Original Research Article

Comparison of hematological parameters among newborns with meconium stained amniotic fluid and clear amniotic fluid

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ABSTRACT

Background: Meconium stained amniotic fluid is an indirect marker of fetal hypoxia. Fetal hypoxia affects bone marrow and affects production of cell lines. Indirect marker of erythropoietin activity is nucleated red blood cells count. Aim of the study is to compare hematological parameter in newborn born with meconium stained liquor and clear amniotic fluid. Also, to determine cut off levels of nucleated RBC as marker of fetal hypoxia in meconium stained amniotic fluid (MSAF).

Methods: The study was conducted over a period of 2 years from January 2012 to January 2014 in Department of Pediatrics, Sri Aurobindo medical college and hospital, Indore. One hundred newborns with meconium stained amniotic fluid (study group) and one hundred newborns with clear amniotic fluids (control group) were studied in this period. Cord blood samples were collected and hematological parameters were compared.

Results: Hemoglobin % and WBC counts were seen in study group in comparison to control group, which is statistically significant (P<0.05). The reported NRBC/100 WBC between the two groups was statistically analyzed. The mean NRBC count in study group were 8.09±6.09 which was statistically higher than the corresponding values in control group 2.07±2.25 (P<0.05).

Conclusions: Study concluded that the hemoglobin levels , WBC and NRBC counts in babies with meconium stained amniotic fluid is higher than those babies delivered with clear amniotic fluid and NRBC can be taken as a surrogate marker of hypoxia in a resource limited setting.

Keywords: Gestational age, Hypoxia, Hemoglobin, Meconium stained amniotic fluid, Nucleated red blood cells, White blood cells

INTRODUCTION

Meconium is a muco-viscid odorless substance and is the first stool passed by the newborn. It is known to be present in the fetal ileum by as early as 10-16 weeks of gestation. Meconium stained amniotic fluid is an indirect marker of fetal hypoxia. Fetal hypoxia stimulates fetal evacuation of meconium. One of the consequences of chronic fetal hypoxia is increased erythropoiesis caused by erythropoietin stimulation. It has been observed that hypoxia causes stimulation of hematopoietic system as evidenced by the rise in levels of erythropoietin. This rise in erythropoietin levels translates into the erythroid precursors (NRBC) into the circulation of fetus. NRBCs are immature erythrocytes, commonly found in the peripheral blood of newborns at birth. Elevated nucleated RBCs in neonates are increasingly considered as markers of chronic intrauterine hypoxia as seen in meconium stained amniotic fluid. Little information exists on hematologic status of infants with meconium aspiration. Early identification and intervention in newborns with MSAF will improve the outcome and...
avoid the need for expensive and invasive treatment. Simple tests like nucleated red blood cells (NRBCs) could supplement costly tests like erythropoietin etc. as a marker of fetal hypoxia in a resource limited setting.

The aim of this study was to find out the co-relation between meconium stained amniotic fluid and hematological parameters (hemoglobin levels, WBC and NRBC). Since NRBC counts is less expensive than erythropoietin assay we sought to determine the correlation between neonatal NRBC counts and meconium stained amniotic fluid to associate NRBC as an important indicator of meconium aspiration syndrome.

METHODS

The study was conducted over a period of 2 years from January 2012 to January 2014 in Department of Pediatrics, Sri Aurobindo medical college and hospital, Indore (M.P.). All newborns delivered in our institution during the study period were included in the study. Babies born to mothers with risk factors (diabetes mellitus, pre-eclampsia, hypertension, chorio-amnionitis) were excluded from study. Also, babies with low birth weight, intra-uterine growth retardation and pre-natal or post-natally diagnosed congenital heart disease were excluded from the study. One hundred newborns with meconium stained amniotic fluid and one hundred newborns with clear amniotic fluids were studied in this period.

After taking necessary permission from ethical committee, parents of the newborn were explained verbally in detail about the aims of the study, procedures, risks / benefits involved, etc. A written informed consent was obtained from the parents and/or his/her legally acceptable representative. Immediately after delivery of the newborn, 2 ml of umbilical cord blood was collected into vacutainer with interior walls coated with K-3 EDTA and sent for analysis. Hemoglobin percentage and white blood cell count was determined using automated hematologic blood cell counter (Model LH750 Analyzer, Beckman Coulter, USA). A thin blood smear was made and nucleated red blood cell (NRBC) counts per 100 white blood cells were determined manually.

All report along with baseline data characteristics were filled in the self-made Performa. The newborn was followed till discharge / death / LAMA, etc. All the data required for the study was collected during this time period. Calculated descriptive statistics for all quantitative variables was used. Student’s t test was used to compare averages (mean NRBC in study vs. control group). Statistical Package for Social Sciences software Version 16.0.0 was used.

RESULTS

In the study, 100 newborns delivered with meconium stained amniotic fluid (study group) and 100 newborns born with clear amniotic fluid (control group) were assessed. In the present study, among controls 41 were females and 59 were males. Among the study group 35 were females and 65 were males. In the study a total of 157 newborn were delivered by normal vaginal delivery and 43 newborns by LSCS.

| Baseline characteristics | Study group (mean±SD) | Control group (mean±SD) |
|--------------------------|-----------------------|-------------------------|
| Maternal age (years)     | 21.21±2.35            | 21.41±2.63              |
| Gestational age (weeks)  | 38.89±1.14            | 38.59±0.99              |
| Sex of infant            |                       |                         |
| Male (%)                 | 65                    | 59                      |
| Female (%)               | 35                    | 41                      |
| Birth weight (kg)        | 2.82±0.30             | 2.76±0.23               |

Base line characteristics were comparable among two groups. Gestational age in weeks was significantly higher in study group compared to control group. (Table 1)

| Hematological Parameter                          | Min  | Max  | Mean  | SD   | p value |
|--------------------------------------------------|------|------|-------|------|---------|
| Hemoglobin (gm%)                                 |      |      |       |      |         |
| Control group                                    | 13.8 | 19.2 | 15.67 | 1.22 | p < 0.05|
| Study group                                      | 14   | 20   | 16.20 | 1.43 |         |
| WBC (per cu mm)                                  |      |      |       |      |         |
| Control group                                    | 7600 | 17800| 11561.50| 1755.00| p < 0.05|
| Study group                                      | 9700 | 20400| 13450.00| 2544.01|         |

Table 2 shows comparison of hematological parameters among study and control group. The mean hemoglobin levels in control group were 15.67±1.22 gm% and in study group were 16.2±1.43gm%. Similarly, mean white blood cells in control group were 11561.5±1755 per cubic mm and in study group were 13450±2544 per cubic mm. In the study, hemoglobin percent and white blood cell count were found to be significantly raised among
newborn born with meconium stained liquor as compared with clear amniotic fluid.

Table 3: Range of nucleated red cells in study and control group.

| NRBC (%) | Study | Control |
|----------|-------|---------|
| 0-5      | 45    | 91      |
| 6-10     | 28    | 9       |
| 11-15    | 13    | 0       |
| 16-20    | 9     | 0       |
| 21-25    | 5     | 0       |
| Total    | 100   | 100     |

Table 3 highlights the range of nucleated red cells in study and control group. The reported NRBC/100 WBC between the two groups was statistically analyzed. In study group 27% babies had NRBC count >10 but none in control group had NRBC >10.

Table 4: Association between NRBC values in control and study group.

| NRBC | Min | Max | Mean | SD | p value |
|------|-----|-----|------|----|---------|
| Control group | 0 | 9 | 2.07 | 2.25 | < 0.05 |
| Study group | 1 | 25 | 8.09 | 6.09 | |

This table shows that the mean NRBC value in the study group to be significantly higher when compared with the controls. The mean NRBC count in study group were 8.09±6.09 which was statistically higher than the corresponding values in control group 2.07±2.25 (P<0.05) (Table 5).

Table 5: Values of NRBC in study group with reference to meconium aspiration syndrome.

| Study group | Meconium aspiration syndrome | N | Mean | SD | p value |
|-------------|-------------------------------|---|------|----|---------|
| NRBC        | Absent                        | 89 | 6.49 | 4.18 | < 0.05 |
|             | Present                       | 11 | 21.00 | 2.57 | |

The table shows us that in the study group (babies delivered through meconium stained amniotic fluid) the mean NRBC counts were significantly higher, i.e., it was 21.00 in babies with meconium aspiration syndrome (11 babies) whereas in babies without meconium aspiration syndrome the mean NRBC count was 6.49. The mean NRBC value in study group in newborns with meconium aspiration syndrome was 21.00±2.57 which was statistically significant to those without meconium aspiration syndrome 6.49±4.18 (P<0.05).

DISCUSSION

Authors aimed to compare the nucleated RBC count in neonates born with clear amniotic fluid and those with meconium stained amniotic fluid and to associate NRBC as indicator of MAS. A prospective analysis was conducted on 200 term, appropriate for gestational age infants born of uncomplicated pregnancies. The babies were divided into two groups according to character of amniotic fluid observed upon delivery. The study group consisted of 100 babies delivered by clear amniotic fluid and control group consisted 100 babies delivered by meconium stained amniotic fluid. In study group 32% suffered from different morbidities as hypoxia (32), respiratory distress (9), MAS (11), ventilation (11) and HIE (2) Deaths (5)

There was a significant difference in Hb% and WBC counts between study and control groups the mean hemoglobin levels in control group were 15.6±1.22 gm% and in study group were 16.2±1.43gm%. Similarly, mean white blood cells in control group were 115±1.5±1755 per cubic mm and in study group were 135±2544 per cubic mm. In the study, hemoglobin percent and white blood cell count were found to be significantly raised among newborn born with meconium stained liquor as compared with clear amniotic fluid.

In our study, babies with meconium stained amniotic fluid had higher cord blood NRBC counts than babies with clear amniotic fluid. This finding supported that neonates with meconium stained amniotic fluid suffered from fetal hypoxia since NRBC count is related to hypoxic situation. Various other studies have also demonstrated similar findings Dollberg et al, Tungalag et al, Darkanesh et al. 8-10

The mean NRBC values in this study group was 8.09±6.09 which was comparable to other studies. Hanion-Lundberg et al, studied 93 newborns with meconium stained liquor and found the mean NRBC count as 9.27±12.63.11 Similarly, McCarthy et al, 33 newborns with meconium stained liquor and found the mean NRBC count as 7.8±7.4.12 In this study group, authors have found 11 newborns with meconium aspiration syndrome.

The mean NRBC count in these 11 newborns was found to be 21.00±2.57, which is higher than the newborns with clear amniotic fluid. Our results support previous studies which indicate neonate with MSAF suffered from chronic fetal hypoxia as demonstrated by elevated fetal NRBC levels.

It can be seen that study group with meconium stained amniotic fluid had evidences of asphyxia (low apgar scores) resulting in higher cord blood NRBCs compared to the newborns with clear amniotic fluid. NRBC can be taken as a surrogate marker in a resource limited setting, to evaluate hypoxia in such a situation. With these findings it can be concluded that the NRBC counts in babies with meconium stained amniotic fluid is significantly higher than those babies delivered with clear...
amniotic fluid and it can be taken as a marker to evaluate hypoxia in such a situation in a resource limited setting.

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**Ethical approval: The study was approved by the Institutional Ethics Committee**

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