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Detection, Retrieval and Analysis of Fetal Nucleated Red Blood Cells in Maternal Blood: A Preliminary Study

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ABSTRACT

Objective To detect and retrieve fetal nucleated red blood cells (fetal NRBC) from maternal blood by magnetic activated cell sorting (MACS). and analyze the fetal NRBC by polymerase chain reaction (PCR).

Study Design A descriptive study.

Setting Antenatal clinic, Department of Obstetrics and Gynecology, Pramonkutklao Hospital. Department of Pathology, Srinakharinwirot University.

Subjects Healthy pregnant women at 7th-12th weeks of gestation.

Method The enrichment of the fetal NRBC from maternal blood was performed by MACS method. The Fetal NRBC were detected and retrieved by micromanipulation under light microscope and using the PCR technique to determine the purity of the fetal cells.

Main outcome measurement The number of fetal NRBC detected in maternal blood and the number of maternal cell contamination.

Results Total 13 cases between 7-12 weeks of gestation were studied. The mean gestational age was 9.46 weeks (S.D. 1.8) and the mean maternal age was 27.46 years (S.D. 3.0). The fetal NRBC were detected in all cases with the mean of 16.07 cells (S.D. 6.9). The maternal cell contamination was found in 2 over 6 cases (33.33%). There were 7 cases of PCR failure in the early period of this study.

Conclusion Fetal NRBC were able to identified in maternal circulation in first trimester by MACS method. This technique is simple, fast, noninvasive and low costs. The procedure for retrieval and analysis of the fetal cells need to be improved to overcome the problem of maternal cell contamination, before using this technique for prenatal screening or prenatal diagnosis.

Key words: Fetal nucleated red blood cells, maternal blood, MACS, micromanipulation, PCR

Fetal cells have long been known to be present in the maternal circulation. The fetal cells that can be identified are trophoblasts, lymphocytes, granulocytes and nucleated red blood cells (erythroblasts).[1] The best candidate fetal cells that can be clinically useful in prenatal diagnosis is nucleated red blood cells (NRBC) because of their short life span and are unlikely to persist from the previous pregnancy rarely present in adult circulation but common in the fetus, and also express the transferrin receptor antigen which make it

possible to use cell-sorting technique to enrich sample from the maternal blood.[2] The fetal cells in maternal circulation are estimated to be 1 in 105 and 1 in 108 and are also increasing in the aneuploidy fetus.[3] The two most common method for fetal cells enrichment and separation are Fluorescence activated cell sorting (FACS)[4-9] and magnetic activated cell sorting (MACS).[9-12] Recently the prenatal diagnosis of chromosome abnormalities (trisomy 21,18 and 13) and single gene defect (Thalassemia, sickle cell anemia, Duchen muscular dystrophy,) using fetal cells in maternal blood has been reported.[13-17] The effects of gestational age on the detection of fetal nucleated red blood cells in maternal blood was studied.[18] The different methods to identify and separate fetal cell are undergoing investigation. As current procedures are not sufficiently sensitive and specific enough this transition into clinical application has therefore not yet taking place. Since the MACS method seem to be cheaper and more easy to perform when compare to the FACS method. Our aim of the study is therefore to detect fetal nucleated red blood cells (NRBC) in maternal blood in the first trimester by using the MACS method.

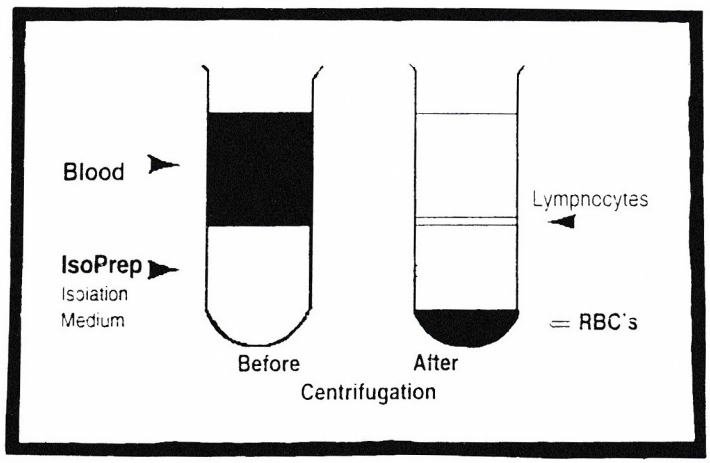
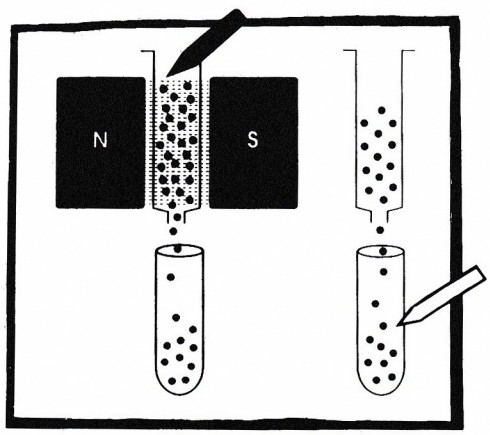
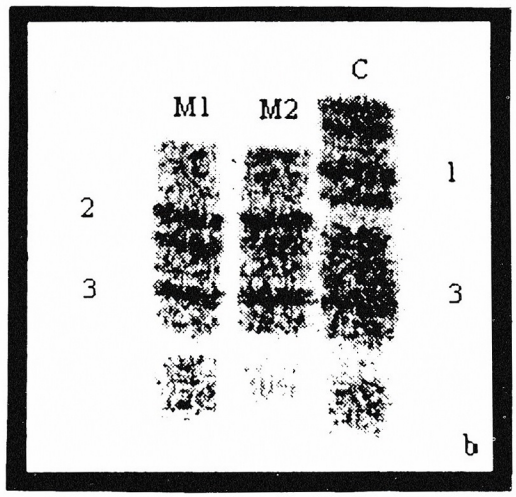


Fig.1. Density gradient seperation of mononuclear cells with Isoprep solution.



Black arrow = Fetal NRBC + anti CD71 + Magnetic bead.
Open arrow = Fetal NRBC

Fig. 2. Fetal cells enrichment by Magnetic cells sorting.



Using D22S539 Primer
M = maternal lymphocyte.
C = Fetal NRBC

Fig. 3. PCR analysis of fetal and maternal cells.

Materials and methods

With informed consent a venous blood sample (15-20 ml.) was collected in EDTA from a singleton and non complicated pregnancy at 7-12 weeks of gestation. Totally 13 patients were enrolled in this study. This protocol was approved by the hospital ethical committee. The gestational age was calculated as completed week from the first day of the last menstruation and confirmed by fetal ultrasono graphy. Ten ml. of maternal blood was diluted with 10 ml. of normal saline solution and was layered on top of 5ml. Isoprep solution (Robbins Scientific). The sample was then spun at speed 800 x g for 30 minutes at room temperature. The mononuclear cell layer was isolated and washed with buffered solution (Fig.1.) The cell pellet was then incubated with anti CD71 antibody (Becton Dickinson) and magnetic microbead (Miltenyi Biotech) at temperature 4 degree celcius for 15 minutes. The cells were washed with 500 microlitre of buffered solution twice (1 X PBS; 0.5% BSA, 2mM EDTA). The magneticbead labeled cell suspension was pipetted on top of a MiniMACS column and the unlabeled cells were washed and eluted with 500 microlitre buffered solution. The labeled cells were then collected by pushing them out of the column using 1 ml. buffer solution and a plunger (Fig.2). Slides were prepared from these cells using a cytospin (Shandon) at 500 rpm for 6 minutes. The slide were then stained with Wright's stain. The slides were examined by inverted microscope, the positive fetal nucleated red blood cells were located and count. The fetal nucleated red blood cells were individually scraped by using a glass needle controlled by a micromanipulator (ependorf) under microscope (Zeiss). The scraped cells were collected in 7 µl distilled water and prepared for the PCR technique as described by Bauner.[19] The primer used was D22S539 (research genetics) that was specific for chromosome 22. The PCR technique was performed on the fetal nucleated red cells and maternal lymphocyte for comparing.

Result

The fetal nucleated red blood cells were identified from maternal blood in all 13 cases with the range of 7-28 cells (mean 16.07 cells). The characteristics of the study population and the numbers of detected fetal nucleated red blood cells in the maternal blood are shown in table1.

Table 1. Fetal nucleated red blood cells in maternal blood

Patient no.	Age (years)	Gestation (weeks)	Parity	No. of fetal NRBC.
1	27	7	2	7
2	22	8	2	20
3	27	8	1	10
4	28	7	2	21
5	29	10	2	9
6	29	8	1	28
7	31	11	2	15
8	25	12	1	20
9	25	9	3	24
10	30	12	1	8
11	33	9	3	23
12	27	10	1	10
13	24	12	2	14
Mean	27.46	9.46	1.76	16.07
S.D.	3.01	1.85	0.72	6.98

The primer D22S539 were used for PCR analysis of fetal and maternal cells (fig 3.)

Procedure failure was found in 7 cases. Only 6 in total 13 cases were able to performed the study with confirmed the fetal cell origin in 4 cases and the maternal cells contamination in 2 cases (33.3%).

Discussion

In the present study, we studied 13 cases of normal pregnant women of 7-12 weeks of gestation. The enrichment steps of fetal nucleated red blood cells in the maternal are first by density gradient separation of mononuclear cells with isoprep solution, second by labelled fetal nucleated red blood cells with anti-Cd71 (Transferrin receptor) and third by separation the labelled cells by magnetic activated cells sorting (MACS). This technique was found to be very successful in identifying the fetal nucleated red blood cells in maternal blood. The technique is easy and does not need any expensive instruments. This technique can be an alternative method in the future for enrichment fetal nucleated red blood cells for a non-invasive prenatal diagnosis. The nucleated red blood cells that are found in maternal blood can be either fetal or maternal origin. The maternal cell contamination in our study was 33.3%, by using the PCR technique performed on pooled fetal nucleated red blood cells that was retrieved by micromanipulation. The problem of maternal cell contamination can be isolved by analyzing single cell instead of pooled cells, and then concluded that majority of cells are of fetal origin. The PCR technique for DNA study needs to be improved especially for a single cell study. More studies in large scale cocerning their sensitivity, specificity and accuracy should be investigated before implication as a prenatal diagnosis test.

In conclusion, fetal nucleated red blood cells were able to be identified in maternal blood during first trimester in all cases of this study by using the density gradient separation with isoprep solution. The Magnetic activated cell sorting (MACS) of anti-CD71 labeling cells is highly effective for enrichment of the fetal nucleated red blood cells.

Acknowledgements

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