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Isolation of Fetal Cells from Maternal Circulation Using Magnetic Cell Separation

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ABSTRACT

Prenatal diagnosis employs a variety of techniques to determine the health of fetus. Without knowledge gained by prenatal diagnosis, there could be an untoward outcome for the fetus or the mother or both. There are a variety of non-invasive and invasive techniques available for prenatal diagnosis. Each of them can be applied only during specific time periods during the pregnancy for greatest utility. All current methods of fetal karyotyping are invasive and carry a definite, albeit small, procedure-related risk. Because of this and testing costs, only women older than 35 years who have a greater risk for fetal aneuploidy are currently offered prenatal testing. The isolation and analysis of fetal cells from maternal blood would allow non-invasive prenatal genetic screening and diagnosis. One method that is currently being explored involves culturing fetal cells. Developing conditions which allow the number of fetal-derived cells to expand in culture and the number of maternally derived cells to be suppressed in culture may lead to a new selection process for obtaining fetal cells. In this paper the isolation of fetal cells using magnetic cell sorter methods was discussed. It was found that 5000 cells were present in 28 million maternal cells.

Keywords: Prenatal diagnosis, fetal cells, markers for fetal cells , isolation of fetal cells and culturing of fetal cells

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INTRODUCTION

World Health Organization (WHO) recently reported more than 270000 deaths world-wide during the first 28 days of life. March of Dimes (MOD) reported that 7.9 million births (6% of total births) occur annually worldwide with serious birth defects. 94% of these births occur in the middle and low-income countries[1]. According to WHO and MOD meeting report, birth defects account for 7% of all neonatal mortality. India stands sixth place in the world population and with ethnical, geographical and genetic diversity[2]. In several Indian communities, birth defects are moderately high due to consanguineous marriage in families. The occurrence of birth defects in India is 6-7% which translates to around 1.7 million birth defects annually[3]. The common birth defects include congenital heart disease (8-10 per 1000 live births), congenital deafness (5.6-10 per 1000 live births), and neural tube defects (4-11.4 per 1000 live births)[4]. These birth defects are clinically deceptive at birth; other defects may be diagnosed later[5-8]. Common genetic disorders occur at birth are Down syndrome, sickle cell disease and ß-thalassemia etc[9-10]. These disorders are due to gene mutations or chromosomal abnormalities.

Cells from very early developmental stages such as the colony forming definitive erythroid progenitors (BFU-E) can be isolated from umbilical cord blood using CD34[11-13]. To enrich and identify erythroid cells from later developmental stages, surface markers such as CD71, glycophorin A, CD36 and intracellularly expressed hemoglobin's have been used[13].

MATERIALS AND METHOD

Selection of the subjects

A total of 18 subjects were participated in the study. All the subjects were regular out-patients in the department of Gynecology and Obstetrics, Andhra Medical College, Visakhapatnam. Willingness to participate in the study was taken from the subjects. The protocol was thoroughly studied and approved by Institutional Ethics Committee (IEC) of Andhra Medical College, Visakhapatnam.

Collection of samples

20ml of peripheral blood was collected in EDTA coated tubes after obtaining informed consent from 18 primigravidae at 11 to 16 weeks of gestational age.

Separation of Mononuclear cells

Blood samples were diluted 1:2 with phosphate buffer saline (PBS). Diluted sample was overlaid on HiSep 1077 and centrifuged at 7000 rpm for 30 min. The mononuclear cells (MNCs) layer was separated. The MNCs were subjected to magnetic cell sorter method (MACS)

Enrichment of fetal cells by Magnetic Cell Sorting

MNCs were labeled with CD45 magnetic beads: Mononuclear cell suspension was centrifuged at 1500 rpm for 5min. Supernatant was discarded and pellet was re-suspended in 80µl of buffer per 10^7 cells. To this mixture 20µl of CD 45 micro beads was added. The cell suspension mixed well and kept for incubation for 15 min at 4°C to 8°C. The Cd45 negative cells were isolated using MS and LS columns. To the CD45 negative fraction, CD71 and CD36 magnetic microbeads were added. CD71 and CD36 positive fraction in CD45 negative cells were isolated using MS and LS columns. The CD45-CD71+CD36+ cell population is known to be the fetal origin in maternal circulation.

The methodology followed for isolation of fetal cells from maternal circulation was shown in Figure 1.

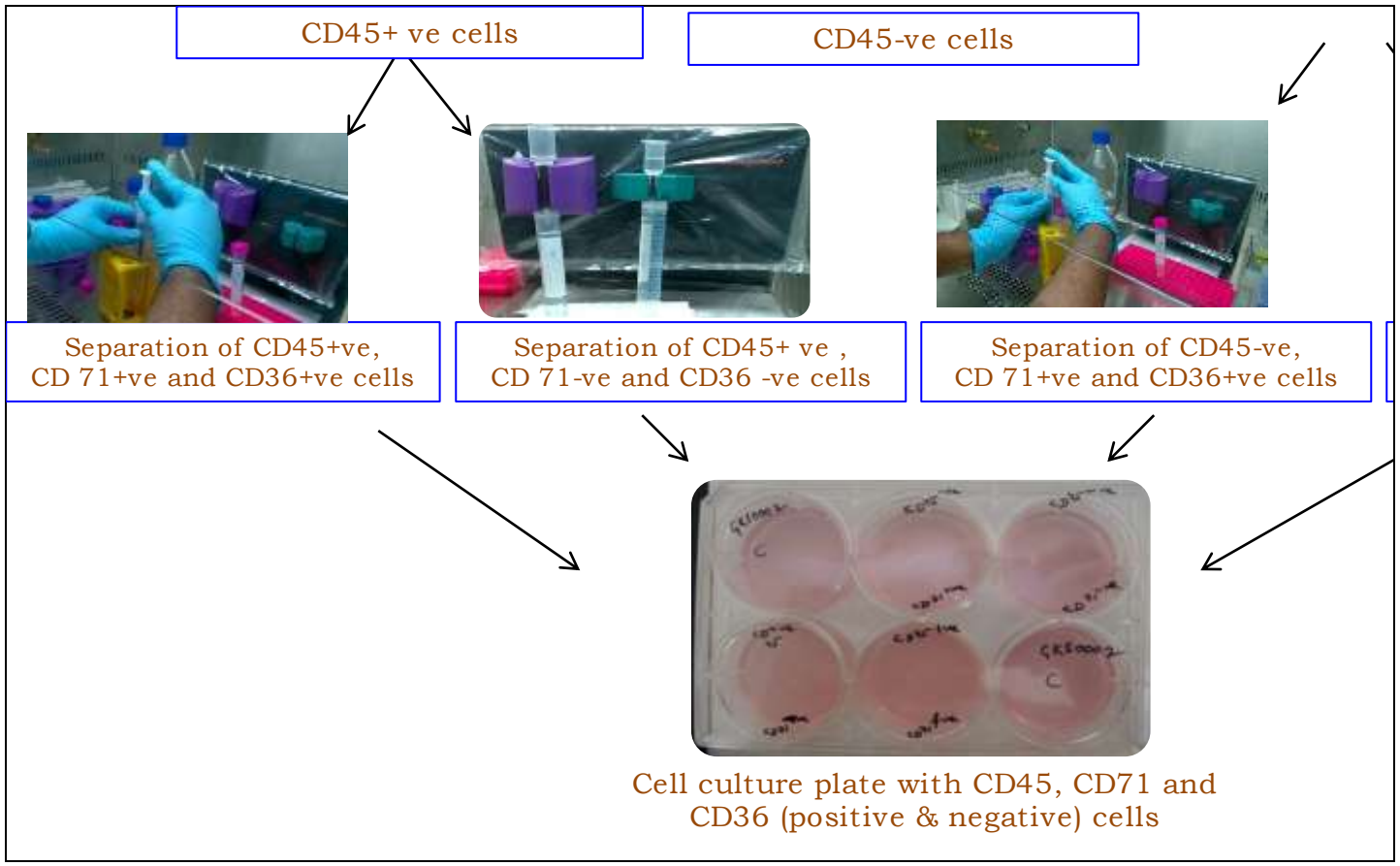

Figure 1 Isolation of CD71 positive and CD36 positive cells from CD45 Negative fraction

RESULTS AND DISCUSSION

Isolation of fetal cells from maternal circulation

The fetal cells were isolated from maternal circulation as describe previously. The average age of the subjects was 24 ± 2.32. The mean of the subjects was 24 years. The minimum age of the

subjects was 20 and maximum age of the subjects was 28. The range of the age groups in the study was 8. The average number of MNCs isolated from maternal circulation was 28.6 ± 4.38 million cells. The average number of fetal cells (CD45-CD71+CD36+) was 5.0 ± 0.660 × 10^3 cells per 20 ml. The percentage of occurrence of fetal cells in maternal circulation was 0.018 ± 0.004 (Table 1). This indicates that the occurrence of fetal cells in maternal circulation was very low.

CONCLUSION

In this paper, the standardized protocol for isolation fetal cells in maternal circulation was mentioned. The protocol infers the number of fetal cells in maternal circulation. The phenotype of fetal cells in maternal circulation was found to be CD45-CD71+CD36+. The occurrence of fetal cells in maternal circulation was found to be very low. This creates big venture in the science of fetal cells and prenatal diagnostics tools to develop ex vivo expansion techniques for fetal cells.

Table 1: Number of mononuclear cells and fetal cells in maternal circulation

Subject No.	Age	Total MNCs isolated (x10^6) per 20mL	Fetal cells isolated (x10^3) per 20mL	% of fetal cells occurrence
1	22	30	5.1	0.017
2	28	27	4.2	0.016
3	24	32	6.4	0.02
4	25	25	4.5	0.018
5	28	22	5.8	0.026
6	22	31	4.7	0.015
7	20	32	4.8	0.018
8	25	34	4.5	0.013
9	21	35	5.2	0.015
10	23	30	3.9	0.013
11	27	27	4.6	0.014
12	22	33	5.1	0.015
13	25	24	5.8	0.024
14	26	25	5.9	0.024
15	24	22	4.7	0.021
16	25	33	5.5	0.017
17	22	30	4.5	0.015
18	23	22	5.3	0.024
Mean ± SD	24 ± 2.32	28.6 ± 4.38	5.0 ± 0.660	0.018 ± 0.004
Median	24	30	4.95	0.017
Min	20	22	3.9	0.013
Max	28	35	6.4	0.026
Range	8	13	2.5	0.013

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