation period ranged between 14-20 weeks of pregnancy.

The above Table shows that female sex was predicted by amniotic fluid in 13 cases, whereas the actual sex as found later on revealed only 12 female foetuses one being male thus giving an accuracy of 92.4%. The incorrectly predicted sex was a male foetus as was found at the time of abortion. This was a case of threatened abortion who on conservative treatment failed to continue the pregnancy. We presume that this error was due to admixture of maternal vaginal cells in the sample of amniotic fluid obtained (Lein *et al*, 1960) and Agarwal and Devi (1976) agree with us.

Table II shows the analysis of those 70 cases (Group B whose gestation period

TABLE I
Sex Determination in Group A

Total No. of cases	Predicted sex from amniotic fluid	Sex at the time of MTP abortion or delivery	No. of incorrect prediction	Percentage of correct prediction
Female (F)	F—13	F—12 M— 1	1	92.4%
Male (M)	M—23	M—27 F— 0	In 4 cases no diagnosis could be made because of improper staining	In 23 100% predicted cases

Overall accuracy 97.5%.

TABLE II
Sex Determination in Group B

Total cases	Predicted sex by amniotic fluid	Sex as found out at birth	No. of incorrect predictions	Percentage of correct prediction
Female (F)	F—27 3 ?	F—30 M— 0	In 3 cases no diagnosis could be made because of improper study	100% in predicted 27 cases
Male (M)	M—40	M—40	—	100%

Overall accuracy 100%.

was above 20 weeks. Our first 3 cases belonged to Group B, 2 being 20 weeks, and 1, 24 weeks of pregnancy. We could not diagnose the sex in only of these cases because of improper staining and our lack of experience.

In 27 cases we found out the foetal sex to be female which was confirmed by the phenotype at the time of birth and in 40 cases we predicted male foetus which also was found to be correct at the time of birth thus giving an overall accuracy of 100%.

In the present series we diagnosed a female foetus when chromatin mass was present in 15-60% of amniotic fluid cells nuclei as has also been observed by Hingorani *et al* in 1976. We predicted a male foetus when the sex chromatin limits ranged between 0.10% of amniotic fluid cells which is quite in agreement with Keymer *et al* (1957). We feel that this variation present in the count of the evidence of sex chromatin in the amniotic fluid cells observed by different workers is due to different stains and staining techniques. Hingorani *et al* (1976) agree with us.

We found a considerable variation in the shape and size of chromatin material in the nuclei. Makowski *et al* (1956) have made similar observations.

The number of pyknotic cells in the amniotic fluid smears increased on an average three to four fold in still births. Makowski *et al* (1956) have also report-

ed the similar findings. This is probably due to pyknosis of exfoliated somatic cells due to foetal death.

Summary and Conclusions

Transabdominal needle puncture of the foetal membranes for collection of amniotic fluid is not dangerous as has also been confirmed by Alvarez and Caldeyro (1950) and Kemer *et al* (1957). When sex chromatin was seen in the nuclei of 15-60% of amniotic fluid cells, the female foetus was diagnosed and a male foetus was predicted when sex chromatin ranged between 0.10%. Various workers differ in the range of percentage of chromatin positive cells which is probably due to different stains and for e.g. Fuelgen's technique show sex chromatin in a higher proportion of cell nuclei as has been reported by Keymer *et al* (1957).

The crucial point of the method is the avoidance of admixture of maternal cells. If the puncture is made through the uterine wall, a needle with a stylet should be used and if the amniotic fluid is collected by rupture of membranes per vaginum, a catheter should be inserted into the bag of water. The specimen should be fixed immediately after collection to avoid centolysis.

Although the entire process is quick and simple and does not caarry any risk to the mother or the baby, yet its use can not be justified for merely satisfying the curiousity of the parents nor for terminating a pregnancy if the predicted sex of the foetus is not agreeable to the parents. It is definitely important for deciding termination of pregnancy in certain sex linked disorders. However, if the results are confirmed in animals, it might become of great importance in veternary practice. But this method has certainly opened a new field for academic research.

References

1. Agarwal, S. and Devi, P. K.: J. Obstet. & Gynaec. India. **26**: 245, 1976.
2. Makowski, E. L., Prem, K. A. and Kaiser, I. H.: Science, **123**: 542, 1956.
3. Moore, K. L., Graham, M. A. and Barr, M. L.: Surg., Gynaec. & Obstet. **96**: 641, 1953.
4. Polani, P. E., Hunger, W. F. and Lennox, B.: Lancet, **2**: 120, 1954.
5. Wilkins, L., Grambach, M. M. and Vanwxr, J. J.: J: Clin. Endocrinol. **14**: 1270, 1954.