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Assay of nucleated red blood cells in the umbilical cord blood to assess the severity of outcome among the neonates born with meconium stained amniotic fluid

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Abstract

Background and Objectives: Meconium stained Liquor is an alarming perinatal condition of the foetus which is associated with high rates of perinatal mortality. Nucleated red blood cells (NRBCs) are released from the foetal bone marrow in response to increased erythropoietin situations of hypoxia. The objectives of the study were to compare the level of nucleated red blood cells between neonates born with meconium stained amniotic fluid (cases) and clear amniotic fluid (controls) and to find out the association between nucleated red blood cells level and poor outcomes among neonates with meconium stained amniotic fluid.

Materials and Methods: The study was case control study conducted in neonatology unit of paediatrics department among 50 neonates with meconium stained amniotic fluid as cases and 50 neonates with clear amniotic fluid as controls for a period of 2 years. Informed consent was obtained from each study participant. The data was collected using a pretested semi structured interview schedule and NRBCs level were estimated using 2 ml umbilical cord blood collected from each study participant. The data collected was entered into excel sheet and analysed using SPSS version 23.

Results: The NRBCs among the cases was found to be more than among the controls and the difference was statistically significant. The mean NRBCs level were found to be more among those with poor Cry/Suck/Activity. The mean NRBCs level among those who had received oxygen support was more than those who had not received any. Similarly, among those who expired the mean NRBCs level was more.

Conclusion: To conclude increased NRBCs level among the neonates with meconium stained amniotic fluid could indicate poor outcome.

Keywords: Nucleated red blood cells (NRBCs), neonate, meconium stained amniotic fluid (MSAF), oxygen support, Cry/Suck/Activity.

1. Introduction

India as a developing economy is focused on improving quality and standards of health care in all perspectives^[1]. Among the various dimensions of health, maternal and child health is one of the major components requiring due attention and focus. A healthy mother and a healthy child as a unit contribute to the health and wellbeing of the entire country^[2]. A woman during the pregnancy is expected to deliver a normal and healthy child with adequate primary care and support. However, in certain cases of high-risk pregnancies, due to various maternal and other risk factors, the pregnancy becomes complicated resulting in adverse prenatal and postnatal outcomes^[3].

Meconium Stained amniotic fluid is an alarming perinatal condition of the foetus which is associated with high rates of perinatal mortality. The incidence of MSL ranges between 7% to 22% and they contribute to the neonatal mortality of up to 0.05%^[4]. Meconium Stained Amniotic Fluid (MSAF) progresses to Meconium Aspiration Syndrome (MAS) which develops in 10% of the MSL neonates^[5]. When the respiratory distress becomes irreversible, the neonatal death ensues resulting in loss of the pregnancy. MAS are classified as mild, moderate and severe where mild MAS is defined as neonates requiring <40% of oxygen for <48 hours. Moderate MAS is a condition where there is requirement of more than 40% oxygen for more than 48 hours with no air leak. Severe MAS is a condition requiring assisted ventilation for more than 48 hours and this is often associated with persistent pulmonary hypertension of the newborn (PPHN)^[6]. At the pathological level it has been postulated that vagal stimulation from umbilical cord compression results in fetal hypoxia causing increased peristalsis and relaxation of anus which leads to passage of meconium^[7].

Nucleated red blood cells (NRBCs) are premature precursor cells of the red blood cells which are released from the fetal bone marrow in response to increased erythropoietin situations of hypoxia [8]. Studies have demonstrated that increased fetal counts of NRBCs are indicative of Intra uterine growth retardation (IUGR) and fetal distress [9]. Nevertheless, the accuracy of NRBCs in predicting Meconium aspiration has been seldom evaluated. Identifying the cause and effect relationship between NRBCs in the cord blood cells and the Meconium aspiration could lead to early detection of Meconium aspiration before the onset of symptoms and also help in preventing complications associated with MAS thereby minimising the Perinatal mortality. The objective of the present study was to compare the level of nucleated red blood cells between neonates born with meconium stained amniotic fluid (cases) and clear amniotic fluid (controls) and to find out the association between nucleated red blood cells level and poor outcomes in neonates born with meconium stained amniotic fluid.

Methodology

The study was case control study carried out in neonatology unit of rajah Muthiah medical college and hospital, Annamalai University, Chidambaram for a period of two years between September 2018 to August 2020. Ethical committee clearance was obtained from the institute's ethical committee. The cases were those neonates born with meconium stained amniotic fluid and controls were neonates born with clear amniotic fluid. 50 cases and controls, respectively were included into the study following sample size calculation and the study participants were selected by convenient sampling. A structured proforma was used to collect data on the neonates like mode of delivery, Birth weight, APGAR score at 1, 5 and 10 minutes, resuscitation details was recorded. Immediately after delivery 2ml of umbilical cord blood was collected using a syringe into a vial containing EDTA. Hemoglobin (HB) and white blood cell count (WBC) per mm³ were determined using automated hematologic blood cell count. A thin blood smear was made and NRBCs per 100 WBC was determined manually. For making smear, two clean glass slides were taken and a drop of the sample was placed towards one end. A spreader glass slide was placed at 30° - 45° inclination to the sample and in one uniform motion the drop of blood was smeared onto the rest of the slide. The slide was allowed to dry and then covered with Leishman's stain. After 3min the

stain was carefully diluted with distilled water and mixed on the slide by gently blowing on the surface. The slide was allowed to take in the stain for 15 min and then washed in a gentle stream of tap water. The dried smear was focused under high power of microscope and the number of nucleated red blood cells was counted against the number of white blood cells until 100 white blood cells were counted, NRBCs less than 10% was considered normal and NRBCs equal or more than 10% was considered abnormal. Data was entered and analysed using SPSS version 21. The descriptive statistics was expressed in terms of mean and standard deviation. The prevalence of nucleated RBC'S was expressed in percentages. Categorical variables were analysed using chi square test and continuous variables were analysed using independent sample t-test. P-value <0.05 was considered statically significant.

Results

Among the cases, 56% were males and among controls, 48% were males. Among the cases, 76% had birth weight between 2.5 to 3Kgs and among controls, 74% had birth weight between 2.5 to 3Kgs. 44% and 40% had parity of 2 among cases and controls, respectively. 6% and 8% had breech presentation among cases and controls, respectively. 50% had normal vaginal delivery among cases and 54% had normal vaginal delivery among controls. Variables like sex, birth weight, parity, presentation and mode of delivery were found to be equally distributed between cases and controls. APGAR score at 1 min, 5mins and 10mins were found to be low in cases than in the controls and the difference was found to be statistically significant (Table 1).

Among the cases, 48% had NRBCs level of more than or equal to 13, 32% between 10 and 12 while among the controls none had NRBCs level of 13 or more and 10% had NRBC level of 10 to 12. The NRBCs per 100 WBCs were found to be more among the cases than when compared to the controls and the difference was statistically significant (Fig 1).

Among the cases, the mean NRBCs level among those with poor Cry/Suck/Activity was more than those with fair and good Cry/Suck/Activity. The difference was statistically significant. Those who had received oxygen support were found to have increased mean NRBCs level than those who had not received any. Among the cases those neonates expired had significantly higher mean NRBCs level (Table 2).

Table 1: Distribution of baseline characteristics

Characteristics		Cases		Controls		P value
		N	%	N	%	
Sex	Male	28	56	24	48	0.423
	Female	22	44	26	52	
Birth weight (in Kgs)	2.5-3	38	76	37	74	0.817
	>3	12	24	13	26	
Parity	1	21	42	21	42	0.841
	2	22	44	20	40	
	3	7	14	9	18	
Presentation	Breech	3	6	4	8	0.695
	Cephalic	47	94	46	92	
Mode of delivery	NVD	25	50	27	54	0.885
	AVD	4	8	3	6	
	LSCS	21	42	20	40	
Mean APGAR score	1 min	5.46	2.47	7.30	0.76	0.001
	5 mins	7.20	1.80	8.68	0.47	0.001
	10 mins	8	1.30	8.96	0.198	0.001

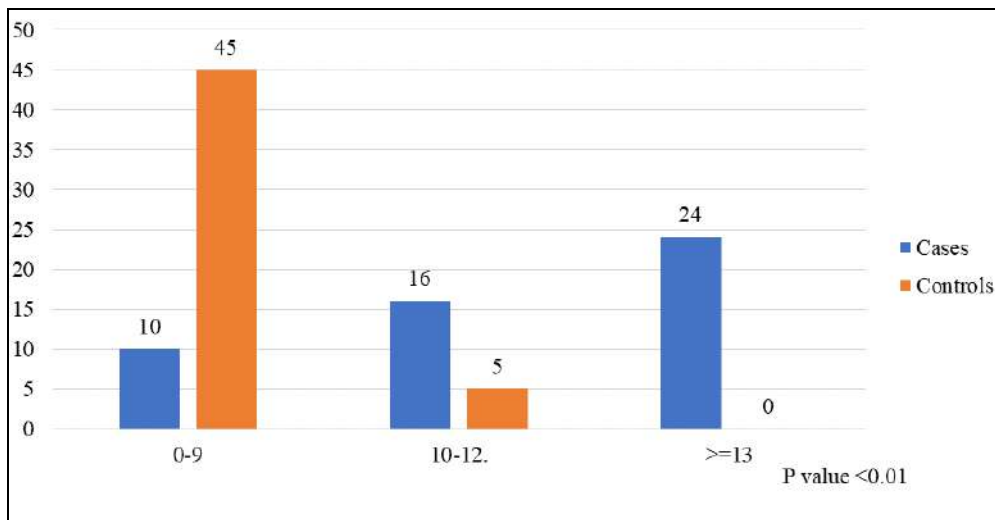


Fig 1: Distribution of NRBCs per 100 WBCs

Table 2: Comparison of outcome and NRBCs among the cases

Variables	Cases		NRBCs per 100 WBCs		P value	
	N	%	Mean	SD		
Cry/Suck/Activity	Poor	14	28	12.85	1.83	<0.05
	Fair	27	54	11.55	2.35	
	Good	9	18	6.55	2.87	
Oxygen support	Hood	10	20	10.4	0.51	<0.05
	CPAP	14	28	13.57	1.28	
	CPAP+MV	4	8	13.00	0	
	MV	4	8	13.7	0.5	
	None	18	36	8.33	3.37	
Outcome	Alive	45	90	10.75	3.22	<0.05
	Dead	5	10	13.40	0.54	

Discussion

The present study was case control study carried out among 100 term neonates of which 50 were cases with Meconium Stained Amniotic Fluid (MSAF) and 50 were controls with clear amniotic fluid. The baseline characteristics like distribution of sex, birth weight, parity, presentation and mode of delivery were found to be similar between the cases and controls. Mean APGAR score for neonates in the cases group was 5.46±2.47, 7.20±1.80 & 8.00±1.30 at 1 minute, 5minutes & 10 minutes respectively. Whereas in the controls group it was 7.30±0.76, 8.68±0.47 & 8.96±0.19 at 1 minute, 5minutes & 10 minutes respectively. Moreover poor APGAR score was seen among 44% of cases group. In the present study, out of 80% elevated NRBCs level in cases group, NRBCs level between 10-12 cells per WBCs and ≥ 13 cells per 100 WBCs were observed among 32% and 48% of neonates respectively compared to elevation of 10% neonates in control group which was between 10-12 cells per 100 WBCs. The observed difference was statistically significant. In a study done by Darakhaneh *et al* in Turkey, cord blood NRBCs count per 100 WBCs was found to be 11.18 in acute fetal distress group compared to 24.43 in chronic fetal distress group.¹⁰ Similar findings were observed in another study done by Ghosh *et al*.¹¹ Increased NRBCs level in the present study was also found to be associated with poor Cry/Suck/Activity, oxygen support and death among neonates with meconium stained liquor. Studies have indicated that elevation in NRBCs level is an indication of inflammatory response, mediated by Interleukin-6 (IL-6) and these inflammatory mediators

trigger perinatal asphyxia in these neonates¹². Nucleated red blood cells (NRBC) are premature precursor cells of the red blood cells which are released from the fetal bone marrow in response to increased erythropoietin in situations of hypoxia⁹.

The role of NRBCs level has been widened from a marker of inflammatory response to a predictor of morbidity and mortality in the neonates. Elevated NRBCs have a significant role in predicting various adverse neonatal outcomes including poor apgar score, fetal hypoxia, respiratory distress and MAS. In addition, it has been considered that NRBCs accentuate the morbidity and mortality in the presence of meconium stained amniotic fluid and the present study has elucidated on the predictive role of meconium aspiration syndrome, which further worsens the prognosis.

Conclusion

Increased NRBCs level by itself is a predictor of meconium aspiration syndrome and increased NRBCs level among the neonates with meconium-stained liquor could also indicate poor outcome. The study emphasizes the need to place NRBCs level evaluation as a routine protocol to reduce neonatal mortality.

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