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A Study of Nucleated red blood cells in cord blood of Neonates born with Meconium-stained amniotic fluid with Respiratory Symptoms and in those born with clear amniotic fluid

[1]Dr Maggidi Ajay Reddy, Pediatrics Resident, Department of Pediatrics, Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth (Deemed to be University, Karad, Maharashtra, India

[2]Dr G.G. Joag, Professor, Department of Pediatrics, Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth (Deemed to be University, Karad, Maharashtra, India

Corresponding Author: Dr G.G. Joag, Professor, Department of Pediatrics, Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth (Deemed to be University, Karad, Maharashtra, India

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Abstract

Introduction: Meconium is composed of urine, lanugo, hair, vernix, desquamated epithelial cells from the mouth, skin, alimentary tract, vernix and gastrointestinal tract secretions swallowed during fetal life. Infants born with meconium aspiration syndrome are at increased risk of fetal hypoxia, evidenced by increased rates of abnormalities indicated by fetal monitoring in labor, low neonatal apgar scores, and fetal deaths. One of the consequences of chronic fetal hypoxia is increased erythropoiesis caused by erythropoietin stimulation. Little information exists on hematologic status of infants with meconium aspiration. 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

Aim: To find correlation between NRBC/100WBC in cord blood of neonates born with meconium stained amniotic fluid with respiratory symptoms and in those born with clear amniotic fluid.

Objectives of the study 1. To compare the number of NRBC/100WBC in the cord blood of newborns with meconium-stained amniotic fluid with respiratory symptoms and in those born with clear amniotic fluid. 2. To compare the number of nucleated RBCs as indicator of severity of respiratory symptoms associated with meconium stained amniotic fluid.

Material and Methods: Twelve new-borns born with meconium-stained amniotic fluid with respiratory symptoms (Group A). Twelve new-borns born with meconium-stained amniotic fluid without respiratory symptoms (Group B) and Twelve new-borns born with

clear amniotic fluid without respiratory symptoms (Group C) in Krishna Hospital, Karad were enrolled in this study. A prospective comparative study was carried out over 18 months on 36 newborns.

Results: The study found that Group A had the highest mean NRBC count/100 WBC (13.58±3.11), while Group C had the lowest mean NRBC count/100 WBC (4.58±2.71).Intergroup comparison showed a statistically significant difference in NRBC counts/100 WBC between Group A, Group B, and Group C.($p<0.005$)In Group A, all newborns had respiratory distress, 9 newborns had mild respiratory distress, 2 newborns had moderate respiratory distress and 1 had severe distress. Group A had higher (NRBC count/100WBC $\geq$ 10) NRBC count/100 WBC when compared with group B & group C. This shows correlation between NRBC count/100WBC and severity of respiratory distress associated with meconium-stained amniotic fluid.

Conclusion: To determine markers of intrauterine hypoxia, which is a major contributor to adverse neonatal outcomes, In the present study, NRBC counts were considered. This simple laboratory test of NRBC counts can be performed in a resource limited setting. It is cost effective in predicting meconium aspiration.

Keywords: Meconium Aspiration Syndrome, Erythropoietin, Intrauterine Hypoxia, NRBC count/100WBC, Respiratory Distress, Meconium-Stained Amniotic Fluid, Apgar Scores.

Introduction

Meconium in Latin ‘menium’ means poppy juice, and in Greek mkion- poppy. The word meconium is derived from the Greek word “meconium-arion” meaning opium like.’ It was so named by Aristotle because of the belief that this opium like substance promoted fetal sleep during gestation.[2] Meconium is known to be present in the fetal ileum by as early as 10-16 weeks of gestation.[3]Meconium can be defined as the first stools passed by a newborn, meconium usually passed within 24 hours of birth by more than 90% of newborns.[16] Meconium is composed of urine, lanugo, hair, vernix, desquamated epithelial cells from the mouth, skin, alimentary tract, vernix and gastrointestinal tract secretions swallowed during fetal life.[2]Meconium aspiration syndrome (MAS) is defined as development of respiratory distress soon after birth in a neonate born through meconium stained amniotic fluid (MSAF) with characteristic radiological changes and whose symptoms cannot be otherwise explained.[16]The most common signs in meconium aspiration syndrome are tachypnoea, bradycardia, cyanosis, grunting, retractions, hypotonia at birth.

Meconium aspiration syndrome (MAS) is one of the common causes responsible for neonatal respiratory distress.[16] Meconium aspiration syndrome (MAS) is a life-threatening respiratory disease affecting some neonates born through meconium-stained amniotic fluid (MSAF).[16] The prevalence of meconium aspiration syndrome (MAS) among neonates in India varies across different studies and regions. In a recent study conducted at a rural tertiary centre in Tamilnadu, the incidence of meconium aspiration syndrome among newborn with meconium stained amniotic fluid (MSAF) was found to be 12.8%.[18] In a recent study conducted at Shri Vasant Rao Naik Government medical college, Maharashtra, it was found that meconium stained amniotic fluid complicates delivery in approximately 8% to 25% of live births, of which nearly 5% of the neonates born through meconium stained amniotic fluid develop meconium aspiration syndrome.[16] MSAF is found to be associated with many maternal and neonatal risk factors, and it is

one of the indicators of fetal distress.[16] For meconium aspiration syndrome to develop in a newborn, fetus should pass meconium inuteroatleast 3-4 hours before the delivery. Infants born with meconium aspiration syndrome are at increased risk of fetal hypoxia, evidenced by increased rates of abnormalities indicated by fetal monitoring in labor, low neonatal apgar scores, and fetal deaths.[1]One of the consequences of chronic fetal hypoxia is increased erythropoiesis caused by erythropoietin stimulation. Little information exists on hematologic status of infants with meconium aspiration.[6]

Early identification and intervention in newborns with MAS 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. Nucleated red blood cells (NRBCs) are immature erythrocytes, commonly found in the peripheral blood of newborns at birth. Researchers claim that elevated NRBCs in infants is related to intrauterine hypoxia.[7]The association between chronic intrauterine hypoxia and erythropoietin levels has also been established.[5]

Infants with meconium aspiration syndrome (MAS)with respiratory symptoms were found to have higher absolute nucleated RBC counts than infants with asymptomatic meconium aspiration and clear amniotic fluid babies. These findings support the theory that infants with significant meconium aspiration syndrome suffered from fetal asphyxia.[6] Since Counting NRBC in cord blood is less expensive than erythropoietin assay we sought to determine the correlation between NRBC counts in cord blood and meconium-stained amniotic fluid with severity of respiratory symptoms in newborn.

Material and Methods

SOURCE OF DATA: Twelve newborns born with meconium-stained amniotic fluid with respiratory symptoms (Group A). Twelve newborns born with meconium-stained amniotic fluid without respiratory symptoms (Group B) and Twelve newborns born with clear amniotic fluid without respiratory symptoms (Group C) in Krishna Hospital, Karad were enrolled in this study.

All the newborns are inborn that is born in Department of Obstetrics and Gynaecology KIMS, Karad.

Study Setting: Tertiary care hospital (Krishna Hospital, Karad)

Type of study: Hospital-based prospective comparative study.

Study Duration: 18 months (June 2022-November 2023) The study protocol was approved by the institutional ethics committee and the study was performed following good clinical practice guidelines. Informed consent has been obtained for the study from the parents of the neonates who were included in the study.

Sample Size: 36 newborns fulfilling the inclusion criteria were included in this study during the study period June 2022 to November 2023

Method of Collection of Sample: Immediately after delivery 2 ml of umbilical cord Artery blood was collected in a vial containing EDTA. Hemoglobin percentage and white blood cell count were determined using an automated hematologic blood cell counter (3-part Nihon Kohden MEK6420P analyzer). A thin blood smear was made, stained with Leishman stain, air dried and nucleated red blood cell (NRBC) counts per 100 white blood cells were determined manually in the pathology lab in Krishna Hospital.

Inclusion Criteria

1. Term New born babies (37 weeks,0 days of gestational age - 41 weeks, 6 days gestational age) 1.
2. Termnewborn babies with Normal birth weight 2.5kg-3.5kg 2.
3. Termnewborn babies Born via Elective LSCS or Vaginal delivery

Exclusion Criteria

1. Mothers with following conditions: during pregnancy or labour Diabetes Mellitus, Hypertension, Preeclampsia
2. COPD, history of smoking, drugs or alcohol abuse during pregnancy
3. Placental abruption or Placenta previa
4. Maternal heart, kidney, lung, or other chronic condition
5. Newborns with congenital anomalies & chromosomal anomalies
6. LBW babies and IUGR
7. Instrumental delivery

Statistical Analysis

Statistical analysis was done with Statistical Package for Social Sciences (IBM SPSS Statistic for window, version 21) at 95% CI and 80% power to the study. Kolmogorov-Smirnov and Shapiro Wilk test was done to check for normal distribution of the data. Data being normally distributed parametric test were applied for statistical analysis. Descriptive statistics was performed in terms of Mean, Std Deviation, Frequency and percentage. One Way ANOVA followed by Tukey's post hoc test was applied to compare the findings between and within groups respectively. Chi square test was applied to analyze qualitative data. Statistical significance was calculated at $p<0.05$ and $p<0.001$ was considered highly significant.

Results

Table 1: Descriptive statistics for Mean Gestational age, birth weight, haemoglobin WBC count, NRBC/100 WBC and ESR

		N	Mean	Std. Deviation	Minimum	Maximum
Gestational Age (weeks)	Group A	12	39.2500	.87126	38.00	40.30
	Group B	12	38.8917	.63741	38.10	40.20
	Group C	12	38.9333	.69848	38.00	40.00
Birth weight (KG)	Group A	12	2.8733	.18480	2.60	3.20
	Group B	12	2.9475	.17147	2.70	3.30
	Group C	12	2.8542	.12595	2.60	3.10
Hemoglobin (%)	Group A	12	16.48333	1.231284	14.600	18.600
	Group B	12	16.72500	1.454851	14.600	19.200
	Group C	12	15.91667	1.983034	13.600	19.200
WBC count (Cu.mm)	Group A	12	15825.0000	3194.91784	9600.00	21200.00
	Group B	12	15333.3333	3706.58709	8700.00	22400.00

	Group C	12	13095.8333	2390.55512	8300.00	16900.00
NRBC/100wbc	Group A	12	13.5833	3.11764	10.00	22.00
	Group B	12	4.6667	3.17185	.00	10.00
	Group C	12	4.5833	2.71221	.00	10.00

Mean gestational age of the newborns in Group A was 39.25±0.87 weeks, Group B was 38.89±0.63 weeks and Group C was 38.93±0.69 weeks. Mean Birth weight of the newborns was 2.87±0.18 kgs,2.94±0.17 kgs, and 2.85±0.12 kgs in Group A, Group B and Group C respectively. Mean Haemoglobin of the Newborns in Group A was 16.48±1.23%, Group B was 16.72±1.45% and Group C was 15.91±1.98%.Mean White blood cell (WBC) count of Group A was 15825±3.194 /cu mm, Group B was 15333.33±3706.58 /cu mm and Group C was 13095±2390.555 /cu mm.Mean (NRBC count/100 WBC) was 13.58±3.11 for Group A, 4.66±3.17 for Group B, and 4.58±2.71 for Group C.Mean ESR was 8.33±3.25mm at the end of 1 hour, 6.66±2.46 mm at the end of 1 hour and 5.±00 mm at the end of 1 hour of Group A, Group B and Group C Newborns respectively.

Intergroup comparison showed no statistically significant difference in Gestational age, birthweight, haemoglobin count, WBC count, between Group A, Group B and Group C respectively. (p>0.05) However NRBC count/100 WBC and ESR showed statistically significant difference between Group A, Group B and Group Crespectively. (p<0.05)

Intragroup comparison was done using Tukey's post hoc test. There was no statistically significant difference observed in the gestational age (weeks) Hb %, birth weight and WBC count when the intragroup comparison was done between Group A, Group B and Group C respectively. (p>0.05) Intragroup comparison of NRBC count/100 WBC showed statistically significant difference between Group A and Group B and between Group A and Group C respectively. (p<0.05) There was no statistically significant difference observed in NRBC count between Group B and Group C.(p>0.05)

Table 2: Mode of delivery of Group A, Group B and Group C

			Groups			Total	P value
			Group A	Group B	Group C		
Mode of Delivery	LSCS ivo DCDA twins	Count	0	0	2	2	<0.001**
		% within Groups	0.0%	0.0%	16.7%	5.6%	
	LSCS ivo fetal distress	Count	5	0	0	5	
		% within Groups	41.7%	0.0%	0.0%	13.9%	
	LSCS ivo maternal request	Count	0	0	2	2	
		% within Groups	0.0%	0.0%	16.7%	5.6%	
	LSCS ivo MSL	Count	0	3	0	3	
		% within Groups	0.0%	25.0%	0.0%	8.3%	
	Lscsivomsl & fetal distress	Count	5	2	0	7	

		% within Groups	41.7%	16.7%	0.0%	19.5%	
	Lscsivoprevlscs	Count	1	2	1	4	
		% within Groups	8.3%	16.7%	8.3%	11.1%	
	NVD	Count	1	5,	7	13	
		% within Groups	8.3%	41.7%	58.3%	36.1%	
Total		Count	12	12	12	36	
		% within Groups	100.0%	100.0%	100.0%	100.0%	

(*statistical significance at p<0.05 and high statistically significant difference at** p<0.001)

Intergroup comparison showed a highly statistically significant difference in Mode of delivery between group A group B group C respectively. (p value<0.01).

Table 3: Outcome in Group A, Group B, Group C

Outcome	Group A	Group B	Group C
Discharge	11	12	12
Death	1	0	0

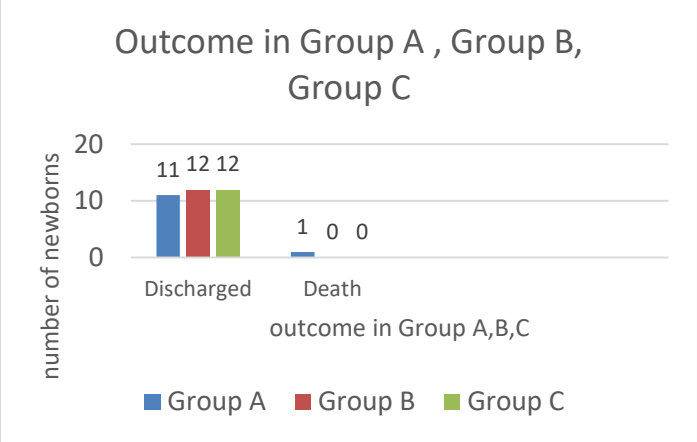

Figure 1

Table 3 & Figure 1 shows the distribution of outcome in the three groups which is as follows: Group A has a slightly lower discharge rate (92%) compared to Groups B and C (both 100%). Group A is the only group with recorded deaths, with a mortality rate of 8%. Groups B and C have no recorded deaths and all newborns were discharged. The data indicates that Groups B and C had uniformly positive outcomes with 100% discharge rates and no deaths, while Group A had discharge rate (92%) and 8% mortality

Table 4: Respiratory Support needed in Group A, Group B and Group C

			Groups			Total	
			Group A	Group B	Group C		P value
Respiratory Support	CPAP	No of newborns	7	0	0	7	<0.001**
		% within Groups	58.3%	0.0%	0.0%	19.4%	
	HFNC	No of newborns	4	0	0	4	
		% within Groups	33.3%	0.0%	0.0%	11.1%	
	mechanical ventilation	No of newborns	1	0	0	1	
		% within Groups	8.3%	0.0%	0.0%	2.8%	

	Nil	No of newborns	0	12	12	24	
		% within Groups	0.0%	100.0%	100.0%	66.7%	
Total		No of newborns	12	12	12	36	
		% within Groups	100.0%	100.0%	100.0%	100.0%	

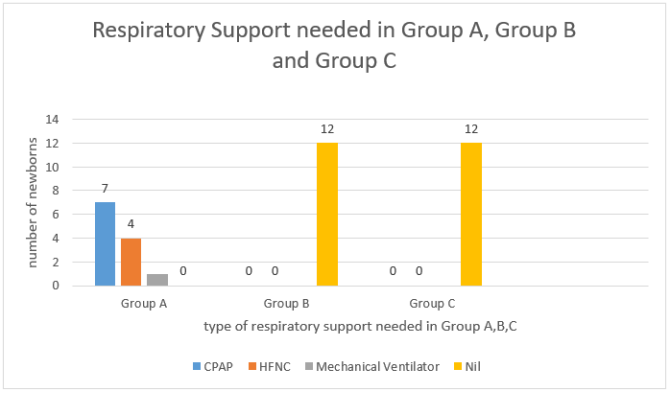

Figure 2

Table 4 & Figure 2 shows the distribution of Respiratory support needed in the three groups which is as follows: In Group A, CPAP was required for 7(58.3%) newborns, HFNC was required in 4(33.3%) newborns, and Mechanical ventilation was required in 1(8.3%) newborn respectively. In Group B and Group C, no respiratory support was required respectively. This observation was found to be statistically highly significant between Group A, Group B, and Group C ($p<0.001$).

Table 5: Correlation of NRBC counts/100 WBC in Group A, B, C with mode of delivery

NRBC counts/100 WBC	LSCS	NVD	Total(n-36)
GROUP A (13.5±3.11)	11(30.5%)	1(2.7%)	12
GROUP B (4.6±3.17)	7(19.4%)	5(13.8%)	12
GROUP C(4.5±2.71)	5(13.8%)	7(19.4%)	12

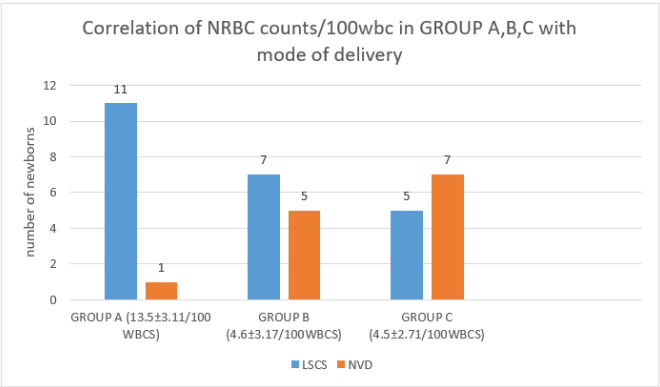

Figure 3

Table 5 & figure 3 shows Group A has the highest mean NRBC counts/100 wbc (13.5±3.11). The majority of deliveries in this group were via LSCS (11 LSCS vs. 1 NVD). Group B has moderate mean NRBC counts/100 wbc (4.6±3.17). Deliveries were more evenly split between LSCS and NVD (7 LSCS vs. 5 NVD). Group C has the lowest NRBC counts/100 wbc (4.5±2.71) and the majority of deliveries were NVD (7 NVD vs. 5 LSCS)

Table 6: Correlation of NRBC counts/100 WBC in Group A, B, C with type of respiratory support given

NRBC counts/100 WBC	CPAP	HFNC	Mechanical Ventilation	No Support	Total(n-36)
GROUP A (13.5±3.11)	7	4	1	0	12
GROUP B (4.6±3.17)	0	0	0	12	12
GROUP C(4.5±2.71)	0	0	0	12	12

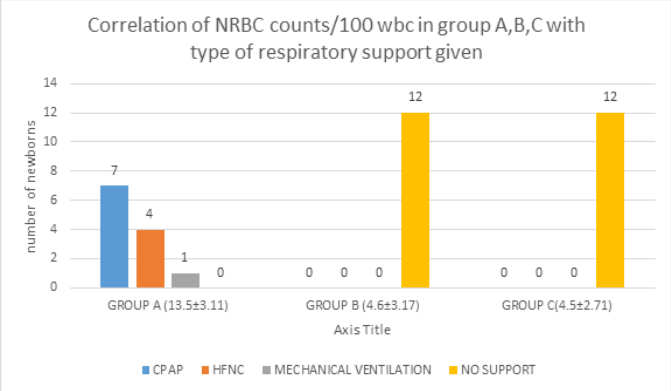

Figure 4

Table 6 & figure 4 shows Group A has higher NRBC count/100 WBC (13.5±3.11) and requires significant respiratory support: 7onCPAP, 4 on HFNC, and 1 on mechanical ventilation. Groups B and C have lower NRBC counts/100 WBC (4.6±3.17 and 4.5±2.71, respectively) and nonewborn in these groups required respiratory support.

Table 7: Correlation of number of NRBC count/100 WBC in Group A with severity of respiratory distress. (Downes score)

Group A NRBC count/100 WBC	Mild respiratory distress	Moderate respiratory distress	Severe respiratory distress	Total (n=12)	P Value <0.001*
10-13	6	0	0	6	
14-18	3	2	0	5	
19-22	0	0	1	1	

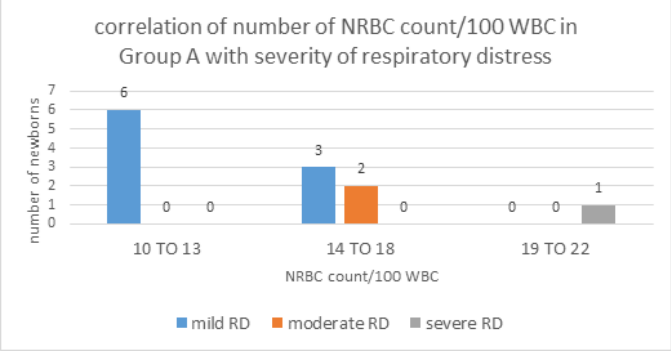

Figure 5

Table 7& figure 5 shows the distribution of number of NRBC counts/ 100 WBC in Group A shows a trend where higher NRBC counts are associated with increased severity of respiratory distress. Most mild cases fall within the lower NRBC count range (10-13), while the severe case is in the highest NRBC count range (19-22). The **p-value**< 0.001 indicates a highly significant association between number of NRBC count and the severity of respiratory distress.

Discussion

Meconium stained amniotic fluid occurs more commonly in term or post term pregnancies and occurs rarely prior to 34 weeks of gestation. In a post term fetus MSAF may result with rising motilin levels and normal gastrointestinal function, vagal stimulation produced by cord or head compression, or in utero fetal stress. In the presence of fetal stress, gasping by the fetus can result in aspiration of meconium before, during, or immediately following delivery. Severe MAS appears to be caused by pathological intrauterine processes, hypoxia, acidosis and infection.[17]When meconium is aspirated into the lungs, meconium may stimulate the release of cytokines and vasoactive substances that result in cardiovascular and inflammatory responses in the fetus and newborns. Meconium itself, or the resultant chemical pneumonitis, mechanically obstructs the small airways, causes at

electasis, and a 'ball valve' effect with resultant air trapping and possible air leak. Aspirated meconium leads to vasospasm, hypertrophy of the pulmonary arterial musculature and pulmonary hypertension that leads to extra pulmonary right to left shunting through the ductus arteriosus or the formaenovale, resulting in worsened ventilation perfusion(V/Q) mismatch and severe arterial hypoxemia. Approximately one third of the newborns with MAS develop persistent pulmonary hypertension of the newborn(PPHN), which contributes to mortality associated with this syndrome. Aspirated meconium also inhibits surfactant function. Enzymatic and sterol component of meconium disrupt the surfactant phospholipids and limit the ability of surfactant to lower surface tension.[17]

Meconium aspiration syndrome is a common occurrence in developing countries where optimum antenatal and natal care services are still inadequate especially in rural areas. Krishna hospital and medical research centre, Karad caters to the needs of the rural population. In the present study newborns with meconium stained amniotic fluid with respiratory symptoms had higher cord blood NRBC counts/100 WBC than newborns with meconium stained amniotic fluid without respiratory symptoms and those born with clear liquor.

These findings supported that newborns with meconium stained amniotic fluid with respiratory symptoms suffered from fetal hypoxia since increase in NRBC count/100 WBC is related to hypoxic situation. Various other studies have demonstrated higher NRBC count / 100 WBC in perinatal asphyxia. In the present study we have attempted to analyze various parameters related to MSAF with respiratory symptoms and its effects on cord blood NRBC count / 100 WBC. As gestational age advances, chances of MSAF to occur are more because intestinal parasympathetic innervation and myelination increases in later stage of gestation, suggesting that the rising incidence of MSAF may be due to the maturation of fetal intestinal peristalsis. There are also higher chances of fetal stress as pregnancy advances thus contributing to increased meconium production and MSAF.

In the present study mean Gestational age of Group A was 39.25±0.87, Group B was 38.89±0.63 and Group C was 38.93±0.69. There was no statistically significant difference in gestational age (weeks) between Group A, B & C. In the study conducted by Dollberg et al[6] (in 2001) mean gestational age in symptomatic meconium aspiration group was 40.2±1.2weeks, asymptomatic meconium aspiration group was 39.9±1.0 weeks and control group was 39.8±1.3 weeks. There was no statistically significant difference in gestational age between three groups. This observation is similar to our study. In the study conducted by Darkhaneh et al[10] (in 2008) mean gestational age in the study group was 39.64±1.09 weeks & in the control group was 38.96±0.99 weeks. There was no statistically significant difference in gestational age between study and control groups. This observation is similar with our study. Thus it is seen that the group having MSAF with respiratory symptoms (group A) has highest mean gestational age and hence correlates with the observation that as gestational age advances, there are more chances of MSAF.

In the present study mean birth weight of Group A was 2.87±0.18 kg, Group B was 2.94±0.17 kg and Group C was 2.85±0.12 kg. There was no statistically significant difference in birth weights (kg) between Group A, B & C. In the study conducted by Darkhaneh et al[10] (in 2008) mean birth weight in the study group was 3.42±0.42 kg

& in the control group was 3.29±0.38 kg. There was no statistically significant difference in birth weights between study and control groups. This observation is similar to our study. Mean birth weight of term newborns in a study conducted by Divyaet al[14] (in 2014) was 3.07±0.45 kg. In the study conducted by Dollberg et al[6] (in 2001) mean birth weight in symptomatic meconium aspiration group was 3.27±0.52 kg, asymptomatic meconium aspiration group was 3.308±400 kg and control group was 3.16±0.16 kg. There was no statistically significant difference in birth weights between three groups. This observation agrees with observations in our study.

We found that Mean cord blood haemoglobin of the Newborns in Group A was 16.48±1.23%, Group B was 16.72±1.45% and Group C was 15.91±1.98%. There was no statistically significant difference in cord blood hemoglobin between Group A, B & C. In the study conducted by Darkhaneh et al[10] (in 2008) mean cord blood Hb in the study group was 14.71±2.01% & in the control group was 14.03±1.71%. There was no statistically significant difference in cord blood hemoglobin between study and control groups. our findings are in accordance with findings of Darkhaneh et al. Mean cord blood hemoglobin of term newborns in a study conducted by Marwaha et al[11] (in 2010) was 16.2±1.5%. The mean cord blood hemoglobin in our study was 16.48±1.23%,16.72±1.45% and 15.91±1.98% in Group A,B and C respectively. This observation is comparable to observations in our study.

In the present study Mean White blood cell (WBC) count of Group A was 15825±3.194 /cu mm, Group B was 15333.33±3706.58 /cu mm and Group C was 13095±2390.555 /cu mm. There was no statistically significant difference in white blood cell (WBC) count between Group A, B & C. Group A with highest mean WBC count had higher NRBC count/100WBC. In the study conducted by Darkhaneh et al[10] (in 2008), mean WBC count in the study group was 12013.32±3411.82/cu mm & in the control group was 11033.33±3532.16/cu mm. Study group with highest mean WBC count had higher NRBC count/100 WBC and there was no statistically significant difference in WBC count between study and control groups. This observation is similar with our study. In the study conducted by Dollberg et al[6](in 2001) mean WBC count in symptomatic meconium aspiration group was 23700±12100/cu mm, asymptomatic meconium aspiration group was 22700±6400/cu mm and control group was 22500±5800/cu mm. Symptomatic meconium aspiration group with highest mean WBC count had higher NRBC count/100 WBC and there was no statistically significant difference in WBC count between three groups. our study observation also agrees with these findings.

We observed that, mean NRBC count/100 WBC was 13.58±3.11 NRBC count/100WBC for Group A, 4.66±3.17 NRBC count/100WBC for Group B, and 4.58±2.71 NRBC count/100 WBC for Group C. Newborns with meconium stained amniotic fluid with respiratory symptoms had higher NRBC count/ 100 WBC. There was a statistically significant difference in NRBC count/ 100 WBC between Group A, B & C. In the study conducted by Darkhaneh et al[10] (in 2008) mean NRBC count/100 WBC in the study group was 8.67±6.54 NRBC count/100WBC & in the control group was 3.88±3.92 NRBC count/100WBC. Study group had higher NRBC count/100WBC compared to control group and there was a statistically significant difference in NRBC count/ 100 WBC between study & control

groups. Our study also found similar observation. The study group of Darkhaneh et al[10] (in 2008) also showed that MSAF newborns had higher NRBC count/100WBC.our findings are in accordance with findings of Darkhaneh et al.[10] In the study conducted by Dollberg et al[6](in 2001) mean NRBC count/100 WBC in symptomatic meconium aspiration group was 0.007x109/L, asymptomatic meconium aspiration group was 0.004x109/L and control group was 0.003x109/L. Newborns with symptomatic meconium aspiration had higher NRBC count/100WBC and there was a statistically significant difference in NRBC count/ 100 WBC between three groups. This observation is similar to our study. Dollberget al[6] (in 2001) also demonstrated in their study that symptomatic meconium aspiration group had higher NRBC count/100WBC. This observation is comparable to observation in our study.

In the present study, in Group A, CPAP (continuous positive airway pressure) was required for 7(58.3%) newborns, HFNC (high flow nasal cannula) was required in 4(33.3%) newborns, and Mechanical ventilation was required in 1(8.3%) newborn respectively. In Group B and Group C, No respiratory support was required respectively. This observation was found to be statistically highly significant between Group A, Group B, and Group C ($p<0.001$). The study conducted by Peter et al[12]noted that 20-30% of the newborns with MAS required CPAP i.e., 1/3rd of the newborns with MAS required CPAP. Up to quarter of newborns requiring intubation with MAS received CPAP before and/or after their period of invasive ventilation. In their study percentage of CPAP required in newborns was less because many other modes of ventilation strategies were used in their study. In the present study, percentage of newborns requiring mechanical ventilation was less probably because of small sample size.

We observed, mean NRBC count/ 100 WBC in relation to normal vaginal delivery (NVD) & lower segment caesarean section (LSCS) was 4.36±2.59 NRBC count/100 WBC & 7.19±2.57 NRBC count/100WBC respectively. In the study conducted by Hanlon-Lundberg et al[4] (in 1997) mean NRBC count/100WBC in relation to normal vaginal delivery (NVD)& lower segment caesarean section (LSCS) was 8.19±9.74 NRBC count/100 WBC & 9.27±12.63NRBC count/100 WBC respectively. In the study conducted by McCarthy et al[8] (in 2006) mean NRBC count/100WBC in relation to normal vaginal delivery (NVD) was 9.3±10.5 NRBC count/100WBC & lower segment caesarean section (LSCS) was 7.8±7.4 NRBC count/100WBC.32 Mean NRBC count/100 WBC in association with mode of deliveries in our study was lower compared to Hanlon-Lundberg et al[4]& McCarthy et al[8]. This observation is different from our study. This could be because, indication of LSCS may have differed and present study was conducted in rural area, where as the other quoted studies were conducted in urban areas.

In the present study, incidence of respiratory distress in association with MAS was 33% and death occurred in 2.77% newborns. In the study conducted by Pushpa et al[9] (in 2007) incidence of respiratory distress in association with MAS was 13.4% & death was 2%. There was comparable percentage of deaths between our study & study done by Puspha et al[9] (in 2007) though the incidence of respiratory distress was slightly higher in our study which might be because of differences in sample size.

In the present study, severity of respiratory distress in MSAF newborns with respiratory symptoms was

classified based on Downes score. Mild was seen in 9(75%) newborns, moderate was seen in 2(16.6%) newborns, and severe was seen in 1(8.3%) newborn. In the studies conducted by S.N. singhet al[13] and Ravindranath et al[15],both studies used Downes score to assess severity of respiratory distress in MSAF newborns with respiratory symptoms. In the study conducted by S.N. singh et al[1] (in 2012), severity of respiratory distress of newborns with MSAF with respiratory symptoms was mild in 30(30.9%) newborns, Moderate in 46(47.4%) newborns, and severe in 21(21.6%) newborns. These observations are not similar with our study. In the study conducted by Ravindranath et al[18] (in 2017), severity of respiratory distress of newborns with MSAF with respiratory symptoms was mild in 42(72.41%) newborns, Moderate in 14(24.13%) newborns, and severe in 2(3.44%) newborns. These observations are similar with our study. In the present study, number of NRBC counts/ 100 WBC in Group A shows a trend where higher NRBC counts are associated with increased severity of respiratory distress. Most mild cases fall within the lower NRBC count range (10-13), while the severe case is in the highest NRBC count range (19-22).

Conclusion

In the present study, in Group A comprising of newborns born with MSAF & had respiratory symptoms, the NRBC counts were found to be significantly higher with a mean of 13.58±3.11 than compared to Group B & Group C who had a mean NRBC level of 4.66±3.17 & 4.58±2.71 respectively. In the present study, number of NRBC counts/ 100 WBC in Group A shows a trend where higher NRBC counts are associated with increased severity of respiratory distress. Most mild cases fall within the lower NRBC count range (10-13), while the severe case is in the highest NRBC count range (19-22). To determine markers of intrauterine hypoxia, which is a major contributor to adverse neonatal outcomes, In the present study, NRBC counts were considered. Meconium Aspiration Syndrome is a common entity and is one of the causes of significant morbidity and mortality in the newborn period especially in rural areas. In this comparative study of three groups and their relation to NRBC count, the NRBC counts were significantly higher in Group A. Based on all these observations, it may be concluded that the NRBCcounts in Group A newborns are statistically significant compared to Group B and Group C, and it may be taken as a marker to evaluate hypoxia in such situations. This simple laboratory test of NRBC counts can be performed in a resource limited setting. It is cost effective in predicting meconium aspiration.

Limitations

Sample Size: The study includes a small sample size of 36 newborns (12 in each group). A larger sample size would provide more robust and generalizable results. **Single Center Study:** The study was conducted in a single tertiary care hospital (Krishna Hospital Karad). Results from a single center may not be generalizable to other settings or populations. Short Study Duration: The study duration was 18 months, which may limit the ability to observe long-term outcomes and variations. **Selection Bias:** As all the newborns are inborn (born in the same hospital), there might be selection bias affecting the generalizability of the results to other populations or settings No Long-term Follow-up: The study does not mention long-term follow-up of the neonates to assess the impact of NRBC counts on later health outcomes.

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