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DEMONSTRATION OF FOETAL CELLS BY CHROMOSOME ANALYSIS OF MATERNAL BLOOD

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ABSTRACT

Chromosome analysis of peripheral blood cultured cells was carried out on 42 pregnancies to prove the Y-chromosome originated in the transplacental foetal cells. The forecast of the foetal sex was confirmed after birth in 41 cases, that is, 22 gravidae were supposed to be male foetus and 19 females.

The rate of agreement between the prenatal diagnosis and the postnatal sex was 85.3%. The misinterpretation of the antenatal genetic diagnosis seems to have been due to several cytogenetic and methodologic factors. But in the present results, it is sufficient to indicate that the foetal cells, possibly the lymphocytes, in the maternal circulation pass through the placenta during the gestations. This phenomenon between mother and foetus would particularly evoke immunological interest.

INTRODUCTION

The determination of foetal sex before delivery has reliably been achieved by means of detection of sex chromatin, Y-fluorescence technique and chromosome analysis from amniotic cell culture[1]. On the other hand, the antenatal diagnosis of foetal sex by the peripheral blood cultured cells from the pregnant women was first reported by Walknowska *et al.* in 1969[2], and has been described by a few investigators since then[3)-6)].

During the course of the cytological analysis of several malignant diseases in our laboratory, the presence of the foetal cells, presumptively the lymphocytes, was confirmed in the peripheral blood of pregnant women. The fact that the foetal cells which preexisted in the maternal circulation gave us the suggestion that the foetal cells passed through the placenta.

Therefore, chromosome analysis was made on 42 pregnancies to demonstrate the foetal cells. This paper summarizes the antenatal results of the chromosome analysis on 42 gravidae, and the postnatal outcome of 41 deliveries

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during the period from April 1970 to October 1971.

MATERIALS AND METHODS

Specimens were taken from the peripheral blood of 42 women, from 20 to 32 years of age, by venipuncture to determine the sex of the foetus from 14 to 44 weeks gestations. The chromosome preparations were made by using the modified method described by Moorhead *et al.*[7], and were stained with Giemsa. In this series, the well spread metaphase chromosome in each case was photographed and karyotyped from 25 to 116 in number.

RESULTS

The results of the chromosome analysis of the peripheral blood cultured cells and of the postnatal sex on the 42 deliveries are summarized in Table 1. The modal chromosome number was 46 in all cases without double mode (56-96%, average 85%). The karyotype analysis manifested Y-chromosome in 22 cases out of the 42 (Fig. 1). The forecast of the foetal sex was confirmed after birth on 41 cases, that is, 22 cases were supposed to be male and 19 cases female. The 19 male-infants were born from the 22 pregnant women who were supposed to have male-foetus according to the antenatal detection of the peripheral blood cultured cells (86.4%), and sixteen female-infants from the 19 gravidae who were expected to be female (84.2%).

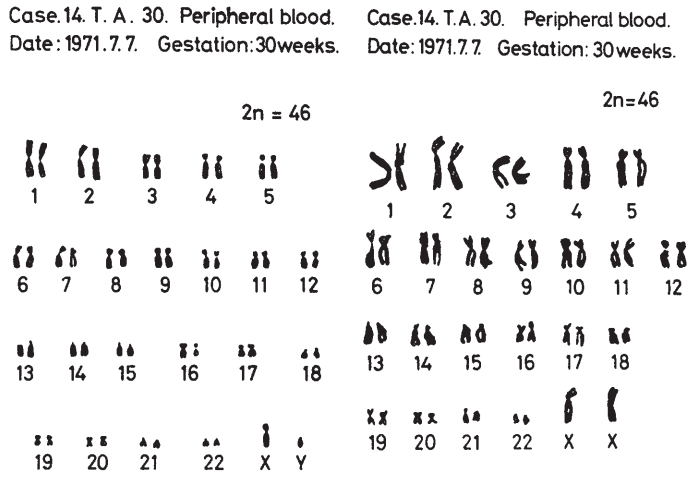

FIG. 1. Karyotypes of metaphase chromosomes in Case 14. The distinct Y-chromosome was confirmed in the peripheral blood cultured cells in the pregnant woman, and the delivery resulted in a normal male-infant.

TABLE 1. Results of Antenatal Diagnosis and of Postnatal Outcome During the Period from April 1, 1970 to October 26, 1971

Case No.	Age	Gestation (Weeks)	No. of cells analysed	No. of Y-chromosome	Confirmation of sex	
					Antenatal	Postnatal
1	27	14	86	—	XX	F
2	24	16	77	5	XY	M
3	31	16	35	1	XY	M
4	28	16	25	1	XY	M
5	28	20	58	—	XX	*
6	24	24	79	—	XX	M
7	33	28	30	2	XY	F
8	25	30	74	—	XX	F
9	23	32	55	2	XY	M
10	28	35	113	1	XY	M
11	21	35	103	—	XX	M
12	20	36	53	1	XY	M
13	25	36	66	—	XX	M
14	30	36	54	4	XY	F
15	30	36	116	—	XX	M
16	28	36	55	1	XY	M
17	27	36	59	—	XX	M
18	25	36	54	1	XY	F
19	23	37	62	1	XY	M
20	23	39	94	—	XX	F
21	25	40	50	—	XX	F
22	28	40	51	—	XX	F
23	28	40	64	1	XY	F**
24	23	40	65	1	XY	M
25	32	40	76	—	XX	F
26	22	40	75	—	XX	F
27	27	40	67	1	XY	M
28	22	40	109	1	XY	M
29	29	40	100	—	XX	F
30	22	40	88	1	XY	M
31	30	41	71	—	XX	F
32	28	41	83	—	XX	F
33	25	41	78	—	XX	F
34	27	41	102	—	XX	F
35	27	42	104	1	XY	M
36	28	42	66	1	XY	M
37	25	42	57	1	XY	M
38	25	43	48	—	XX	F
39	24	43	105	1	XY	M
40	22	43	29	1	XY	M
41	21	43	81	—	XX	F
42	28	44	96	2	XY	M

* Missing. ** Stillborn infant.

The rate of agreement between the postnatal sex and the antenatal diagnosis was 85.3%. The neonatals whose sex did not accord with the antenatal assessment were observed in only 6 cases; Case 6, 7, 11, 15, 19 and 23. Notwithstanding karyological observation of Y-chromosome, these women subsequently gave birth to female-infants in Case 7, 19 and 23. On the contrary, in spite of Y-chromosome observed in the analysed cells, the pregnancies terminated in boys in Case 6, 11 and 15.

The incidence of the observed Y-chromosome referred to the genetic diagnosis in the karyologically analysed cells varied from 0.9% to 7.4% (average 2.6%), with the highest rate of 7.4% in Case 14.

DISCUSSION

In only 6 out of the total cases, the postnatal sex was not in agreement with the antenatal assessment. In spite of observation of the Y-chromosome in Cases 7, 19 and 23, females were delivered. The reason for the misdiagnosis could not definitely be explained. However, it might be considered that the difference was caused by somatic mutation during the culture for 72 hours. Besides, if the pregnant women had previously given birth to a male-infant, and/or had also been given blood transfusions, the continued presence of the foetal cells which passed via the placenta and were transfered into the maternal blood stream may have caused the variant outcome. The above fact might be considered possible because of the extended lifespan of the lymphocyte[9].

Nevertheless, two of the cases among the three exceptions were primiparae, and they had no previous miscarriages. The genetic misdiagnosis in Cases 6, 11 and 15, in which the Y-chromosome was not observed, might be due to the insufficiency of the cells observed. If sufficient cells genetically analysed were examined further, the antenatal misdiagnosis of the Y-chromosome would be avoided.

In view of the experimental results so far achieved, it was not easy to indicate the gestation time when the foetal cells passed through the placenta and attained the maximum number in the maternal circulation of the pregnant women. There were some cases, in which the high frequency of Y-chromosome was observed in the analysed metaphase ohromosomes; 6.7% in Case 7, 6.5% in Case 2 and 7.4% in Case 14 respectively. These figures were remarkably high, but they must not be taken to directly express the number of foetal cells in the maternal blood, since the foetal lymphocytes may have divided more frequently than the maternal lymphocytes during the culture for 72 hours in the presence of the phytophemagglutinin. The prononced difference may also be due to methodological factors.

The present cytogenetic findings would raise several questions particularly in regard to the identification of what appeared to be "46 XY" cells in the maternal circulation when the woman was carrying a male-foetus. The prompt questions were whether or not the presumptive Y-chromosome was misconceived from the following chromosomal abnormalities; (1) a small chromosome similar to the Y-chromosome morphologically which resulted from the breakage of other chromosome. (2) G-trisomy which was derived from the chromosomal nondisjunction of the G-group in the cell-division. (3) mosaicism of the "XX/XY" in the maternal somatic cells. (4) somatic mutation during the culture

for 72 hours, and/or artifact in the preparation.

It is consequently necessary to identify whether the presumptive Y-chromosome is the true Y-chromosome or not, before it is concluded that the presumptive Y-chromosome is originated in the male foetal cells in the maternal circulation.

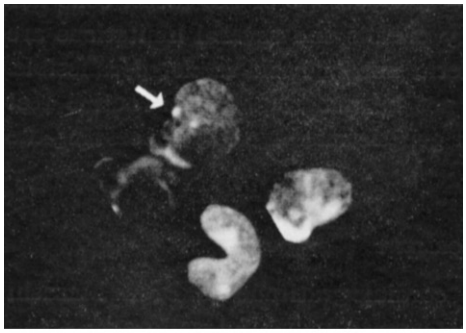

FIG. 2. Nucleus of the presumptive lymphocyte in the maternal circulation showed the bright fluorescence pattern emanating from Y-chromosome of the foetal origin (arrow).

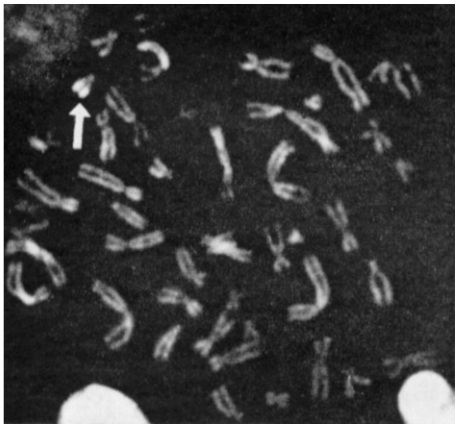

FIG. 3. Metaphase chromosome showed the bright fluorescence of the long arm of Y-chromosome (arrow).

It was indicated, however, in our different experiment (unpublished data) that the F-body could be demonstrable on the streak preparations of the maternal blood stained with quinacrine dehydrochloride under the fluorescence microscope, because the differential fluorescence of the long arm of the Y-chromosome was detectable in the interphase nuclei (Fig. 2)[9)10)]. The fluorescence pattern after staining with quinacrine mustard in the cultured cells of the same maternal blood could also manifest the large Y-chromosome resulted from the male foetal cells that had entered into the maternal circulation via the placenta simultaneously (Fig. 3)[11)12)].

Therefore, it may be permissible to state that the results of the present investigation indicate the passage of the foetal cells through the placenta. The present results could also be useful in the further study of the physiological conditions during pregnancy. The immunological interest would also be evoked by the transplacental passage of the foetal cells.

There were a few cases which had chromosomal abnormality in the analysed specimens, but the results did not reflect the abnormal babies genetically. So further investigation is necessary to determine whether or not the characteristic chromosome abnormality in the cultured peripheral blood cells of the pregnant women could detect the postnatal genetic defects of Down's syndrome, Kleinfelter's syndrome and so on. Thus the chromosome

analysis of the cultured peripheral blood cells of the pregnant women made the determination of the foetal sex before birth possible with high reliablity. Our procedures could also be performed without any apparent maternal and/or foetal complications, compared with the method of antenatal diagnosis with amniotic cell culture. But the transplacental passage of the foetal cells has been a subject of much debate, and many pitfalls and several limitations are prevailing. In addition, the clinicogenetic misdiagnosis is not permissible. Therefore, the present procedure should not be undertaken as a routine test because of the possibility of the prenatal genetic misdiagnosis concerning the detection of the foetal sex.

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