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ASSOCIATION OF CORD BLOOD NUCLEATED RED BLOOD CELL COUNT WITH PERINATAL ASPHYXIA, IT'S SEVERITY AND IMMEDIATE OUTCOME

M. Phill Dissertation submitted to

NATIONAL BOARD OF EXAMINATION

In partial fulfillment of the requirements
For the award of the degree of

DIPLOMATE OF NATIONAL BOARD IN PEDIATRIC

BY

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DISSERTATION

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(PAEDIATRICS)

2018

Submitted by

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Certificate

This is to certify that **Dr. SANDEEP KUMAR** has carried out the work on his thesis entitled **"ASSOCIATION OF CORD BLOOD NUCLEATED RED BLOOD CELL COUNT WITH PERINATAL ASPHYXIA, IT'S SEVERITY AND IMMEDIATE OUTCOME"** under my guidance and personal supervision. All the work related to this project was done by the candidate himself. His approach to the subject has been scientific. He has done this work with dedication and integrity.

This work is recommended for the award of the degree of **Diplomate of National Board (Paediatrics)**, National Board of Examination.

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Declaration by the Candidate

I hereby declare that this dissertation entitled **"ASSOCIATION OF CORD BLOOD NUCLEATED RED BLOOD CELL COUNT WITH PERINATAL ASPHYXIA, IT'S SEVERITY AND IMMEDIATE OUTCOME"** is a bonafide and genuine research work carried out by me under the guidance of Dr. Anupam Chaturvedi M.D. (Paediatrics), Senior Consultant, Department of Pediatrics, Santokba Durlabhji Memorial Hospital cum Medical Research Institute, Jaipur.

This dissertation has not been submitted by me on any previous occasion to any university for the award of any degree.

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Word of Gratitude

The pages that follow, embodying the result of my work on **"ASSOCIATION OF CORD BLOOD NUCLEATED RED BLOOD CELL COUNT WITH PERINATAL ASPHYXIA, IT'S SEVERITY AND IMMEDIATE OUTCOME"** *do bear my name but the indebtedness goes to my esteemed guide. Words would not suffice to express my sincere sense of gratitude and indebtedness for my esteemed teacher and distinguished guide:*

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It has been an honour and privilege to work under him. He has always inspired me to enrich myself by reading more and having a deep knowledge of the subject. I owe him for being so patient in mending my mistakes. He has been a constant source of encouragement to me during this work. A thorough clinician and disciplined human being gifted with a confident and dynamic approach, enthusiasm for academics and an equally deep and sincere concern for his patients.

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Dr. Sandeep Kumar

Word of Veneration

The pages that follow, embodying the result of my work on ***"ASSOCIATION OF CORD BLOOD NUCLEATED RED BLOOD CELL COUNT WITH PERINATAL ASPHYXIA, IT'S SEVERITY AND IMMEDIATE OUTCOME"*** *do bear my name but the indebtedness goes to my esteemed co-guide.*

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Dr. Sandeep Kumar

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Dr. Sandeep Kumar

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Introduction

INTRODUCTION

The term perinatal asphyxia is derived from the Greek word '*a-sphyxos*' meaning, born without an evident pulse.[1]

Perinatal asphyxia refers to a condition during the first and second stage of labour, in which impaired gas exchange leads to fetal acidosis, hypoxemia and hypercarbia.[2]

It is one of the most important cause of fetal distress and is a major cause of neonatal mortality and chronic neurological disabilities in survived neonates.[3]

Out of total 1.2 million neonatal deaths in India every year, 300,000-350,000 infants die due to perinatal asphyxia mostly within first 3 days of life.[1]

Asphyxia is defined by WHO as the failure to initiate and sustain breathing at birth.[4]

The National neonatal perinatal database (NNPD) defines moderate asphyxia as slow/ gasping breathing or an APGAR score of 4-6 at 1 min of age and severe asphyxia as no breathing or an APGAR score of 0-3 at 1 min of age.[5]

Hypoperfusion with concomitant hypercapnea and acidosis contribute to organ damage, the extent of which is determined by the duration and severity of the insult.[6]

Perinatal asphyxia affects multiple organs (central nervous system, renal, hepatic, respiratory, cardiac, gastro-intestinal and adrenals).[6]

The defense mechanism in hypoxic ischemia is based on ability to centralize cardiac output to prioritized organs such as brain, heart and adrenals at the expense of less important organs such as liver, lungs, skin, kidney, muscles.[7]

There are many biochemical markers, [8-12] imaging techniques[13-16] and clinical parameters[17] which can be used to predicts perinatal asphyxia.

The APGAR score is a practical method of systematically assessing newborns immediately after birth. As APGAR score decreases, severity of asphyxia increases which inturn predict poor neonatal outcome.[18]

Nucleated RBCs in the cord blood emerged as a newer indicator of severity of Birth Asphyxia as there number increases with severity of asphyxia.[19]

Importance of NRBCs counts in perinatal asphyxia in term babies:

Although nucleated red blood cells (NRBCs) are rarely found in older children, but they are commonly seen in newborns.[20] Increased NRBC number in cord/peripheral blood of term babies at birth is associated with the hypoxic nature of fetal growth.[21] There are many maternal and fetal conditions which can lead to increased NRBC count in cord blood,[22] acute perinatal asphyxia is the most common among them.[23] Though not clear, but many studies shows that this increase in NRBC counts is in response to increased erythropoietin production.[24-32] NRBCs count decreases as gestational age increases, with the exception of post-term infants which have higher NRBC counts than term infants [33-36]. In the normal neonate, NRBCs are rapidly cleared from the blood stream after birth.[22, 37, 38] The NRBCs in the first few hours of life in healthy term newborn is about 500/mm^3. By 12 hours of age, the

counts fall by about 50% and by 48 hours only 20–30 NRBCs/mm^3 are left. In healthy term newborns, virtually no NRBCs are found after the third or fourth day of life, although they may persist in small numbers up to 1 week in preterm newborns.[22, 38] The NRBC count expressed as count of NRBC/100 WBCs.[22]

Need for the study

The severity of asphyxia at birth can be assessed by APGAR score at 1 min, pH and base deficit in cord blood. In conditions where APGAR score at 1 min and/ or ABG machine with expertise not available then NRBC count can be used as a simple tool to predict the severity and immediate outcome of perinatal asphyxia in term newborns. NRBC count can be done easily in hospital laboratory. So this study was done to find out the values of NRBCs in non asphyxiated and asphyxiated term babies and their correlation with severity of Birth Asphyxia and immediate outcome in asphyxiated babies.

Aims & Objectives

AIMS AND OBJECTIVES

Primary objective:

1. To determine any difference in Nucleated Red Blood Cells (NRBCs) count in asphyxiated and non-asphyxiated term babies.

Secondary objectives:

1. Correlation of NRBCs with the severity of birth asphyxia.
2. Correlation of NRBCs with the immediate outcome of perinatal asphyxia.

Review of Literature

REVIEW OF LITERATURE

In under developed countries perinatal asphyxia remains a major cause of neonatal death and disability.[39]

Perinatal asphyxia affect multiple organs. It can leads to seizures, cerebral edema, hemorrhage, cerebral palsy and developmental delay.[6]

Various maternal and fetal conditions along with low socioeconomic status, inadequate antenatal care are risk factors for perinatal asphyxia.[22, 40]

In 1953 Virginia Apgar [18] described popular scoring system which permitted a quantitative expression of the early postnatal condition of newborns. It was designed to be a guide to the need for resuscitation of newborns. An infant suffering from birth asphyxia has low APGAR score. Over the time, universality and convenience of the APGAR score leads many investigators to adopt it as a marker for birth asphyxia.

In 1994 Thilaganathan et al [41] conducted a study in which they describe relations of umbilical cord arterial pH, APGAR score, leucocytes count and erythroblasts count in three groups of babies delivered via NVD, elective caesarean section and by emergency caesarean section. In the emergency caesarean section group babies the umbilical cord arterial pH was significantly lower and the leucocytes and erythroblasts count were higher than elective caesarean section group babies. Comparison of the emergency caesarean section and spontaneous vaginal delivery groups babies showed significant differences for pH and erythroblasts count, but not for leucocytes count. This study suggested that leucocytosis is a non-

specific response of the fetus to labour, whereas erythroblastosis reflects fetal tissue hypoxia.

In 1996 Korst et al [42] conducted a case control study on 129 newborn to determine whether a relationship existed between the presence of nucleated red blood cells, hypoxic ischemic encephalopathy, and long term neonatal neurologic impairment. Neurologically impaired neonates were divided into three groups.

Group 1 - Persistent non reactive fetal heart rate patterns from admission to delivery (n= 69)

Group 2 - Reactive fetal heart rate on admission followed by tachycardia with deceleration and absent variability (n=47)

Group 3 - Reactive fetal heart rate on admission followed by an acute prolonged decelerations (n =37).

The nucleated red blood cell value and time of nucleated red blood cell disappearance was assessed. Significant difference were obtained between each of three groups of neurologically impaired neonates and normal group (group I- 48.6 +/- 106.9; group II- 11.4 +/- 9.8; group III- 12.6 +/-13.4; $p \leq 0.000002$). Mean nucleated red blood cell values of group I higher (mean 51.5 +/- 108.9) were significantly higher than sum of groups II and III (mean 12.7 +/- 11.9) ($p \leq 0.0005$).The NRBC clearance time of Group I (mean -119 +/- 123 hrs) was also significantly longer than sum of groups II and 111 (mean -59 +/- 64 hrs) ($p< 0.001$). In general, the closer the birth was to the asphyxia event, the lower was the number of nucleated red blood cells. There data suggested that cord blood nucleated red blood cells could assist in the timing of fetal neurologic injury.

In 1999 Buonocore et al [33] conducted a study to know the prognostic value of the nucleated red blood cells count at birth with respect to perinatal brain damage. They assessed the neonatal outcomes in infants who are at high risk of having neurologic damage. They enrolled 337 newborns for the study. The nucleated red blood cell counts at birth, pulsed doppler ultrasonography of the cerebral arteries, cranial fontanels sonogram, and neurodevelopment status were evaluated in these newborns. The nucleated red blood cells count was significantly higher-

1. In neonates with abnormal doppler ultrasonographic parameter for cerebral arteries at 48 to 72 hours after birth than in healthy neonates.
2. In 6 month old infants with sequele of hypoxic ischemic encephalopathy than in healthy infants.
3. 3 year old children with abnormal developmental status than in those with no abnormalities at follow up.

Significant correlations were observed between the nucleated red blood cell counts and gestational age, APGAR score at 1 and 5 minutes, pH and base deficit. There study concluded that nucleated red blood cells count at birth not only reflects a response of the infants to perinatal hypoxia but also a reliable index of perinatal brain damage.

In 1999 Lundberg et al [43] conducted a study on 1561 newborns to know the relationship between nucleated red blood cell counts and other possible markers of fetal hypoxia in term neonates. They prospectively collected umbilical cord blood from all live born babies. Arterial blood was analyzed for pH and base deficit and venous blood was analyzed for nucleated red blood cell count. He reviewed the

medical records for maternal data and neonatal outcomes of gestations of ≥37 week's duration. The mean nucleated red blood cell count per 100 white blood cells was 9.2 ± 18.1 (range, 0-327). Nucleated red blood cell counts were higher in infants with pH <7.20 (P ≤ 0.001). Both patients with respiratory academia and uncompensated metabolic academia had elevated nucleated red blood cell counts (P =.03 and P=.14 respectively). As umbilical artery pH and base excess decreases, nucleated red blood cells became more prevalent. Elevated nucleated red blood cell counts were associated with presence of meconium (P=.020) and neonatal intensive care unit admission. They concluded that elevated nucleated red blood cell counts are associated with meconium stained amniotic fluid, acidemia and neonatal intensive care unit admission (P= 0.24).

In 1999 Yeruchimovich *et al* [44] conducted a study to evaluate whether the absolute nucleated red blood cell (RBC) count is elevated in term, appropriate for gestational age (AGA) infants born to mother who had history of smoking. They compared absolute nucleated RBC counts, in two groups of term, vaginally delivered, AGA infants in the first 12 hours of life. one group babies born to mothers who smoked during pregnancy (*n* 530) and the other group (*n* 530) had normal babies. They excluded infants of women with diabetes, hypertension, alcohol, drug abuse, and infants with heart rate abnormalities, hemolysis, blood loss, or chromosomal anomalies. The median absolute nucleated RBC count in infants of smoking mothers was 0.53109/L (range 0 to 5.0) versus 0.00053109/L (range 0 to 0.6) in nonsmoking controls (*P* <0.002). Regression analysis that included APGAR scores, gestational age, and number of cigarettes smoked per day showed a significant correlation of absolute nucleated RBC count with the number of

cigarettes smoked per day (*P* <.001). At birth, term AGA infants born to mothers who smoked during pregnancy had increased circulating absolute nucleated RBC counts compared with those whose mother did not smoked.

In 1999 Baschat AA et al [45] conducted a study to determine the relationship between the nucleated red blood cells count at birth and the circulatory status of fetuses with intrauterine growth restriction. Eighty-four fetuses were grouped as follows, on the basis of the last examination before delivery.

Group 1 - elevated umbilical artery pulsatility index only

Group 2 - middle cerebral artery pulsatility index >2 SD below the gestational age mean in addition to abnormal umbilical artery pulsatility index

Group 3 - either peak velocity index >2 SD above the gestational age mean in the inferior vena cava and ductus venosus or pulsatile flow in the umbilical vein.

Both groups 2 (median 38.5, range 1-273) and 3 (median 145, range 2-3180) had higher nucleated red blood cells count than group 1 (median 8.5, range 1-270) (P <.05 and P <.005, respectively). The persistence of the nucleated red blood cells count elevation was also longer in groups 3 (median 4 days, range 1-19 days) and 2 (median 2. 5 days, range 1-7 days) than in group 1 (median 1 day, range 1-8 days). The umbilical cord artery bicarbonate level was the strongest independent determinant of the peak nucleated red blood cells count and persistence of nucleated red blood cell elevation (r (2) = 0.27, P <. 001 and r (2) = 0.47, P <.0001).There study concluded that increasing abnormality of arterial and venous flows in fetuses

with intrauterine growth restriction was associated with increasing nucleated red blood cell count at birth. Metabolic acidemia rather than altered PO_2 was the main determinant of the rise in nucleated red blood cells.

In 2000 Saracoglu F et al [46] conducted a study to investigate the variations of nucleated red blood cells (NRBC) count in acute and chronic fetal hypoxia and to establish a cut off value of the number of NRBCs for prediction of fetal acidosis. They prospectively studied 77 pregnant women, and grouped them as acute (n=11) and chronic fetal distress (n=21) and controls (n=45) with no fetal distress. The mean NRBC count in chronic fetal distress group was higher than acute fetal distress. NRBCs count was found to be correlated with umbilical cord pH ($r=-0.57$; $P<0.001$). The cut off value predicting fetal acidosis was determined as 14/100 leukocytes (sensitivity 87%, specificity 81%) by using ROC analysis. There study concluded that the duration and the severity of fetal asphyxia may be predicted by the number of NRBCs per leukocyte.

In 2000 Yeruchimovich M [47] compared absolute nucleated RBCs count during the first 12 hours of life in three groups of term, vaginally delivered infants,

Group I - LGA infants of women with gestational diabetes

Group II - AGA infants of women with gestational diabetes

Group III - AGA infants of non-diabetic women

They excluded infants of women with hypertension, smoking, alcohol or drug abuse, and those with fetal heart rate abnormalities in labor, low APGAR score, hemolysis, blood loss, or chromosomal anomalies. There was no significant differences among study groups in gestational age, gravidity, parity, maternal

analgesia, 1 and 5-minute APGAR score, and lymphocyte counts. Haematocrit and corrected white blood cell counts were significantly higher in LGA infants of women with gestational diabetes than in the other groups. The median nucleated RBC count was significantly higher in LGA infants of women with gestational diabetes (0.56×10^9/L) than AGA infants of women with gestational diabetes (0.13×10^9/L) and controls (0.0005×10^9/L) with p value <0.001. Multiple regression analysis showed that absolute nucleated RBC count was significantly correlated with birth weight (or macrosomia) and maternal diabetic status ($r2 = 0.25$, $P < 0.001$ for the multiple regression, contribution of birth weight ($r2 = 0.19$, and diabetes $r2 = 0.06$). They Concluded that at birth, term LGA infants born to women with gestational diabetes had higher absolute nucleated RBC counts compared with AGA infants born to women with gestational diabetes and infants of non diabetic mother.

In 2002 Vatansever et al [48] conducted a study to assess the pattern of NRBC counts and erythropoetin levels in a group of high-risk neonates under stress conditions and determine the short-term outcome for these babies by using these parameters. There were 69 high-risk neonates and control group included healthy, term infants. Three blood samples were obtained from each infant with in 12 h (initial), 3 days and 7 days after birth to measure NRBC counts and EPO levels. Neonatal and short-term outcomes at 3 and 6 months of age were determined. They found no significant difference among the groups with regard to the initial serum EPO concentrations. The initial NRBCs count were significantly lower in the control group compared with the study group ($P = 0.002$). While there was no significant difference between neonates with good and poor outcome in terms of EPO concentrations of initial samples, a significant difference existed in terms of NRBCs

count (P = 0.038). They concluded that both serum EPO level and NRBC count provide limited clinical benefit in detection of pathological conditions of neonatal period, but NRBCs count determination seems to be especially helpful in predicting short-term neuro-developmental outcome.

In 2003 Ferber A et al [49] conducted a study to evaluate the relationship between fetal heart rate patterns and fetal nucleated red blood cell counts. The study population had 279 singleton term pregnancies. The fetal heart rate pattern was analyzed for reactivity, duration and type of deceleration, and time interval between last acceleration and delivery. The median nucleated red blood cells per 100 white blood cells was 7 (range, 0-158). The univariate analysis indicated that nucleated red blood cells are correlated significantly with abnormal fetal heart rate pattern, time interval between the last acceleration and delivery, fetal growth restriction, the presence of meconium and 1 minute APGAR score $\leq$7. However, the stepwise regression analysis identified the time interval between the last acceleration and delivery, as the only variable that independently predicted elevated nucleated red blood cell count at birth (P <.0001, R (2) = 0.26). Their study concluded that the presence of fetal heart rate accelerations is a reliable predictor of the non hypoxic fetus.

In 2003 Mandel D et al [50] conducted a study, to evaluate whether the absolute nucleated red blood cell (RBC) count was elevated in term, AGA polycythemic infants. They compared absolute nucleated RBC counts (taken during the first 12 hours of life) in term, AGA polycethemic infants (n=29) and with nonpolycythemic infants (n = 37). There study excluded infants of women with

diabetes, hypertension, and alcohol, tobacco, or drug abuse and those with fetal heart rate abnormalities or low APGAR score, hemolysis, blood loss or chromosomal anomalies. They concluded that at birth, term AGA polycythemic infants were having increased indices of active erythropoesis. The hematocrit, RBC count, and absolute nucleated RBC counts were significantly higher and the platelet counts were significantly lower in the polycythemic group.

In 2003 Ghosh et al [17] conducted a study to predict perinatal asphyxia with nucleated red blood cells count in cord blood of newborns. A total 75 babies were enrolled for study. Levels of NRBC per 100 WBC in umbilical venous blood were compared between 26 asphyxiated newborns (group I) and 49 non-asphyxiated newborns (group II). Correlation with neonatal outcomes were also evaluated. The mean ("S.D.) NRBC per 100 WBC in umbilical cord blood of newborns in group I was 16.5"6.4 (range 3–25) whereas that in group II was 8.6"7.01 (range 1–26). This difference was statistically significant ($P \leq 0.001$). A statistically significant negative correlation was present between NRBC level and markers of acute intrapartum asphyxia, APGAR score and umbilical arterial pH (*r*sy0.50, $P \leq 0.001$ and *r*sy0.48, *P*-0.001, respectively). Positive correlation was demonstrated with evidence of chronic antepartum asphyxia, presence of pregnancy induced hypertension and intrauterine growth restriction (*r*s2.66, *P*s0.02). A high NRBC count in umbilical blood was correlated with poor early neonatal outcomes. Their study concluded that the level of NRBC per 100 WBC was correlated, both with acute as well as chronic antepartum asphyxia. Further, it can be used as a reliable index of early neonatal outcomes.

In 2004 Ferns SJ et al [51] conducted a prospective case control study to assess nucleated red blood cell counts in cord blood in a group of asphyxiated infants and to determine its predictive value for short-term outcomes. 56 cases and same number of controls were enrolled for study. Babies were followed up in nursery till discharge. Statistical analysis employed were ANOVA test, logistic and linear regression analysis. There was a significant increase in number of nucleated red blood cells in cases as compared to controls. Low APGAR, cord blood pH and neonatal outcomes were correlated well with nucleated RBC counts. They concluded that nucleated red blood cell count at birth was a useful predictor of severity and short-term outcome of perinatal asphyxia.

In 2006 Silva et al [52] conducted a case-control study to estimate whether neonates with cerebral white matter injury have significant elevations in nucleated red blood cell counts and to estimate their predictive ability in identifying injury. They enrolled 176 infants born with cerebral white matter injury at 23-34 weeks of gestation at a single University hospital. A control was matched to each case using the subsequent delivery within 7 days of that gestational age without brain injury. The gestational age at birth was 27 weeks for both groups, but the cases had a significantly lower birth weight (Mean± SD: 958±306gm) than controls (1038±381 gm, $p \leq 0.001$). The cases had significantly increase in nucleated red blood cells per 100 white blood cells (median, 5^{th} percentile and 95^{th} percentile: 22, 3 and 374 cases compared with 14, 1 and 312 control; $p \leq 0.02$). Markers of chronic hypoxia, such as intrauterine growth restriction and oligohydramnios and markers of acute hypoxia, such as an umbilical arterial pH less than 7.0 or base excess less than 12mmol/L, were both associated with significantly elevated neonatal nucleated red blood cell

count. NRBC count of 18 per 100 WBCs had a sensitivity of 56.9 %, specificity of 57.9 %, positive predictive value of 57.9 % and negative predictive value of 56.9 % in predicting the development of cerebral white matter injury in this matched case-control study. They concluded that preterm neonates with cerebral white matter injury have significant increases in nucleated red blood cell counts. Both acute and chronic hypoxia can increase these counts, which limits their usefulness in determining timing of injury.

In 2006 McCarthy JM et al [53] conducted a study to determine effect of labor on umbilical cord nucleated red blood cell counts. A total of 57-term singleton pregnancies were studied: 33 with elective cesarean sections and 24 with vaginal deliveries. The mean NRBC count from the elective cesarean section group was 7.8+/-7.4 and in vaginal delivery group was of 9.3+/-10.5, which was not significantly different. A value of 22 NRBC/100 WBC defined the upper 95% confidence limit. The correlation between absolute NRBC and NRBC/100 WBC was 0.97. They concluded that although chronic hypoxia is associated with elevated NRBC, the stress of uncomplicated labor does not change the level.

Boskabadi H et al [54] conducted a prospective study between Oct 2006 and December 2008 and concluded that NRBC/100 WBC and/or absolute nucleated red blood cell are simple markers for assessment of severity and early outcomes of perinatal asphyxia. A total of 91 infants (42 asphyxiated and 49 normal neonates) were enrolled for study. The NRBC/100 WBC and absolute NRBC levels in the cord blood of newborns in the case group were 18.63 ± 10.63 and 634.04 ± 100.21 mm^3 respectively and in control group were 3.87 ± 5.6 and 58.21 respectively ($P<0.001$).

A statistically significant negative correlation was present between nucleated red blood cell level and indicators of the severity of perinatal asphyxia (1 min APGAR score and blood pH) (P< 0.001). NRBC showed positive correlation with acidosis and showed poor outcome (p< 0.05).

In 2011 Mohammed LH [55] et al conducted a study to predict hypoxic-ischemic encephalopathy by umbilical cord nucleated red Blood cells and lactate. They enrolled 30 full term babies diagnosed as was having HIE who fulfilled AAP criteria, and compared them with 30 age-matched apparently healthy term neonates. In their study the median of nucleated red blood cells in the asphyxiated group was 15/100 WBCs with a range from 13-53 while the median of NRBCs in control group was 2/100 WBCs. In their study, nucleated red blood cells count was reliable to detect HIE as area under the curve (AUC) was 84% and the best cut off for nucleated red blood cells count to diagnose hypoxia was > 4.5/100 WBCs with a sensitivity of 77%, specificity 83%, PPV 82% and NPV 78% with a diagnostic accuracy of 80%. They concluded that both nucleated red blood cells and lactate could be used as early predictors in diagnosis of hypoxic ischemic encephalopathy being very easy, cheap and non-invasive measure.

In 2013 Goel M et al [56] conducted a study to demonstrate NRBC in cord blood as a marker of perinatal asphyxia. A total of 100 neonates were included in the study, 50 were asphyxiated and 50 were non asphyxiated babies. The mean NRBC/100 WBC in asphyxiated and in control group was 29.5 ± 26.0 (range 7-144 NRBCs/100 WBC) and 5.9 ± 2.6 (range 3-14 NRBCs/100 WBC) respectively (P < 0.01). Using quartile deviation, staging of hypoxic ischemic encephalopathy

(HIE) was done on basis of NRBC count and there was 80% agreement between clinical and NRBC staging of HIE. There was a significant ($P < 0.01$) correlation of the number of NRBC\100 WBC with APGAR scoring, HIE staging and mortality. They concluded that the NRBCs/100 WBCs can be used as a simple marker for the assessment of severity and early outcome of perinatal asphyxia.

In 2014 Cohen MC [57] et al conducted a study and found that increased number of fetal nucleated red blood cells in the placenta of term or near-term stillborn and neonates correlates with the presence of diffuse intra dural hemorrhage in the perinatal period. Two groups (22 cases each) of non-macerated term or near term (36 weeks of gestational age) stillborns or newborns dying in the 1st day of life were studied. One group had IDH (with or without SDH) and the other did not have IDH or SDH. There was a significant association between the diffuse IDH and increased number of NRBCs (Fisher exact test P 5 0.0165). An ROC curve analysis showed that the cut-off number of NRBCs with the highest accuracy was 2.15 NRBCs/high-power field, with 79% sensitivity and 67% specificity. The presence of diffuse IDH was associated with SDH (Fisher exact test, P 50.002). The absence of hypoxic brain change were associated with the absence of diffuse IDH (odds ratio 0.308; P 5 0.039). A significant correlation was present between the release of NRBCs into the placental circulation and the occurrence of diffuse IDH and SDH.

In 2014 Colaco SM et al[1] conducted a study to find the values of nucleated red blood cells (NRBCs) in normal and asphyxiated babies and their correlation with simple clinical parameter and immediate outcomes of such babies. 140 babies were registered for the study, with 70 as cases and 70 as controls. Cord blood was

analyzed and the number of NRBCs was determined. The mean NRBC level in cases and control groups was 17.43 ± 19.86/100 WBCs and 2.97 ± 4.79/100 WBCs respectively (P < 0.0001). 58.57 developed HIE and 41.43% of babies did not develop hypoxic ischemic encephalopathy (HIE). Stage I HIE was seen in 14.28% of the babies, while stage II and stage III HIE were seen in 31.43% and 12.86% babies respectively. The mean NRBCs count was 4.48, 11.10, 25.95 and 45.55/100 WBCs in no HIE and stage I, II and III HIE respectively. The NRBC counts was significantly different in different stages of HIE (P < 0.0001).

In 2014 Mohanty AK et al [19] conducted a study to find correlation of the cord blood NRBC /100 WBC counts with perinatal asphyxia in terms of severity and short-term outcomes. They included a total of 200 neonates with 100 asphyxiated babies (case group) and 100 normal babies (control group). The mean NRBC/100 WBC count was 50.82 ± 23.85 (range from 5 to 106) in case group was and 1.67 ± 1.005 (range from 0 to 13) in control group (p < 0.001). Also a statistically significant correlation was present between severity of asphyxia (stage of HI), poor outcome and higher number of NRBC/100 WBC count (p < 0.001). Cord blood NRBC count was found to be a good predictor of perinatal Asphyxia with sensitivity of 96%, specificity of 98%, positive predictive value of 97.9% and negative predictive value of 96%. They concluded that it was cost-effective measure and did not required any special expertise or any high-tech facilities.

In 2016 Gunawat M et al [58] conducted a study to evaluate the role of cord blood Nucleated Red Blood Cells (NRBC) Count as a predictor of the birth asphyxia in full term newborns. A total of 100 full term newborns in which 50 asphyxiated

(cases) and 50 non asphyxiated (controls) were enrolled for the study. They found that the mean NRBC (NRBC/100 WBC) count was 17.82 ± 19.55 (range from 0-102) in cases group and 1.42 ± 3.26 (range from 0 to 18) in controls group ($p < 0.001$). A statistically significant negative correlation between NRBC and severity of birth asphyxia was present. The sensitivity and specificity of nucleated RBCs to predict the Birth Asphyxia with cord blood pH <7.2 were 66.67% and 90%, respectively. The Cord Blood NRBCs count is a sensitive predictor of birth Asphyxia and it correlates well with APGAR score and cord blood pH to predict the severity of Birth Asphyxia

In 2017 Ganta SJ et al [59] conducted a study to evaluate cord blood nucleated RBC's as a marker for fetal asphyxia. A total 100 babies were enrolled for study (50 asphyxiated, 50 nornal babies). They found that the mean NRBC /100 WBC for cases with birth asphyxia was 11.6 and that of the control group was 5.6. NRBCs count was found to be significantly higher in neonates with low APGAR scores. There was a correlation between the APGAR scores at 1st and at 5 minutes, the degree of hypoxic ischemic encephalopathy and the NRBC counts. They concluded that NRBCs count of the cord blood can be used as an effective tool to confirm perinatal asphyxia.

In 2018 Prabhavathi R et al [60] conducted a study to evaluate cord blood nucleated RBC count as a marker of severity of perinatal asphyxia in newborns. 50 normal newborns as control and 50 newborns with perinatal asphyxia as cases were considered**.** Among 50 cases, 24 had no hypoxic ischaemic encephalopathy (HIE), 17 had stage 1 HIE, 6 had stage 2 and 3 newborns had stage 3 HIE. The mean

APGAR score in cases was 5.34 ± 1.19 whereas for the controls group it was 8.12 ± 0.77 with P value of 0.001 which was statistically significant. The mean NRBC in newborns with APGAR score of 3, 4, 5, 6 were 35.5, 19.9, 20.6 and 12 respectively. The low APGAR scores showed high NRBCs. Mean NRBCs for HIE stage 1, 2, 3 was 19.1, 31.0, and 54.3 respectively. High NRBC count correlated with increasing severity of birth asphyxia. They concluded that NRBC count is an easy, simple and a reliable test to assess the severity of birth asphyxia in resources poor setting.

In 2018 Chavan M et al [61] conducted a study to evaluate umbilical cord blood nucleated red cell count as a marker of perinatal asphyxia. A total of 100 babies were enrolled for study, 50 asphyxiated babies as cases and 50 non asphyxiated babies as controls. The distribution of cord blood pH in cases showed maximum babies (80%) with pH value of <7. 38% of the children were detected to have HIE stage II followed by 26% with stage I and 4% with stage III. At admission, 48 hours and 72 hours, significantly higher number of babies were found to have higher cord blood NRBC count (P<0.001) and the mean cord blood NRBC count was found to be significantly high at all the intervals (P<0.001). Comparison of mean cord blood NRBC count among cases in stage III was significantly high compared to stage II and I (P<0.001) at admission, 48 hours and 72 hours. They concluded that cord blood NRBC can be used as surrogate marker for asphyxia.

Material & Methods

MATERIAL AND METHODS

Study Site:

The present study was conducted at NICU, Santokba Durlabhji Memorial hospital, Jaipur with active collaboration of labour room and operation theatre, Santokba Durlabhji Memorial hospital, Jaipur.

Study population:

New borns delivered at Santokba Durlabhji Memorial hospital, Jaipur

Study Design

This was a hospital based, prospective Case control study.

Sample size with justification:

A total of 75 subjects were taken in each of the two groups. This sample size was adequate to detect an expected difference of 14.46 in mean NRBC count among the two groups and pooled standard deviation of 19.86 (as per Colaco et al [1]). Sample size was calculated at alpha error 0.05 and study power 90%. The calculated minimum sample size was 40 subjects. Considering 10% attrition, the sample size was enhanced and 75 subjects were finally taken for study in each group.

Sample size was calculated using the formula for difference in two independent sample mean

$$N = \frac{2\times \quad 1- \quad 2}{\quad} \quad \frac{+1- \quad ^2}{2} \quad \frac{\times \quad 2}{\quad}$$

Where,

N = Sample size

$_{1-\alpha/2}$ = 95% confidence interval)

Standard normal deviate for Type 1 error (taken as 1.96 for

$1-\beta$ 90% study power)

= Standard normal deviate for Type 2 error (taken as 1.28 for

= Pooled standard deviation (taken as 19.86 as per Colaco et al[1])

d =Minimum expected significant difference in UA/CR ratio among study groups (taken as 14.46 as per Colaco et al[1])

Time frame to address the study:

September 2016 - March 2018.

Inclusion criteria:

Babies fulfilling the following criteria were included in the case group:

1. Term babies (Gestational age of 37 to less than 42 completed weeks)
2. Average for gestational age babies
3. APGAR score <7 at 1 min of life or
4. Normal respiration not established at 1 min after birth or
5. Parents of babies who gave consent for participation of their child in the study.

Gestational age was estimated by LMP and/or New Ballard Scoring System **(Annexure II)** and Fenton growth charts **(Annexure III)** were used for AGA estimation.

Exclusion criteria:

Following newborn babies were excluded from the study-

1. Preterm (<37 weeks of gestation)
2. Post term babies (42 completed weeks or more)

3. Rh Incompatibility
4. Intrauterine growth restricted (IUGR) babies
5. Babies of diabetic and hypertensive (PIH) mother.
6. Babies of mother who had history of
   - Maternal smoking
   - Antepartum hemorrhage
   - Chorioamnionitis
7. Babies with congenital malformations
8. Babies with Chromosomal abnormalities
9. Parents of babies who did not gave consent for participation of their child in the study.

Methodology:

Babies who met all inclusion criteria, after written consent (**Annexure-IV**) from the parents, were recruited for the study. The controls were selected as the next baby delivered after the birth of an asphyxiated newborn.

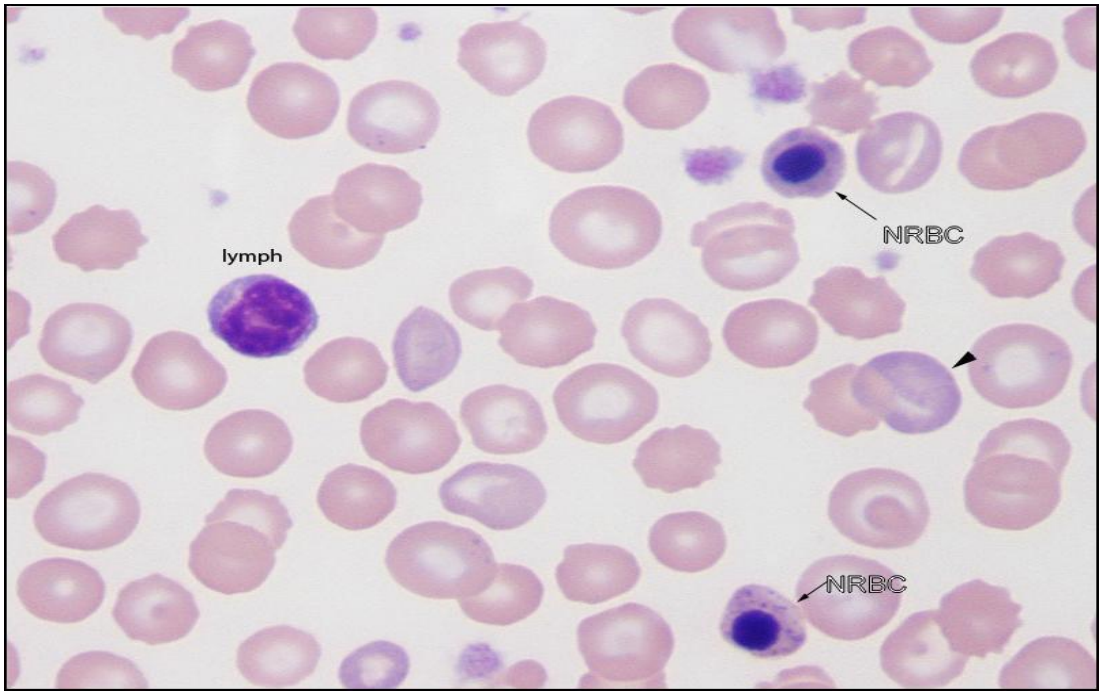
Procedure of cord blood sample

Immediately after birth, 2 ml of cord blood was collected in EDTA vial from placental side of cut cord by milking method, and was send to laboratory for analysis.

Counting of NRBC's:

Samples were processed and analyzed by same blinded pathologist. The blood from EDTA vials was processed by Sysmex XN 1000 Version 6 PART analyzer for obtaining total white blood cells (WBC's), platelet count and

haemoglobin. Thin blood film was prepared on glass slide and stained by Leishman's stain. Absolute NRBC count and NRBC count per 100 white blood cells (WBC) were done manually. Nucleated RBCs and leucocytes were counted till 500 white blood cells (WBCs) and then was reported as NRBC/100 WBCs.



NRBC Microscopic View

(Nucleated red blood cells (NRBC'S or normoblasts) represents the stages of a red blood cell before it matures. The average size of the normoblast is 7-12 µm in diameter. The cytoplasm is pink. The nucleus is pyknotic (a homogeneous blue black mass with no structure).

Assessment of severity of asphyxia:-

The assessment of severity of asphyxia was done by APGAR score and HIE stages.

APGAR Score [18]

The APGAR score is a practical method of systematically assessing newborn immediately after birth with the help of five parameters.

SIGN	**0**	**1**	**2**
Heart rate	Absent	Below 100	Over 100
Respiratory effort	Absent	Slow, irregular	Good, crying
Muscle tone	Limp	Some flexion of extremities	Active motion
Response to catheter in nostril (tested after oropharynx is clear)	No response	Grimace	Cough or sneeze
Color	Blue, pale	Body pink, extremities blue	Completely pink

Birth Asphyxia severity by APGAR score - Birth Asphyxia and its severity were assessed as per criteria given by NNPD Network.[5]

Birth Asphyxia : APGAR score of less than 7 at 1 minute of age.

Moderate Asphyxia : Slow gasping breathing or an APGAR score of 4-6 at 1 min of age.

Severe Asphyxia : No breathing or an APGAR score of 0-3 at 1 min of age.

Hypoxic Ischemic Encephalopathy (HIE) - HIE is the term used to describe neonatal encephalopathy following perinatal asphyxia in newborns.

Sarnat and Sarnat described HIE stages, stated that, with increase in severity of asphyxia stages of HIE increases.[62]

Sarnat and Sarnat staging of HIE [62]:

SIGNS	**STAGE I**	**STAGE II**	**STAGE III**
Level of Consciousness	Hyperalert	Lethargic	Stuporous, Coma
Muscle tone	Normal	Normal	Normal
Posture	Normal	Flexion	Decerebrate
Tendon reflexes/ Clonus	Hyperactive	Hyperactive	Absent
Myoclonus	Present	Present	Absent
Moro reflex	Strong	Weak	Absent
Pupils	Mydriasis	Miosis	Unequal, Poor light reflex
Seizures	None	Common	Decerebration
EEG	Normal	Low voltage changing to	Burst suppression to isoelectric
		Seizure	
		Activity	
Duration	<24 hrs	24 hrs – 14 Days	Days to weeks
Outcome	Good	Variable	Death, Severe Deficits

Immediate outcomes - All the cases were observed for immediate outcomes during the first 24 hours of life and were categorised as neurologically normal or abnormal.

Neurologically normal - Babies who had normal tone and posture, were free from seizures, had good cry, activity and normal neonatal reflexes.

Neurologically abnormal - Babies who had an abnormal tone and posture or poor cry and activity or any abnormal neonatal reflexes at discharge or died.

Hospitalization

After taking relevant history and examination of all the enrolled newborns, only cases (asphyxiated) were admitted in NICU for further monitoring and screening for development of HIE. Controls were kept with their mother. The cases were observed for immediate outcome. Daily progress of cases were recorded on prestructured proforma (**Annexure-I**). The HIE and other complications detected during the hospital stay were managed as per standard protocols. All the cases were followed up till the discharge or death.

Statistical methods:

Categorical / Nominal data was expressed as number and percentage and were analyzed using Chi square test / Fischer exact test as applicable. Continuous data was expressed as mean and standard deviation and difference in mean was analyzed using unpaired t test for two group comparison. One way ANOVA test was used for more than 2 group comparison. Ordinal data was expressed as median and range and was analyzed using Mann Whitney U test. Correlation between two variables was determined using Spearman rank order correlation coefficient. A P value < 0.05 was taken as statistically significant. All statistical analysis was done using statistical software Epi info version 7.2.1.0.

Observation and Results

OBSERVATION AND RESULTS

The present study was done in NICU with active collaboration of labour room and OT, at SDM Hospital, Jaipur. Total 150 newborn babies (75 cases and 75 controls) were enrolled for the study.

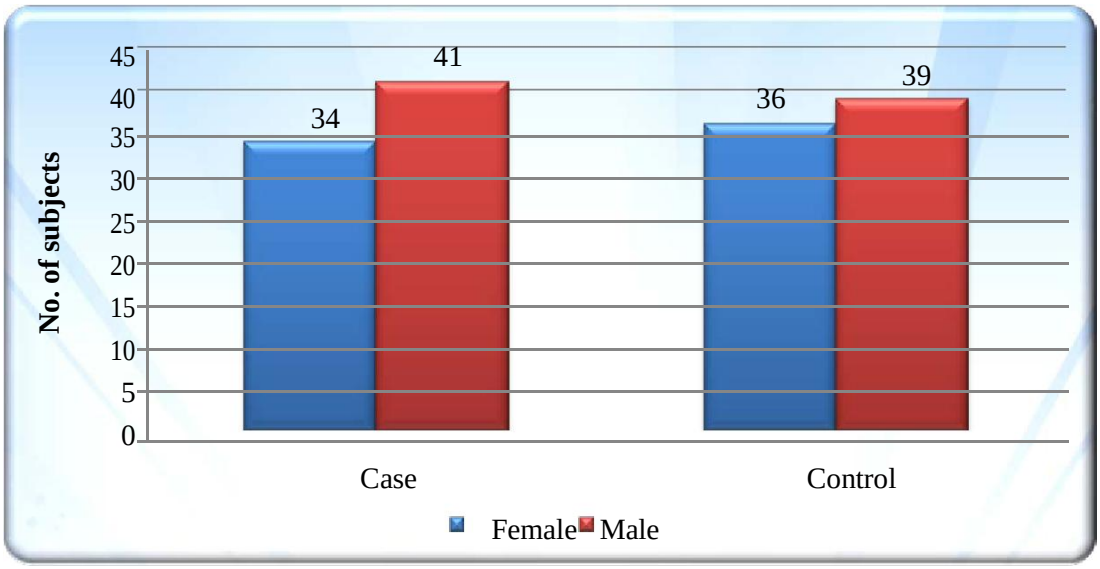
The following observations were made.

Table 1: Gender Distribution of study Groups

Gender	Case		Control		Total	
	N	%	N	%	N	%
Female	34	45.3	36	48	70	46.7
Male	41	54.7	39	52	80	53.3
Total	75	100	75	100	150	100

Chi-square = 0.027 with 1 degree of freedom; P = 0.870 (NS)

The above table shows the gender distribution of study groups. In asphyxia cases males (54.7%) were more than females subjects (45.3%), similarly in controls group males (52%) were more than females (48%). Application of chi square test showed that this difference was not statistically significant and both groups were comparable in relation to their gender distribution (P>0.05).

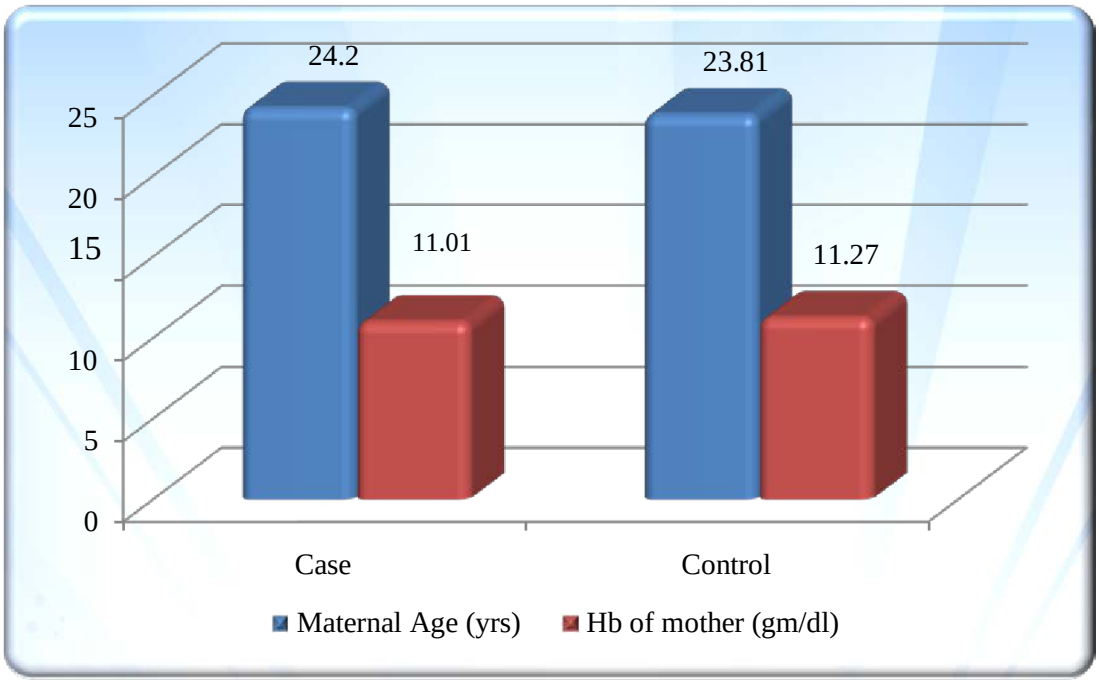


Graph 1: Gender Distribution of study Groups

Table 2: Maternal characteristics of study group

Variable	Case	Control	P Value
Maternal Age (yrs)	24.20 ± 2.62	23.81 ± 1.9	0.302
Hb of mother (gm/dl)	11.01 ± 1.03	11.27± 1.02	0.111

The above table demonstrates that, the mean maternal age was comparable among cases (24.2 years) and controls (23.81 years) groups (P=0.334). The mean maternal haemoglobin among cases (11.01 gm/dl) was not significantly different from controls (11.27 gm/dl). Thus the maternal characteristics were similar among both groups.



Graph 2: Maternal characteristics of study group

Table 3: Mode of delivery and presentation among study Groups

Variable	Sub group	Case		Control		Total		P value
		N	%	N	%	N	%	
Mode of delivery	**LSCS**	45	60	10	13.3	55	36.7	<0.001 (S)
	NVD	30	40	65	86.7	95	63.3	
Presentation	**Breech**	15	20	8	10.7	23	15.3	<0.001 (S)
	Vertex	60	80	67	89.3	127	84.7	

The above table depicts the mode of delivery and presentation among study groups. Most of the asphyxiated cases were delivered by LSCS (60%). In controls group, most subjects delivered by normal vaginal route (86.7%) and only 13.3% delivered by LSCS. This difference in mode of delivery was found to be statistically significant (P<0.001). Higher number of LSCS delivery in asphyxiated babies group can be explained by the fact that this mode of delivery was chosen to prevent further hypoxia in these babies as they already had signs of fetal distress.

Most of the cases (80%) as well as controls (89.3%) had vertex presentation and only 20% of cases and 10.7% of control subjects had breech presentation. This difference in presentation among the two groups was not found to be statistically significant (P>0.05).

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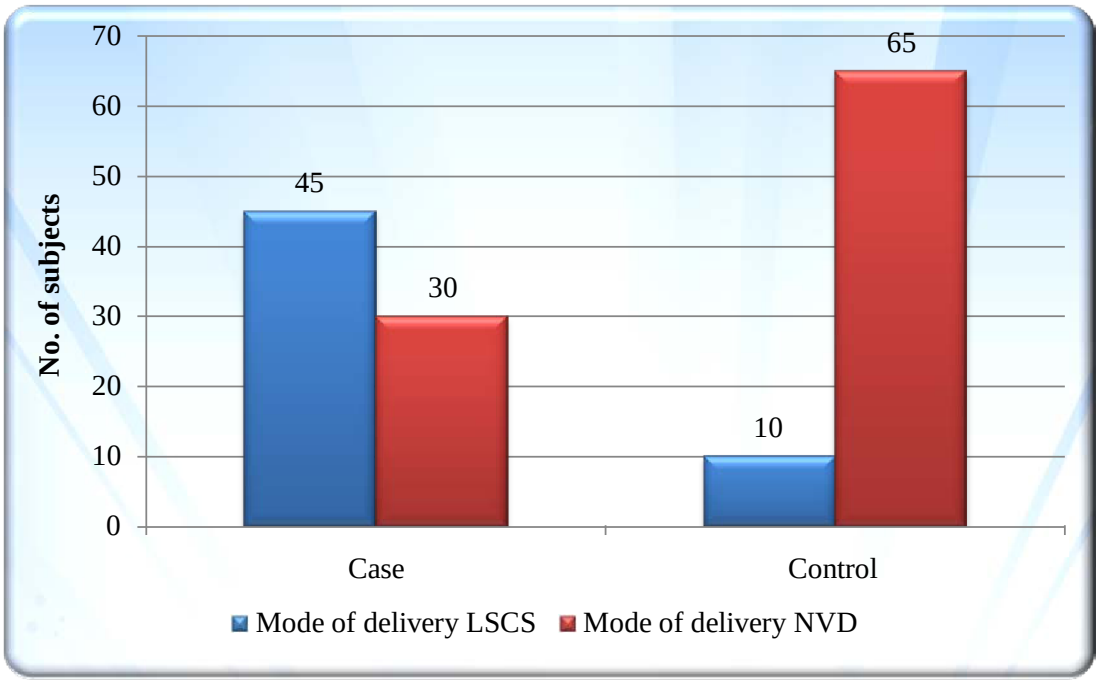
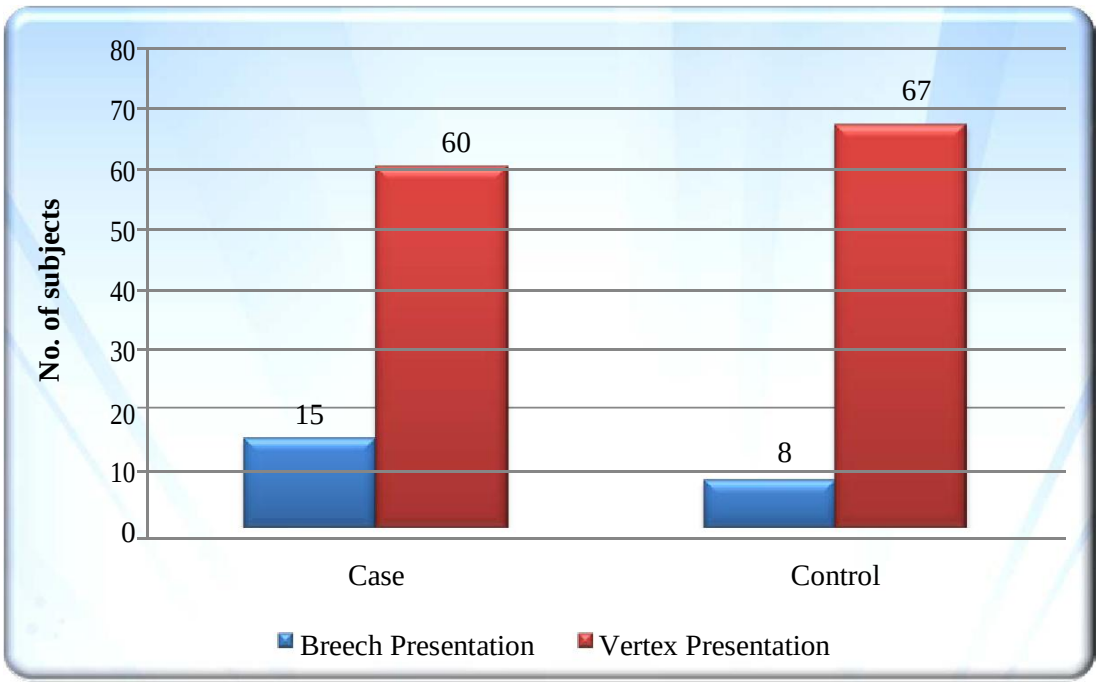


Graph 3(A): Mode of delivery among study Groups



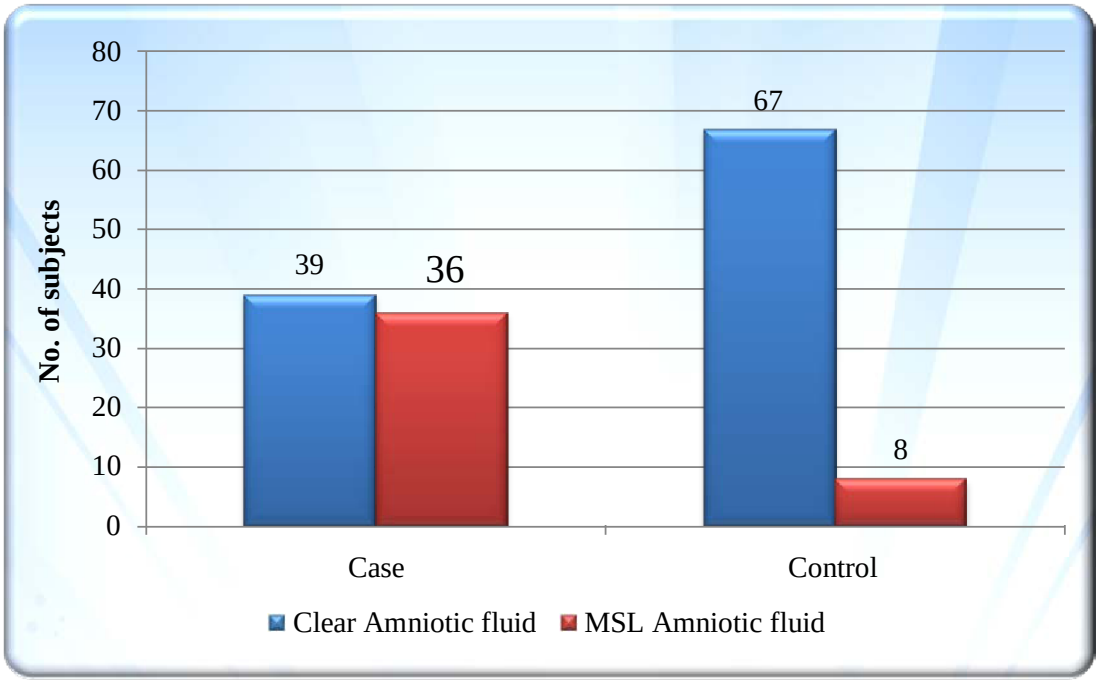
Graph 3(B): Presentation among study groups

Table 4: Type of Amniotic fluid among study Groups

Amniotic fluid	Case		Control		Total	
	N	%	N	%	N	%
Clear	39	52.0	67	89.3	106	70.7
MSL	36	48.0	8	10.7	44	29.3
Total	75	100	75	100	150	100

Chi-square = 23.446 with 1 degree of freedom; P < 0.001 (S)

The above table shows the type of amniotic fluid among study subjects. 48% of asphyxiated cases had MSL and rest 52% cases had clear amniotic fluid. Most of the control subjects (89.3%) had clear amniotic fluid and only 10.7% controls had MSL. This difference was found to be statistically significant on Chi square test application (P<0.001). This is probably because of passage of meconium due to fetal distress in asphyxiated babies.



Graph 4: Type of Amniotic fluid among study Groups

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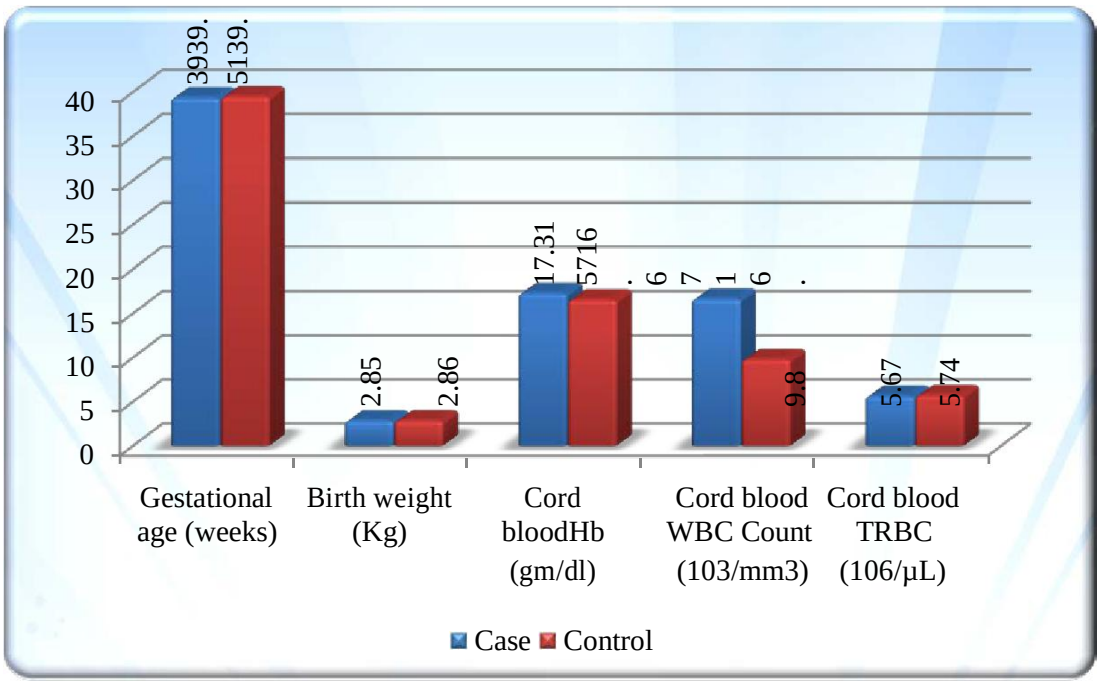
Table 5: Clinical characteristics of study group

Variable	Case	Control	P Value
Gestational age (weeks)	39.39 ± 0.96	39.51 ± 0.84	0.417
Birth weight (Kg)	2.85±0.30	2.86±0.31	0.804
Cord bloodHb (gm/dl)	17.31 ± 2.0	16.57 ± 1.20	0.007 (S)
Cord blood WBC Count (10^3/mm^3)	16.67 ± 5.5	9.8 ± 2.8	<0.001 (S)
Cord blood TRBC (10^6/µL)	5.67 ± 0.63	5.74 ± 0.61	0.499

The above table depicts that the mean gestational age among cases (39.39 weeks) and controls (39.51 weeks) was not significantly different (p>0.05). The mean birth weight was comparable among cases (2.85 Kg) and controls (2.86 Kg) (P=0.804). The Hemoglobin level was significantly higher among asphyxiated cases (17.31gm/dl) as compared to control (16.57gm/dl) group (P<0.05). The WBC count was also found to be significantly higher among cases (16.67 x 10^3/mm^3) as compared to controls (9.8 x 10^3/mm^3). The TRBC count was not found to be significantly different between cases (5.67 x 10^6/µL) and controls (5.74 x 10^6/µL).



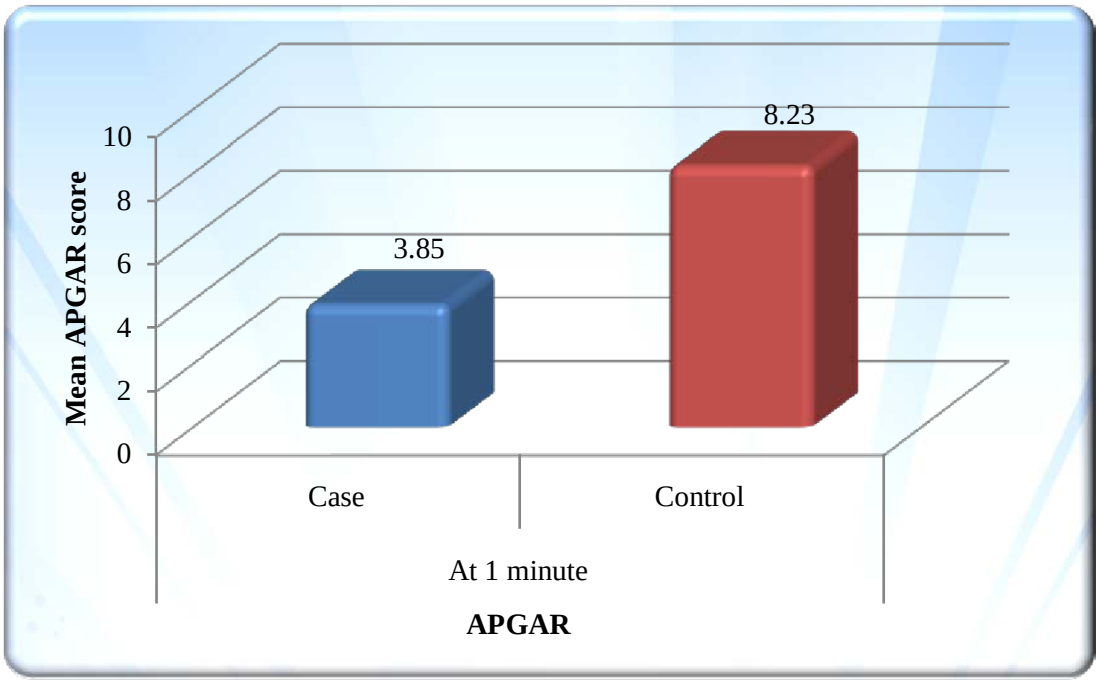
Graph 5: Clinical characteristics of study group

Table 6: Mean APGAR score at 1 minute among study groups

Time	Group	N	Mean	SD	Median	Range	P value*
At 1 minute	**Case**	75	3.85	1.48	4	1 – 6	<0.001 (S)
	Control	75	8.23	0.67	8	7 – 9	

*P value calculated using Mann Whitney rank sum test

This table reveals the APGAR score of study groups at one minute.The median APGAR score at one minute among asphyxia cases was lower (3.85±1.48) as compared to control group (8.23±0.67), and this difference was found to be statistically significant (P<0.001).



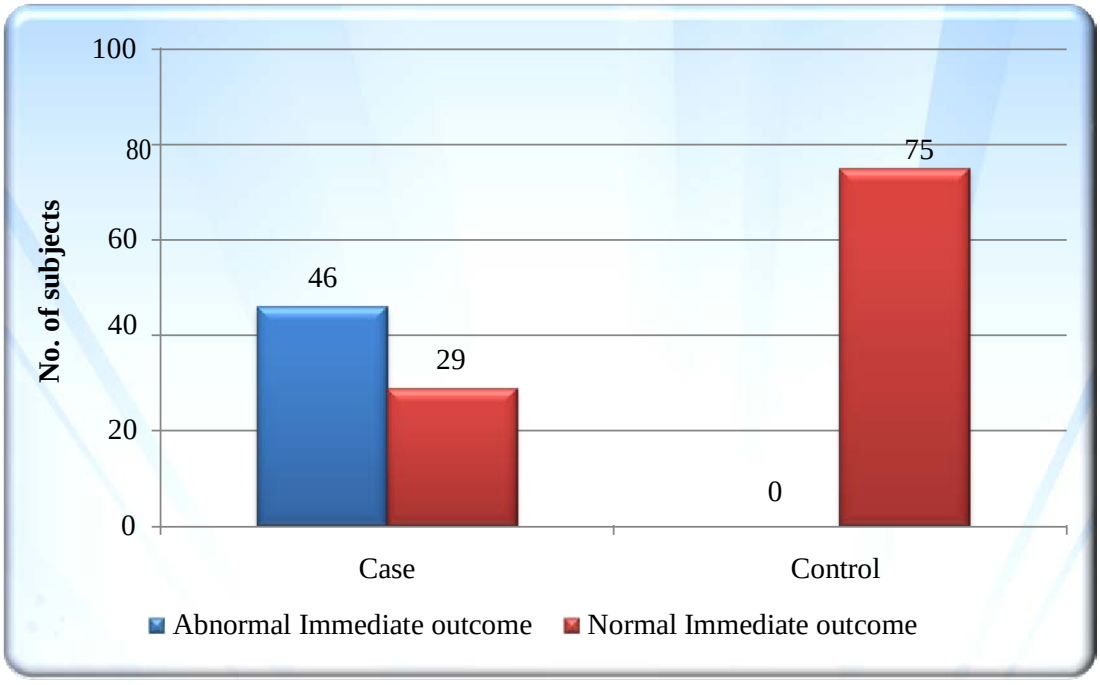
Graph 6: Mean APGAR score at 1 minute among study groups

Table 7: Immediate neurological outcome among study groups

Immediate outcome	Case		Control		Total	
	N	%	N	%	N	%
Abnormal	46	61.3	0	0	46	30.7
Normal	29	38.7	75	100	104	69.3
Total	75	100	75	100	150	100

Chi-square = 63.493 with 1 degree of freedom; P < 0.001 (S)

This table shows that most of the asphyxia cases had abnormal neurological outcome (61.3%) and only 29 (38.7%) cases had normal neurological outcome. All the control subjects had normal immediate neurological outcome. This difference in immediate outcomes among cases and control group was found to be statistically significant (P<0.001). Two babies died within 24 hr and they were included in abnormal group.

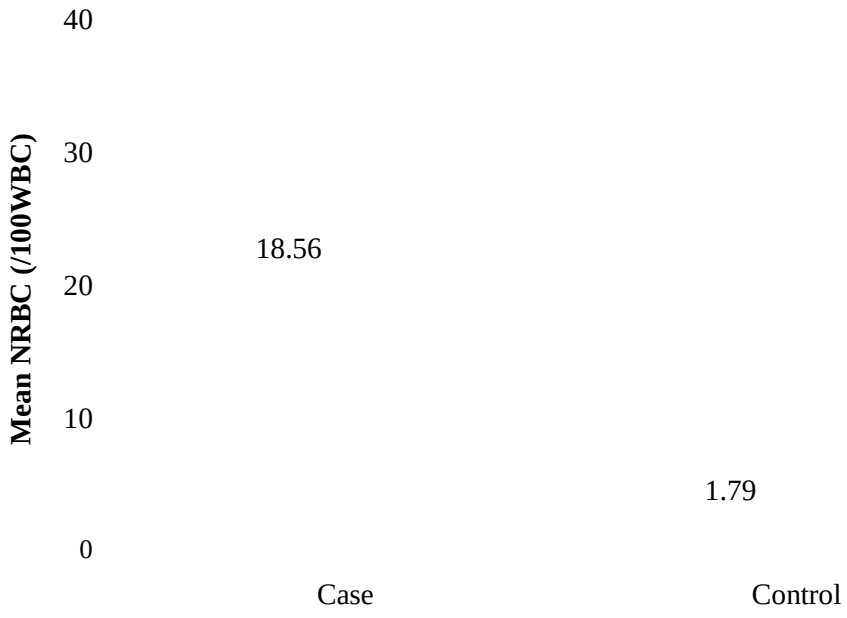


Graph 7: Immediate neurological outcome among study groups

Table 8: Mean NRBC count (per 100 WBC) among study groups

Group	N	Mean	SD	Range
Cases	75	18.56	11.51	5 – 64
Control	75	1.79	1.53	0 – 11
t= 12.509 at 148 degree of freedom; p<0.001 (S)				

This table depicts the NRBC count among study groups.The mean NRBC count among asphyxia cases (18.56/100WBC) was higher as compared to control group (1.79/100 WBC), and this difference was found to be statistically significant (P<0.001). This shows that NRBC count increases with perinatal asphyxia.



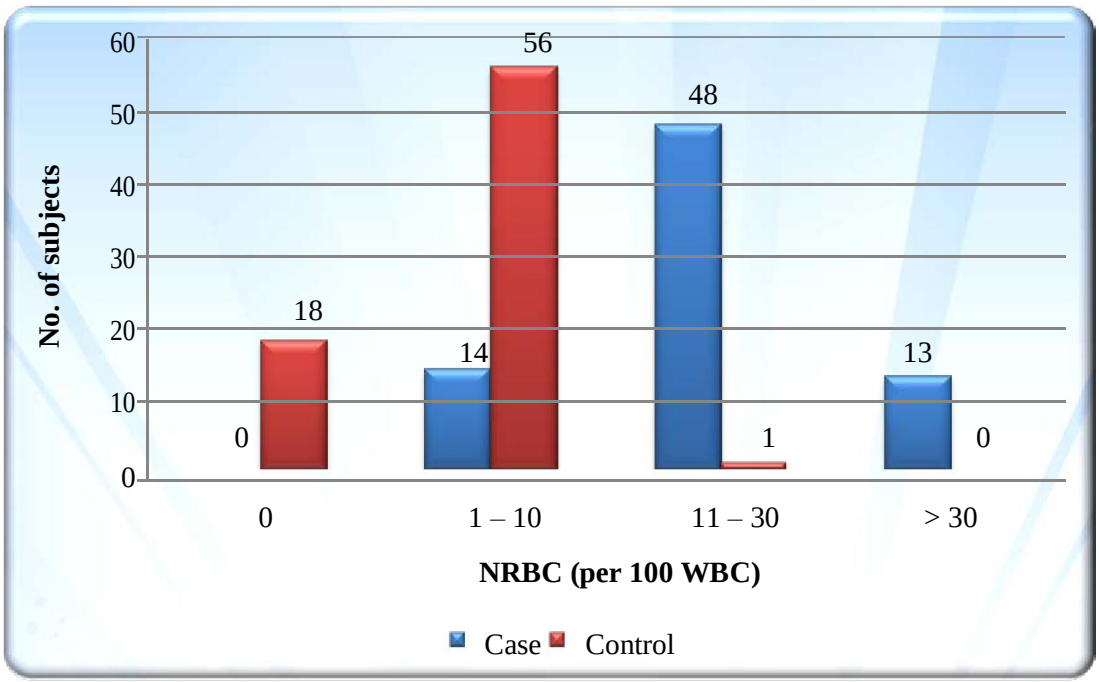
Graph 8: Mean NRBC count (per 100 WBC) among study groups

Table 9: NRBC (per 100 WBC) range among study groups

NRBC (per 100 WBC)	Case		Control		Total	
	N	%	N	%	N	%
0	**0**	0.0	**18**	24	**18**	12.0
1 – 10	14	18.7	56	74.7	70	46.7
11 – 30	48	64	1	1.3	49	32.6
> 30	13	17.3	0	0.0	13	8.7
Total	75	100	75	100	150	100

Chi-square = 101.282 with 3 degrees of freedom; P < 0.001 (S)

This table shows that most of the asphyxia cases (64%) had NRBC in range of 11-30 NRBC/100 WBC followed by in 1-10 NRBC/100 WBC (18.7%) range. 17.3% cases had>30 NRBC/100 WBC. Most of the controls (74.7%) had NRBC count in range of 1-10, while 24% controls did not showed any NRBC and only one control had NRBC count in range of 11-30. This difference in NRBC range among case and control groups was found to be statistically significant (P<0.001).

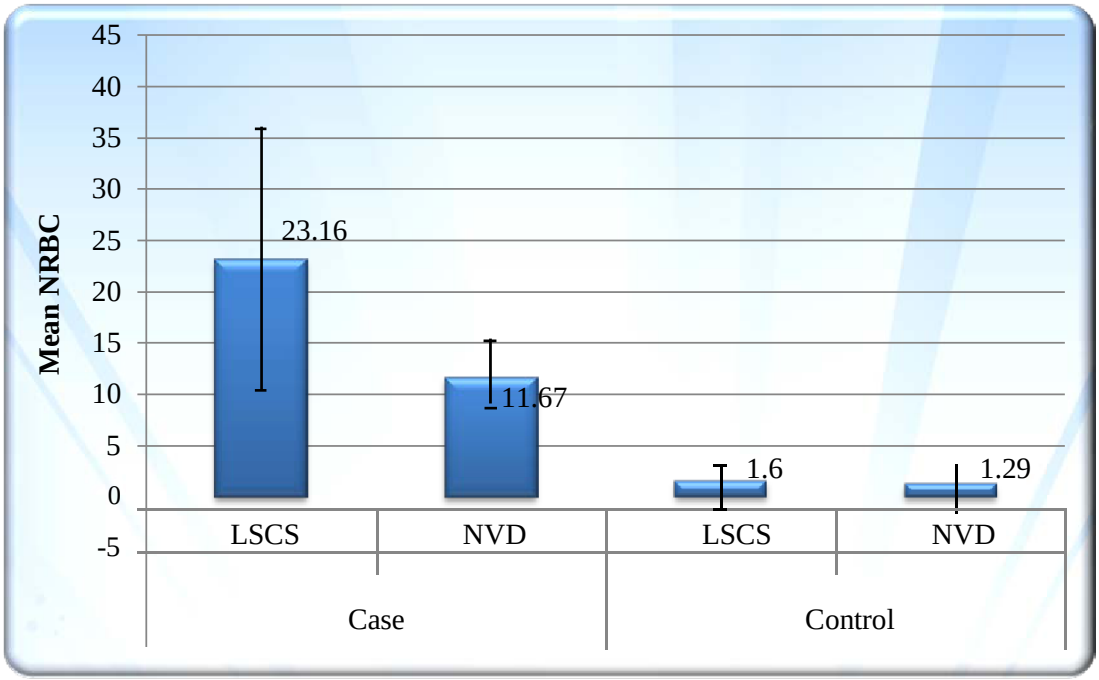


Graph 9: NRBC (per 100 WBC) range among study groups

Table 10: Mean NRBC count with type of delivery in study groups

Type of Delivery	NRBC count in Cases		NRBC count in Controls	
	Mean	SD	Mean	SD
LSCS	23.16	12.77	1.6	1.78
NVD	11.67	2.99	1.29	2.44
P value	<0.001 (S)		0.703 (NS)	

Above table reveals that the mean NRBC count was significantly higher in asphyxia cases delivered by LSCS (23.16/100WBC) as compared to cases delivered by NVD (11.67/100WBC) i.e. NRBC was significantly associated with type of delivery among asphyxia cases. Among controls, the mean NRBC count was not found to be associated with type of delivery (P=0.703).

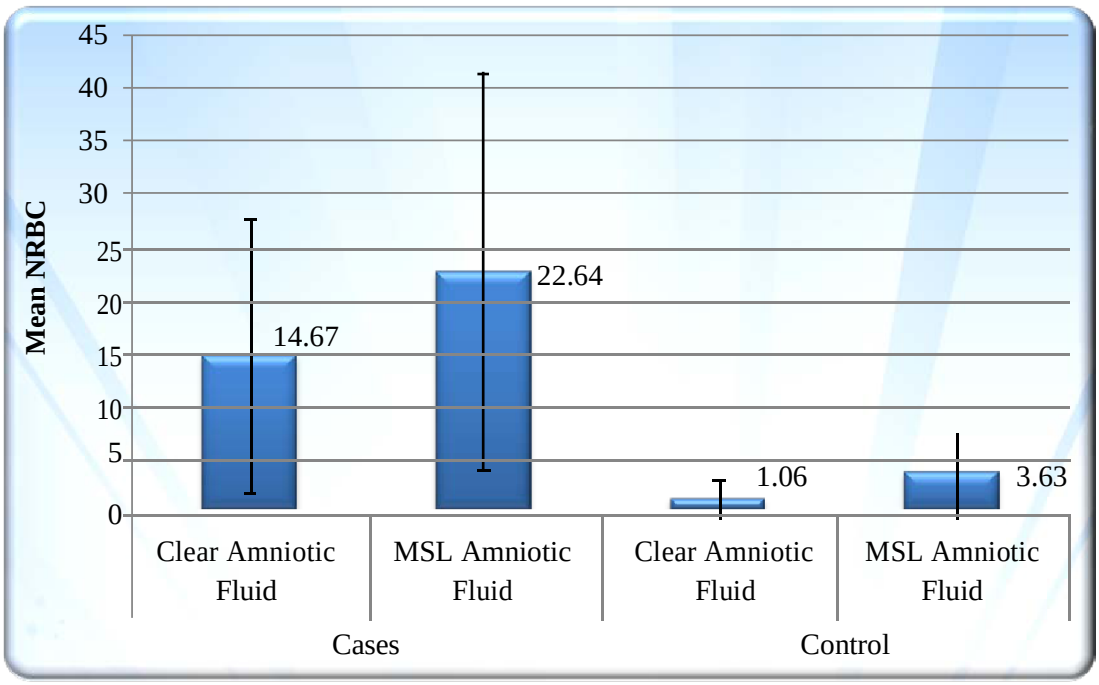


Graph 10: Mean NRBC count with type of delivery in study groups

Table 11: Mean NRBC count in relation to type of amniotic fluid

Type of Amniotic Fluid	NRBC count in Cases		NRBC count in Controls	
	Mean	SD	Mean	SD
Clear	14.67	12.86	1.06	1.95
MSL	22.64	18.69	3.63	3.99
P value	0.034 (S)		0.003 (S)	

This table depicts that the mean NRBC count was significantly higher in asphyxia cases with MSL (22.64/100WBC) as compared to cases with clear amniotic fluid (14.67/100WBC). Similarly the mean NRBC count was significantly higher in controls with MSL (3.63/100WBC) as compared to controls with clear amniotic fluid (1.06/100WBC) i.e. NRBC count was significantly associated with type of type of amniotic fluid among both asphyxia cases (P=0.034) and controls (P=0.003).



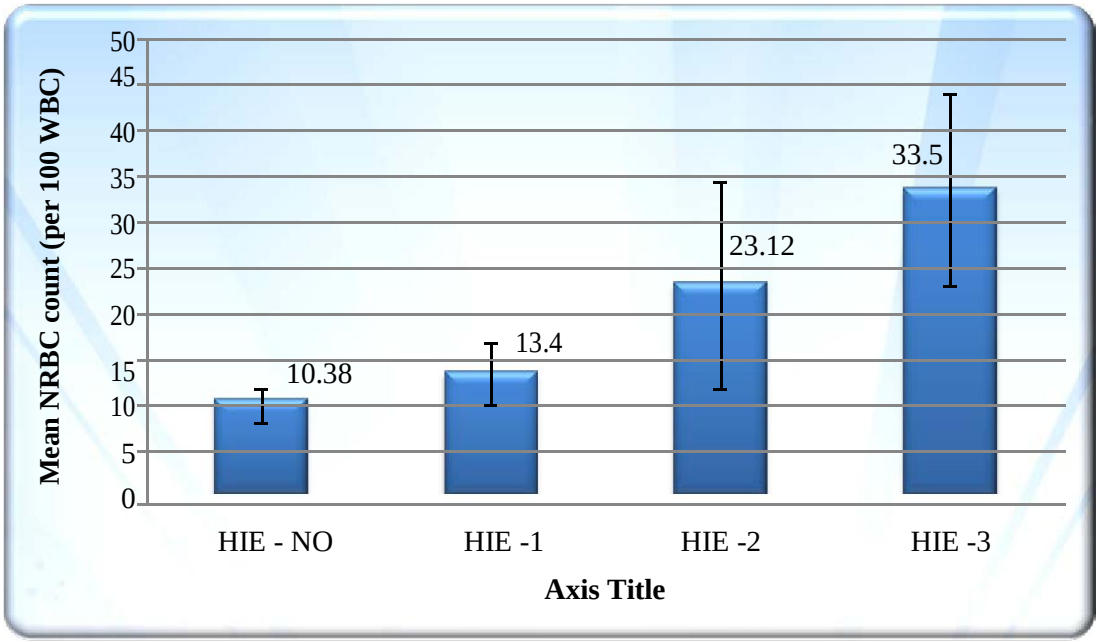
Graph 11: Mean NRBC count in relation to type of amniotic fluid

Table 12: Mean NRBC in Asphyxia cases according to HIE stage

Birth asphyxia	HIE Staging	N	Mean NRBC count	Std. Deviation
Without HIE	NO	29	10.38	2.37
With HIE	I	10	13.4	3.31
	II	24	23.12	11.34
	III	12	33.5	10.48

ANOVA test: F = 28.83 at total 74 degree of freedom; P<0.001 (S)

Above table shows that the mean NRBC was highest among cases with HIE III (33.5/100 WBC) followed by HIE II cases (23.12/100 WBC) and HIE I cases (13.4/100WBC). NRBC count was minimum in cases with no HIE (10.38/100 WBC). This difference in NRBC count in relation to HIE stage among cases was found to be statistically significant (P<0.001). Post hoc Tukey test revealed NRBC count was significantly higher in HIE III versus all other stages (P<0.05). This shows that NRBC counts increases with severity of asphyxia.

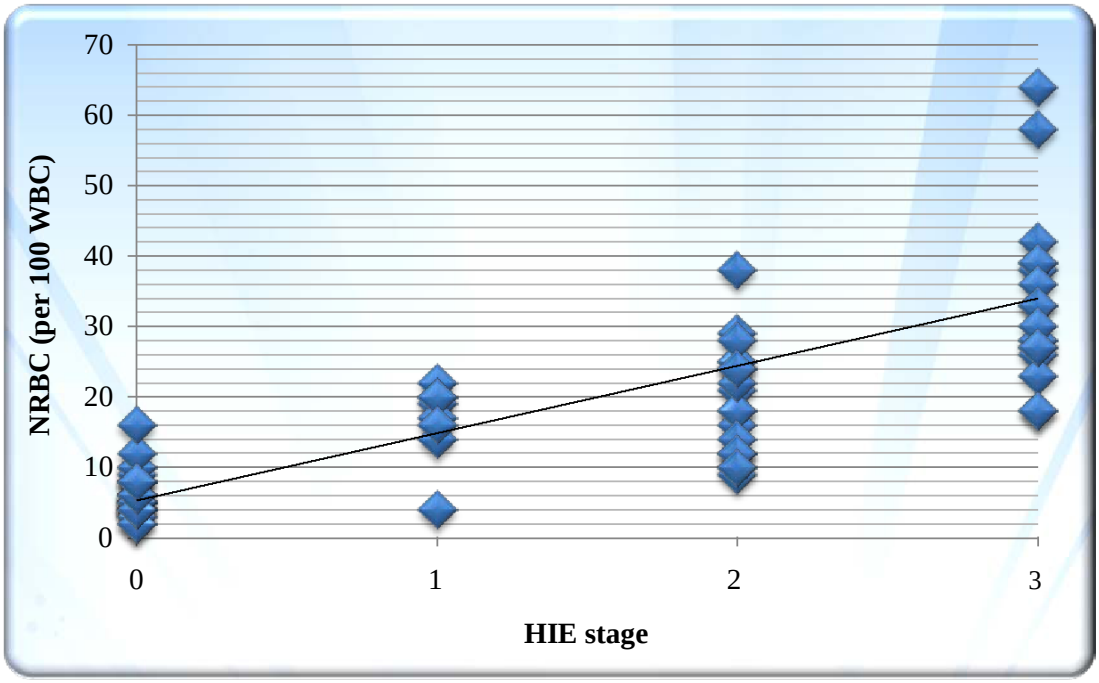


Graph 12: Mean NRBC in Asphyxia cases according to HIE stage

Table 13: Correlation between NRBC and HIE stage of asphyxia cases

Spearmann correlation coefficient	0.833
P value	0.001 (S)

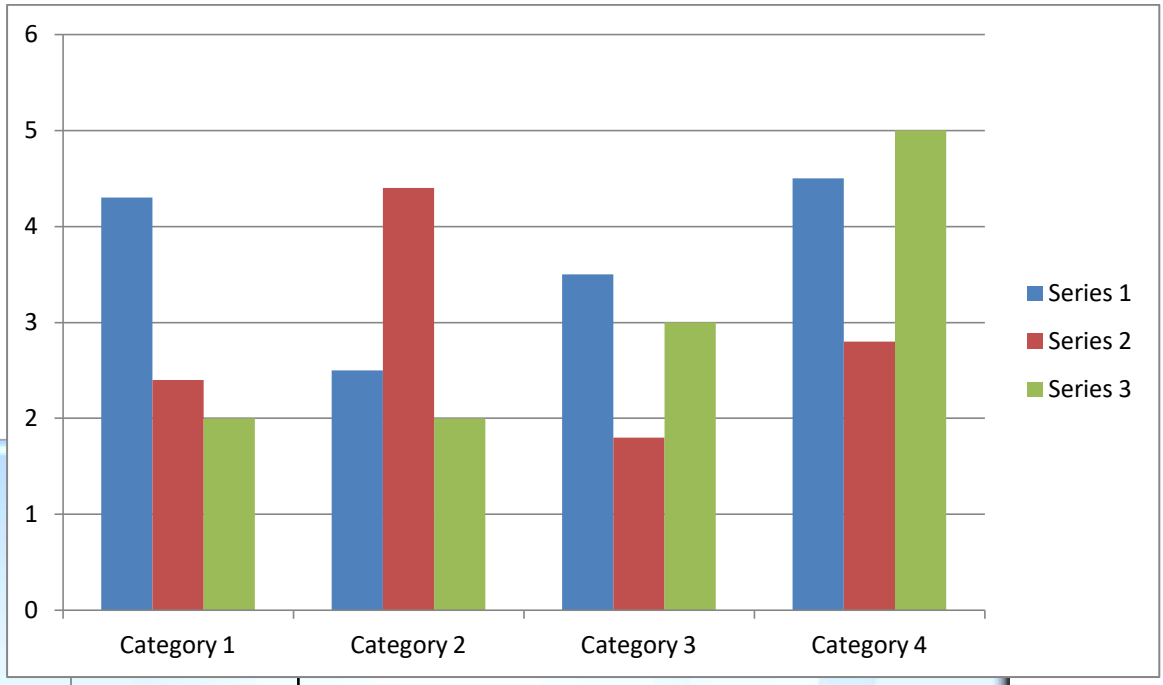
Above table reveals the correlation between NRBC and HIE stage among asphyxia cases. The spearman correlation coefficient was calculated to be 0.833 indicating strong positive correlation between NRBC and HIE (P=0.001) i.e. NRBC increases significantly with increases in HIE stage.



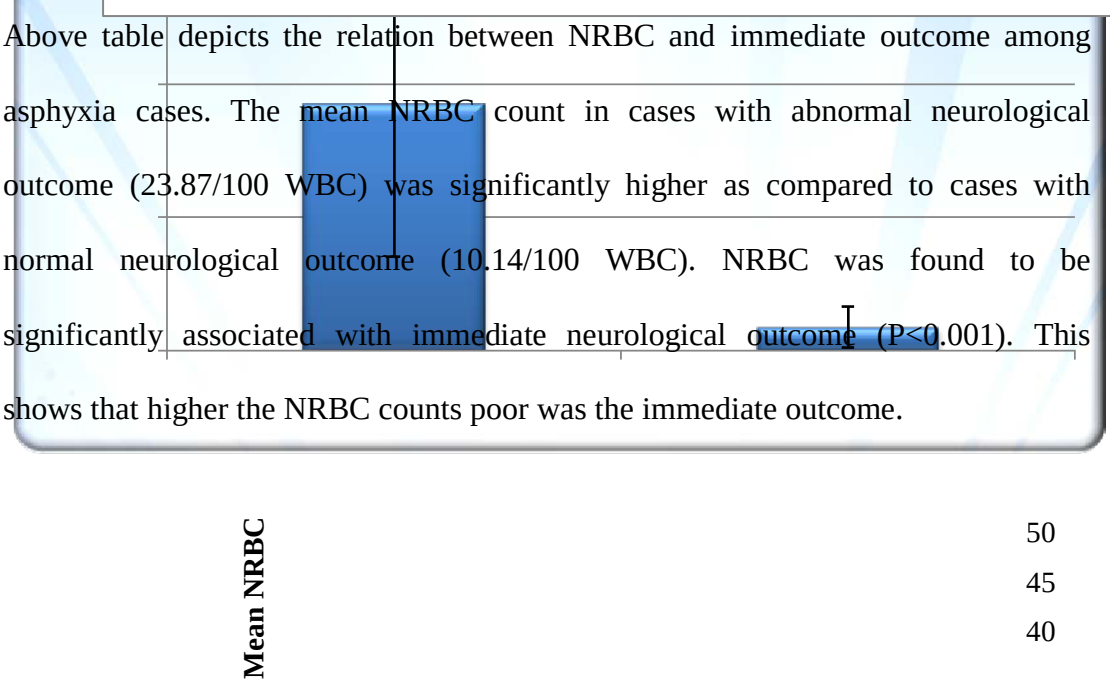
Graph 13: Correlation between NRBC and HIE stage of asphyxia cases

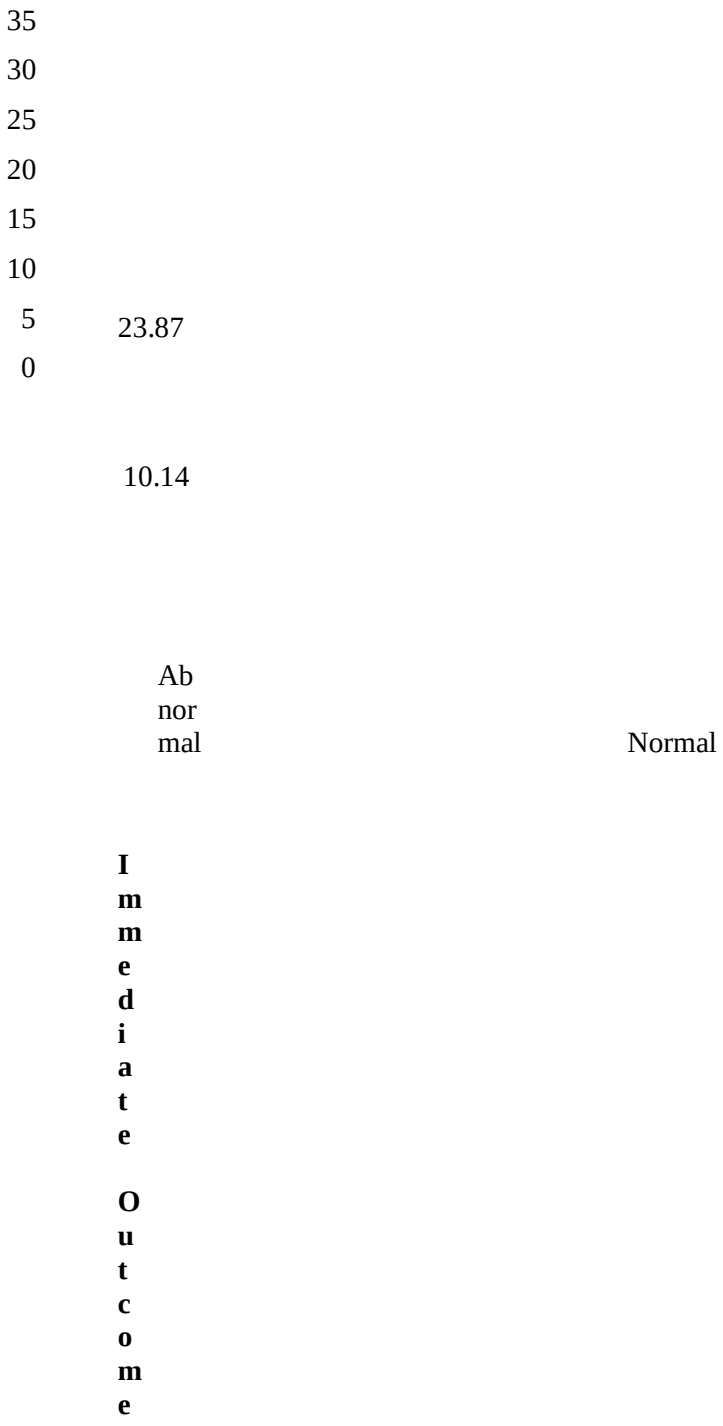
Table 14: Mean NRBC (per 100 WBC) in asphyxia cases in relation to Immediate outcome

Immediate outcome	N	Mean	SD	Median	Range
Abnormal	46	23.87	11.82	21	12 – 64
Normal	29	10.14	2.45	5	5 – 14
t = 6.160 with 73 degrees of freedom; P < 0.001 (S)					



Above table depicts the relation between NRBC and immediate outcome among asphyxia cases. The mean NRBC count in cases with abnormal neurological outcome (23.87/100 WBC) was significantly higher as compared to cases with normal neurological outcome (10.14/100 WBC). NRBC was found to be significantly associated with immediate neurological outcome (P<0.001). This shows that higher the NRBC counts poor was the immediate outcome.



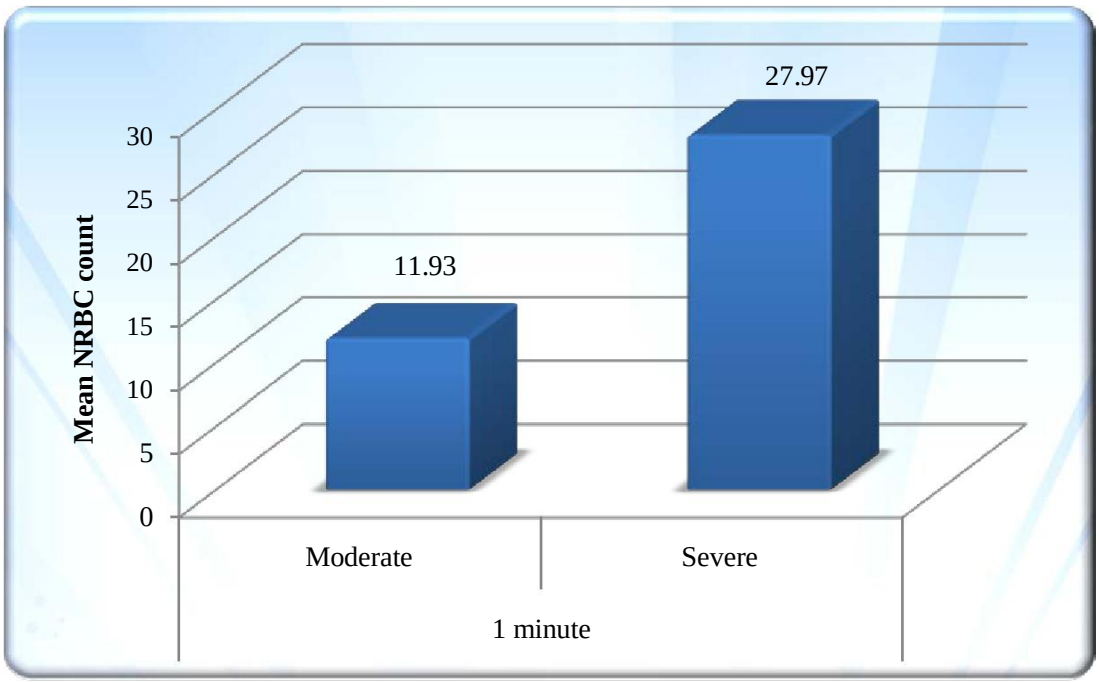


Graph 14: Mean NRBC in asphyxia cases in relation to immediate outcome

Table 15: Mean NRBC count in relation to severity of asphyxia

Time	Asphyxia	N	Mean ± SD	P value
1 minute	**Moderate (APGAR score-4 to 6)**	44	11.93 ± 3.99	<0.001 (S)
	Severe (APGAR score-0 to 3)	31	27.97 ± 12.17	

Above table shows that the mean NRBC was higher among asphyxia cases that had severe asphyxia at one minute (27.97 ± 12.17) as compared to those with moderate asphyxia (11.93± 3.99). NRBC count was found to be significantly associated with severity of asphyxia at one minute (P<0.001).



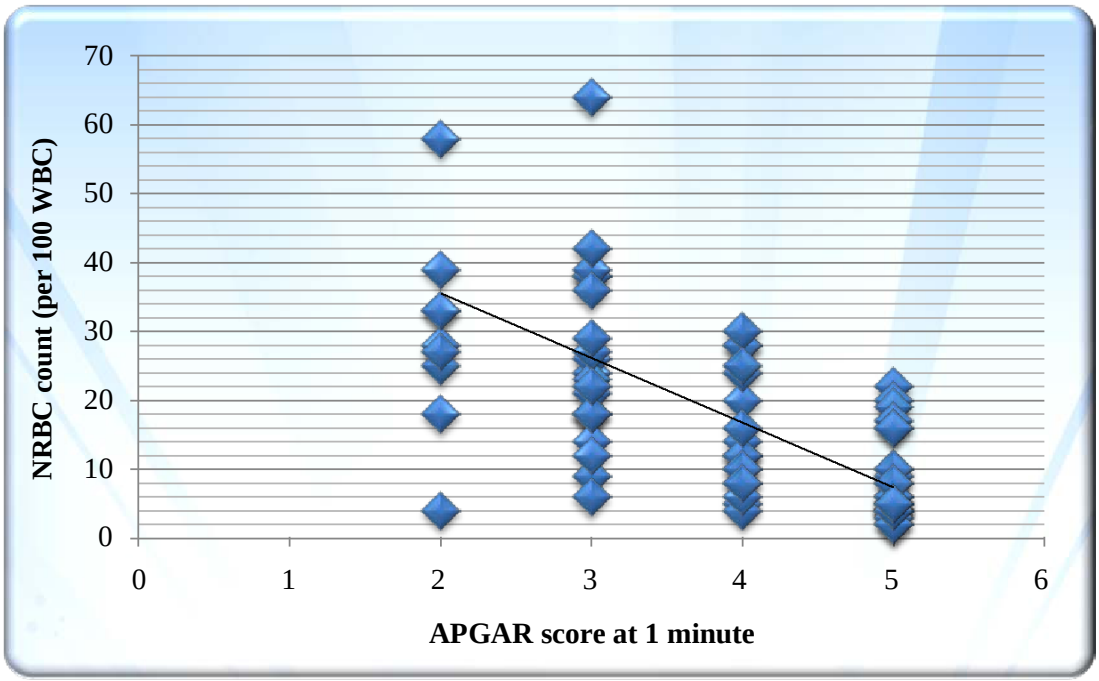
Graph 15: Mean NRBC count in relation to severity of asphyxia

Table 16: Correlation of NRBCs with APGAR score at 1 minutes among asphyxiated cases

APGAR score	ρ value*	p value
APGAR at 1 min	- 0.774	<0.001 (S)

*Spearman correlation coefficient

Above table reveals the correlation between NRBC and APGAR score among asphyxia cases. The spearman correlation coefficient was calculated to be -0.774 for APGAR score at one minute indicates strong negative correlation between NRBC and APGAR score (P<0.001) i.e. NRBC decreases significantly with increasing APGAR score and vice versa.



Graph 16: Correlation of NRBCs with APGAR score at 1 minutes among asphyxiated cases

Discussion

DISCUSSION

The present study was conducted at inborn NICU, Department of Pediatrics, Santokba Durlabhji Hospital, Jaipur. It was a hospital based observational prospective case control study conducted between the period of September 2016 to March 2018.

This study was carried out to evaluate the relation of the cord blood NRBC/100 WBC in predicting the severity of perinatal asphyxia and its immediate outcome.

For this total 150 newborns were enrolled for the study who delivered in labor room and OT, at Santokba Durlabhji Hospital, Jaipur. Out of these babies 75 were enrolled as cases and 75 as controls.

Table 1 shows gender distribution of study groups. In present study in asphyxia cases 54.7 % were males while 45.3 % were females. In control group males (52%) were also more than females (48%). This difference in gender was not statistically significant and both groups were comparable in relation to their gender composition (P >0.05). This sex distribution was similar to study done by **Mohanty et al (2014)** [19] where males and females were 56% and 44% respectively in asphyxiated group and males were 58% and females were 42% in non asphyxiated group.

Table 2 indicates maternal characteristics of the study groups. In this study we studied various maternal clinical parameter in cases and control groups. These

parameters were mean Maternal age and mean Hb of mother. Both these clinical variables were comparable in both the groups without significant difference.

Table 3 shows mode of delivery amongst study groups. In our study, amongst the babies enrolled as cases, 60 % were born by caesarian section (LSCS) and 40 % by normal vaginal delivery (NVD). Where as in controls, 13% babies were born by LSCS and 87% by NVD, so delivery via LSCS was more in number in cases as compared to controls, where as NVD was the main delivery mode in control group. This difference in mode of delivery was found to be statistically significant ($P< 0.001$). Similar results were seen in the study of **Mohanty et al (2014)** [19], where among asphyxiated babies 54% of were born by LSCS and 46% by NVD, and in non asphyxiated babies LSCS was required for 19% babies and 81% of them were born by NVD. **Chavan et al (2018)** [61] reported 46% of the asphyxiated babies (cases) delivered by normal vaginal route compared to 70% of the babies in non asphyxiated (controls) group and LSCS was noted in 38% and 28% of the babies in cases and control groups respectively suggesting high incidence of LSCS in perinatal asphyxia group. **Fern et al (2004)** [51] also found high incidence of emergency LSCS (39.28%) in perinatal asphyxia group, whereas spontaneous vaginal delivery in all controls. In present study most of the non asphyxiated babies delivered vaginally and LSCS was the mode of delivery in most of asphyxiated babies. It may be because LSCS was chosen as a mode of delivery to prevent further hypoxic damage to the fetus which already had the signs of fetal distress as an indicator of intrapartum hypoxia.

Table 4 shows type of amniotic fluid among study groups. In present study, the amniotic fluid was meconium stained in 48% and was clear in 52% case group, where as in control group it was meconium stained only in 11% newborns and was clear in rest 89%. This difference was found to be statistically significant (P<0.001). Similar results were seen in study by **Ghosh et al (2003)** [17] where meconium stained liquor was present in 57.7% of asphyxiated newborns and 24.5% of non asphyxiated newborns. This shows that the rate of occurrence of meconium stained liquor is more in the asphyxiated babies, as the presence of meconium in the amniotic fluid is a sign of intrapartum fetal hypoxia. Most of non-asphyxiated babies were delivered with clear amniotic fluid.

Table 5 shows clinical characteristics of study group. In our study the mean gestational age in cases was 39.39 ± 0.96 weeks and in controls was 39.5± 0.84 weeks. This difference was not statistically significant (P> 0.05). A similar study was done by **Chavan et al (2018)** [61]**,** reported mean gestational age in asphyxiated babies of 39.24 ± 1.13 weeks and in non-asphyxiated babies of 39.48 ± 1.19 weeks. The mean gestational age in these two group was comparable. Similarly **Saracoglu et al (2000)** [46] found mean gestational age of 38.7±2.10 weeks in asphyxiated babies.

In present study mean birth weight of cases was 2.85 ± 0.30 kg and in controls was 2.86 ± 0.31 kg. The mean birth weight was comparable among cases and controls. Similarly **Mohanty et al (2014)** [19] reported mean birth weight of 2.865 ± 0.404 kg in asphyxiated babies and of 2.776 ± 0.405 kg in non asphyxiated babies.

In our study the cord blood mean hemoglobin level of cases was 17.31 ± 2.0 mg/dl and in controls was 16.57 ± 1.20 mg/dl. The hemoglobin level was significantly higher in cases than controls (P <0.007). Similar results was seen in study done by **Colaco et al (2014)** [1] where mean Hb of asphyxiated babies was 16.38 ± 1.90 mg/dL in the cases group and 15.68 ± 1.45 mg/dL in the control group.

The mean WBC count of cord blood in present study was 16.67 ± 5.5 x 10^3 /mm^3 and 9.8 ± 2.8 x 10^3 /mm^3 in the cases and control group respectively. Thus the mean total leukocyte count was significantly higher in the asphyxiated babies as compared to the normal babies (P value<0.001), which is quite comparable with study done by **Boskabadi et al (2010)** [54] who reported mean WBC count in controls as 14.13 ± 8.34 x10^3/mm^3 and in cases as 21.93 ± 15.06 x10^3/mm^3. Similarly **Colaco et al (2014)**[1] found 21,257.79 ± 7,788.9/mm^3 of mean WBC in asphyxiated newborns and of 14,343.78 ± 3,987.3/mm^3 in non asphyxiated newborns. Thus the mean total leucocyte count was significantly higher in asphyxiated babies as compared to the normal babies. This can be attributed due to stress during asphyxia, which lead to increased number of WBC in the blood.

Table 6 represent mean APGAR score at 1 min in study group. In this study the mean APGAR score at 1 minute in case group was 3.85± 1.48 and in control group was 8.23 ± 0.67. The mean APGAR score at 1 minute in asphyxiated cases reported by **Mohanty et al (2014)** [19] was 4 ± 1.2. Similarly **Colaco et al (2014)** [1] reported mean APGAR score of 3.86 ± 0.95 at 1 minute. The mean APGAR score at 1 minute in cases in our study was comparable with the mean APGAR scores reported by **Mohanty et al (2014)** [19] and **Colaco et al (2014)** [1].

Table 7 shows immediate neurological outcomes (assessed at 24 hours) among study group. In our study most of the asphyxia cases had abnormal neurological outcome (61.3%) and only 29 (38.7%) cases had normal neurological outcome. All the control subjects had normal immediate neurological outcome. This difference in immediate outcomes among cases and control group was found to be statistically significant (P<0.001). **Mohanty et al (2014)** [19] found 63% had normal outcome while 37% had poor outcome in asphyxiated cases (assessed for 7 days). Higher number of abnormal immediate outcome in present study was probably due to shorter duration for assessment.

Table 8 depicts mean NRBC /100 WBC among study group. In present study the mean NRBC Count (NRBC/100 WBC) was 17.82 ± 19.55 in the case group and was 1.42 ± 3.26 in the control group. There was statistical difference in mean NRBC in both groups (P value <0.001). Several studies have reported an increased NRBC count in neonatal cord blood following perinatal asphyxia. A study by **Colaco et al (2014)** [1] had the mean NRBC count per 100 WBC of 17.43 ± 19.86 in cases and 2.97 ± 4.79 in controls. **Ghosh et al (2003)** [18] reported mean NRBC per 100 WBC of 16.5 ± 6.4 in asphyxiated newborn and 8.6 ± 7.01 in non-asphyxiated group. **Saracoglu et al (2000)**[46] study found the mean NRBC count/ 100 leukocytes of 11.18 ± 4.92 for acute fetal distress and of 24.43 ± 20.05 for chronic fetal distress group. Both these values were significantly higher than the control group. **Mohanty et al (2014)** [19] reported the mean NRBC count for normal newborn was 1.67 ± 1.05 and for case group it was 50.82 ± 23.85. In present study the mean of NRBC count was higher in case group as compared to controls which supports the previously

suggested view that NRBC's are released in stressful condition of perinatal asphyxia.

Table 9 shows NRBC / 100 WBC range among study group. In our study 19% of the newborns in case group and 98% of the newborns in control group were falling in <11 NRBC/100WBC range group, remaining 81% in cases and 2% controls were falling in ≥ 11 NRBC/100 WBC range group. This justifies the cut off value ≥ 11 NRBC/100 WBC in our study. These results were similar to study done by **Mohanty et al (2014)** [19] where 4% of asphyxiated (cases) and 98% of non asphyxiated (controls) were in the range group of <11 NRBC/100WBC and rest 96% cases and 2 % controls were in the range group of ≥ 11 NRBC/100 WBC.

Table 10 shows mean of NRBC count in respect to the type of delivery in study group. In present study the mean NRBC count in babies delivered by LSCS was 23.16± 12.77 and those by NVD was 11.67 ± 2.99 in cases (P ≤0.001) which is significant and in controls for LSCS it was 1.6 ± 1.78 and for NVD it was 1.29 ± 2.44 (P =0.703 which is not significant). Hence we found that mean NRBC count tends to be on higher side in asphyxiated babies (cases) as compared to non asphyxiated babies (controls). Similar results were found by **Thilaganathan et al (1994)** [41] where statistically significant difference was present (P <0.05) in erythroblast count of babies delivered via NVD and emergency LSCS. In present study, higher mean NRBC in LSCS can be explained on the basis that LSCS was chosen as a mode of delivery in babies with fetal distress.

Table 11 shows mean NRBC count in respect to the type of amniotic fluid. In present study the mean NRBC count in cases with clear amniotic fluid was

14.67±12.86 and for meconium stained fluid was 22.64±18.69 in cases where as in controls for clear fluid it was 1.06 ± 1.95 and those with meconium stained fluid was found to 3.63± 3.99. Similarly **Lundberg et al (1999)**[43] reported that elevated nucleated red blood cell counts were associated with presence of meconium (*P* =.020). Thus in present study it was found that the meconium stained liquor was associated with the increased number of NRBC. These findings correlate very well with the severity of fetal hypoxia and fetal distress.

Table 12 and 13 shows mean NRBC correlation with HIE stages, in asphyxia cases. In our study the mean NRBC count in cases without HIE was 10.38 ± 2.37 while in HIE stages I, II and III was 13.4 ± 3.31, 23.12 ± 11.34 and 33.5 ± 10.48 respectively. Similarly **Boskabadi et al (2010)** [54] reported mean NRBC count of 11.94 in HIE I, 21.08 in HIE II and 29.18 in HIE III. **Colaco et al(2014) 1** found the mean of NRBC count in cases with no HIE as 4.48 ± 6.180, in HIE I as 11.10 ± 9.036, in HIE II as 25.95 ± 17.622 and in HIE III as 45.55 ± 25.88. **Mohanty et al (2014)** [19] also found the higher value of NRBC count, in higher degree of severity of HIE, 15.88 in Stage I, 46.5 in Stage II and 77.12 in Stage III of HIE. **Chavan et al (2018)** [61] found mean NRBC count of 23.38 in HIE I, 48.32 in HIE II and 96.50 in HIE III cases. This shows that the cord blood NRBC count is correlated with degree of asphyxia and HIE. In present study Spearmann correlation coefficient was 0.833, indicating strong positive correlation between NRBC count and HIE ($P \leq 0.001$).

Table 14 shows mean NRBC count in relation to immediate outcome. In present study immediate outcome for Ist 24 hr life was observed and we found that in cases with neurologically abnormal babies, mean NRBC count was 23.87± 11.82

and in neurologically normal babies it was 10.14 ± 2.45. Similar results were observed by **Mohanty et al (2014)** [19] where in neurologically abnormal (death or survived with sequelae) babies the NRBC count was 65.44 which was higher than babies with good outcome (42.14). Similarly **Korst et al (1996)** [42] found mean number of initial nucleated red blood cells were significantly higher in the group of neurologically impaired neonates (30.3 +/- 77.5, range 0 to 732 per 100 white blood cells) than in the control group (3.4 +/- 3.0, range 0 to 12 per 100 white blood cells). In our study babies who died within first 24 hours had mean NRBC of 50. From present study we concluded that with increase in NRBC count, there was increased chances of unfavorable immediate outcome in the form of neurological abnormality or mortality.

Table 15 and 16 represents the correlation of mean NRBC count with severity of perinatal asphyxia. In present study the mean NRBCs count increased with increase in severity of perinatal asphyxia. Severity of perinatal asphyxia was assessed by APGAR score at 1 min. It was found that higher the NRBC counts poor was the APGAR score. The mean NRBC count in moderate asphyxia was 11.93 ± 3.99 and in severe asphyxia was 21.97 ± 12.17 at APGAR score at 1 min ($P \leq 0.001$ which was significant). In present study there was significant negative correlation between NRBC count and APGAR at 1minute (Spearman correlation coefficient was -0.774 with $P \leq 0.001$, which was statistically significant) . Similarly **Colaco et al (2014)** [1] found significant negative correlation in APGAR score and NRBCs, stating that as APGAR scores decreases the NRBCs count increases (r-0.4396). **Lundberg et al (1999)** [43] evaluated the relation between the severity of asphyxia and increased NRBCs by comparing cord NRBCs and APGAR score found that the

NRBC counts increased with progressive decrease in the APGAR scores. Similar results was reported by **Ghosh et al (2003)** [17] where NRBC per 100 WBC were correlated well with APGAR score. The correlation coefficient (*r*) between NRBC per 100 WBC and APGAR score at 1 min was - 0.50 ($P<0.001$). So from present study we found that nucleated RBC count in the cord blood at birth was well correlated with APGAR score.

So in present study we found that cord blood NRBC is good predictor of perinatal asphyxia.

Summary & Conclusion

SUMMARY AND CONCLUSION

SUMMARY

We performed a hospital based prospective case control study conducted at inborn NICU, Department of Pediatrics, at Santokba Durlabhji Memorial Hospital, Jaipur.

The present study was carried out to know the cord blood NRBC count in asphyxiated and non asphyxiated babies and to evaluate correlation of NRBCs with the severity of birth asphyxia and its immediate outcome in asphyxiated babies. A total of 150 term newborns, who were delivered in labour room and OT, at Santokba Durlabhji Hospital, Jaipur were enrolled for study. Out of these newborns, 75 babies who had birth asphyxia were enrolled as cases and 75 normal newborn babies as controls. Cord blood was taken for NRBC count.

Various outcome of the study are summarized as :

1. The male and female ratio in cases and controls was 1.08:1 and 1.14:1 respectively.
2. LSCS was the mode of delivery in 60% of cases and in 13.3 % of controls whereas NVD was mode of delivery in 40% of cases and 86.7% of controls.
3. At the time of birth the amniotic fluid was meconium stained in 48% of the cases and only in 10.7% of the controls.
4. Vertex was the common presentation at time of delivery in most of the enrolled babies i.e. 80% in cases and 89.3% in controls.
5. The mean gestational age in cases and control group was 39.39 ±0.96 and 39.51± 0.84 weeks respectively.

6. The mean birth weight in cases and control group was 2.85 ± 0.30 and 2.86 ±0.31 kg respectively.
7. The mean cord blood haemoglobin in cases and control group was 17.31 ± 2.0 and 16.57 ± 1.20gm/dl respectively.
8. The mean APGAR scores at 1 minute in cases was 3.85 ± 1.48 and in controls was 8.23 ± 0.67.
9. The Mean APGAR score at 1 minute in cases without HIE was 5.38 ± 0.56 whereas in cases with HIE I, II and III was 3.8 ± 0.79, 3.04 ± 0.75 and 1.83 ± 0.39 respectively.
10. The mean cord blood NRBC count/100 WBC was 18.56 ± 11.51 in cases and 1.79 ± 1.53 in controls. This difference was found to be statistically significant (P< 0.001).
11. The mean total leukocyte count ($10^3/mm^3$) in cord blood was 16.67 ± 5.5 in the cases and 9.8 ± 2.8 in the controls. This difference was found to be statistically significant (P< 0.001).
12. 81.3% babies in case group and 1.3% in control group were falling in ≥ 11 NRBC/100 WBC range group, remaining 18.7% babies in case group and 98.7% in control group were falling in the <11 NRBC/100WBC range group. This difference in NRBC range among case and control groups was found to be statistically significant (P< 0.001)
13. The mean NRBC count in cases delivered by LSCS was 23.16 ± 12.77 and those by NVD was 11.67 ± 2.99. Whereas, in controls the NRBC count was 1.6 ± 1.78 in LSCS and 1.29 ± 2.44 in NVD. NRBC was significantly associated with type of delivery among asphyxia cases (P< 0.001).

14. The mean NRBC in cases group with MSL and clear amniotic fluid babies was 22.64± 18.69 and 14.64± 12.86 respectively and in controls group with MSL and clear amniotic fluid was 3.63± 3.99and 1.06 ± 1.95 respectively. NRBC count was significantly associated with type of amniotic fluid among both asphyxia cases (P=0.034) and controls (P=0.003).
15. The Mean NRBC count in cases without HIE was 10.38 ± 2.37whereas in cases with HIE I, II and III was 13.4 ± 3.31, 23.12 ± 11.34 and 33.5 ± 10.48 respectively (P<0.001). There was strong positive correlation between NRBC counts and stages of HIE (Spearmann's correlation coefficient 0.833).
16. Immediate outcome: 38.7% of the cases were neurologically normal while, 61.3% abnormal.
17. The mean NRBC count in neurologically abnormal babies and normal babies was 23.87 ± 11.82 and 10.14 ± 2.45 respectively. NRBC was found to be significantly associated with immediate neurological outcome (P< 0.001).
18. The mean NRBC count in moderate (APGAR 4-6) and severe asphyxia (APGAR 0-3) was 11.93 ± 3.99, 27.97 ± 12.17 respectively (P< 0.001. There was a significant negative correlation of NRBC count with APGAR at 1 minute (Spearmann correlation coefficient - 0.774)

Our study showed that nucleated RBC count in the cord blood at birth was well correlated with APGAR score and HIE stages. So Nucleated RBC count can be used as an indicator to predict the severity of birth asphyxia and immediate outcome.

CONCLUSION

- We conclude from our present study that the NRBC count in cord blood can be considered as a marker of perinatal asphyxia. The level of NRBCs in the cord blood is significantly correlated with the degree of asphyxia and immediate neurological outcome.
- NRBC count can be used as a simple tool to predict the severity and immediate outcome of birth asphyxia in newborns in resource poor country like ours, where APGAR score with expertise and blood gas analysis facilities are not available in majority of places.
- Based on NRBC count babies delivered in primary health care centers can be referred for further care to neonatal intensive care units. And by applying new approaches in treatment like the neuro-protective techniques such as hypothermia, anti-oxidant therapy and other modalities of treatment, the CNS morbidities can be reduced.

RECOMMENDATION

- A simple, cost effective, rapid and non-invasive test of NRBC count, obtained from otherwise discarded umbilical cord blood, provides valuable information about the well being of the newborn at birth and it correlates well with APGAR score and thus should be done in asphyxiated babies immediately after birth.
- Based on present study it is recommended to do cord blood NRBC count in asphyxiated babies which will provide valuable information about well being of the newborn and thus will helps in reducing neonatal mortality as well morbidity.

Limitation of Study

LIMITATIONS

This study had few limitations:

1. We were not able to predict which inclusion criteria was better marker of perinatal asphyxia.
2. We were not able to establish the relationship between NRBC count and acidosis.
3. Parameters regarding the clearance of NRBCs from neonatal blood were not studied.
4. Chronic outcome were not studied as we didn't follow these asphyxiated babies.

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Annexures

Annexures

ANNEXURE I

STUDY PROFORMA

Reg. No. **Name - B/O :** **Father's Name:**

Sex: M / F **Address:**

Date of Birth: **Time of Birth:** **Birth Weight:**

Date & Time of Admission: **Age (In Hours) At Admission:**

Indication for Admission **Provisional Diagnosis on Admis**sion

Mother's Information:

Age :

Gravida : **Parity :** **Live : Abortion:**

LMP: **EDD:**

Hb : **Blood Group:**

PIH: Pre-Eclampsia / Eclampsia

APH: Yes / No

Gestational Diabetes : Yes / No

History of Maternal Smoking: Yes / No

Any Other Major Medical Illness:

During Labour

H/O Leaking: Yes / No, If Yes Then Duration In Hrs:

Amniotic Fluid: Clear/Blood Stained/Meconium Stained/Foul Smelling

Presentation: Vertex/Breech/Transverse

Labour: Spontaneous /Induced

Annexures

Course of Labour: Uneventful/ Prolonged 1st Stage/ Prolonged 2nd stage /Obstructed

Type of Delivery: VD – NVD/AVD –Forceps/Vacuum

LSCS Indication for Caesarean:

At birth:

Cried at Birth Immediately: Yes / No **Birth Weight:**

Gestation Age (Weeks):

Maturity: Preterm/Term/Post Term SGA/AGA/LGA

APGAR : at 1 Minute : at 5 Minutes : at 10 Minute :

Resuscitation Required: Yes / No Type: Oxygen /Bag And Mask/Intubation /Chest Comp/ Drugs……..

General Examination at Admission:

General Condition: Alert / Lethargic / Comatose

Vitals: **HR:** **RR:**

Resp-Regular/Irregular/Gasping/Apnoea/Grunting

Temp: **SpO_2 -** Without O_2: With O_2:

Pre Ductal: Post Ductal: Difference:

CRT >3 Sec : Yes / No

Meconium Stained: Yes/No Tone: **Convulsions:** Yes / No

Cry: Normal/Feeble/High Pitched

Sucking: Good / Poor / No Sucking

Systemic Examination

CNS : Consciousness

AF

Pupil

Annexures

Neonatal Reflexes

Tone

DTR

Respiratory System:

CVS :

Per Abdomen :

Investigations

CBC: Hb: TRBC Count: WBC Count:

DLC Count: HCT: Platelet Count:

Cord Blood PBF Nucleated RBC Count: Per 100 WBC

RFT: B. Urea: S. Creatinine :

S. Electrolytes - Na: K: Ca: S. CRP (Q)

ABG Within First Six Hour Of Birth :

pH PCO_2

PO_2 HCO_3

USG Cranium (If Required):

CT Scan Head (If Required):

Treatment Given

1.

2.

3.

4.

5.

Annexures

Daily Progress During Hospitalisation

SN	Parameters	D1	D2	D3	D4	D5	D6	D7
1.	**Gen. Cond.**							
2.	**Vital:** Heart Rate							
	Respiratory Rate							
	CRT							
	SpO_2							
3.	**CNS :** Consciousness							
	AF							
	Tone							
	Posture							
	Reflexes							
	Moro's							
	Pupils							
	Seizures							
	Sarnat stage							
4.	**Resp. System**							
5.	**CVS**							
6.	**Per Abdomen**							
7.	**T/t:** a.Vasopressor							
	b. Anti Convulsant Drugs							
	c. Blood/ FFP/ Platelets							
8.	**Others:**							
	Feed : IG/Oral							
	Feed Tolerance							
	Urine Output							

Final Outcome And Its Date:

Out Come: LAMA/ Abscond/Discharge / Death

Date : **Hosp. Stay (Days) :**

Final Diagnosis:

Neurological Examination At Discharge:

Normal/Abnormal, if Abnormal then Details

Annexures

ANNEXURE II

NEW BALLARD SCORING CHART FOR GESTATIONAL AGE

Neuromuscular Maturity

	-1	0	1	2	3	4	5
Posture							
Square Window (wrist)	 >90°	90°	60°	45°	30°	0°	
Arm Recoil		 180°	 140°-180°	 110°-140°	 90-110°	 <90°	
Popliteal Angle	 180°	 160°	 140°	 120°	 100°	 90°	 <90°
Scarf Sign							
Heel to Ear							

Physical Maturity

Skin	sticky friable transparent	gelatinous red, translucent	smooth pink, visible veins	superficial peeling &/or rash, few veins	cracking pale areas rare veins	parchment deep cracking no vessels	leathery cracked wrinkled
Lanugo	none	sparse	abundant	thinning	bald areas	mostly bald	
Plantar Surface	heel-toe 40-50mm: -1 <40mm: -2	>50mm no crease	faint red marks	anterior transverse crease only	creases ant. 2/3	creases over entire sole	
Breast	imperceptible	barely perceptible	flat areola no bud	stippled areola 1-2mm bud	raised areola 3-4mm bud	full areola 5-10mm bud	
Eye/Ear	lids fused loosely:-1 tightly:-2	lids open pinna flat stays folded	sl. curved pinna; soft; slow recoil	well-curved pinna; soft but ready recoil	formed & firm instant recoil	thick cartilage ear stiff	
Genitals male	scrotum flat, smooth	scrotum empty faint rugae	testes in upper canal rare rugae	testes descending few rugae	testes down good rugae	testes pendulous deep rugae	
Genitals female	clitoris prominent labia flat	prominent clitoris small labia minora	prominent clitoris enlarging minora	majora & minora equally prominent	majora large minora small	majora cover clitoris & minora	

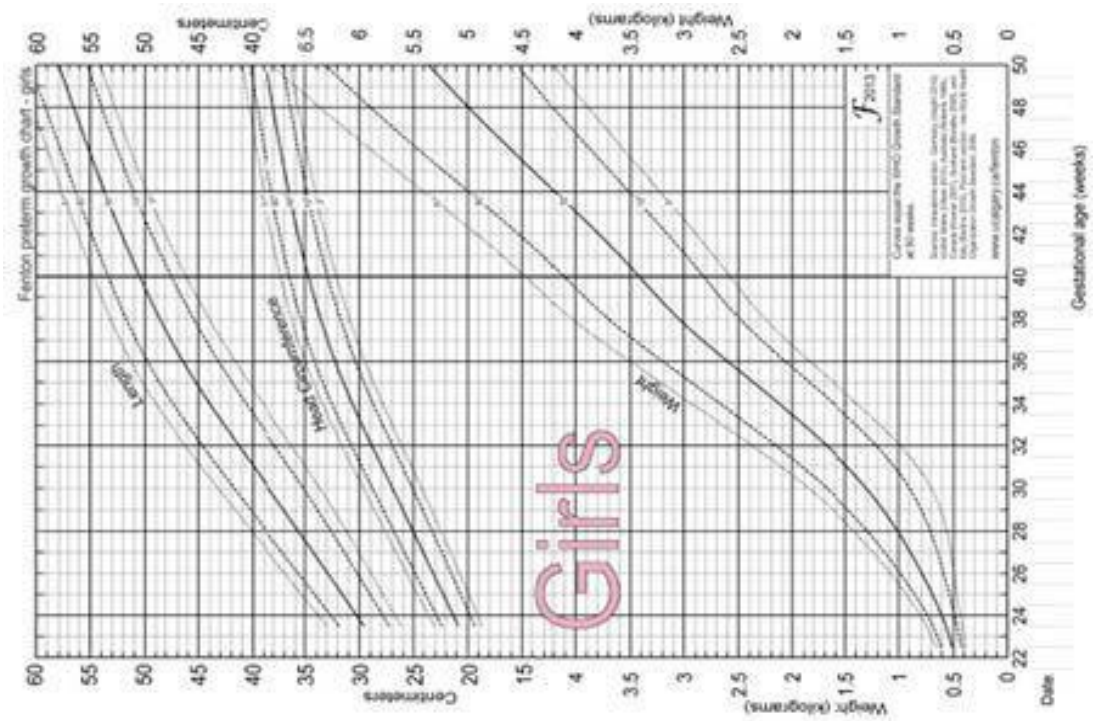
Maturity Rating

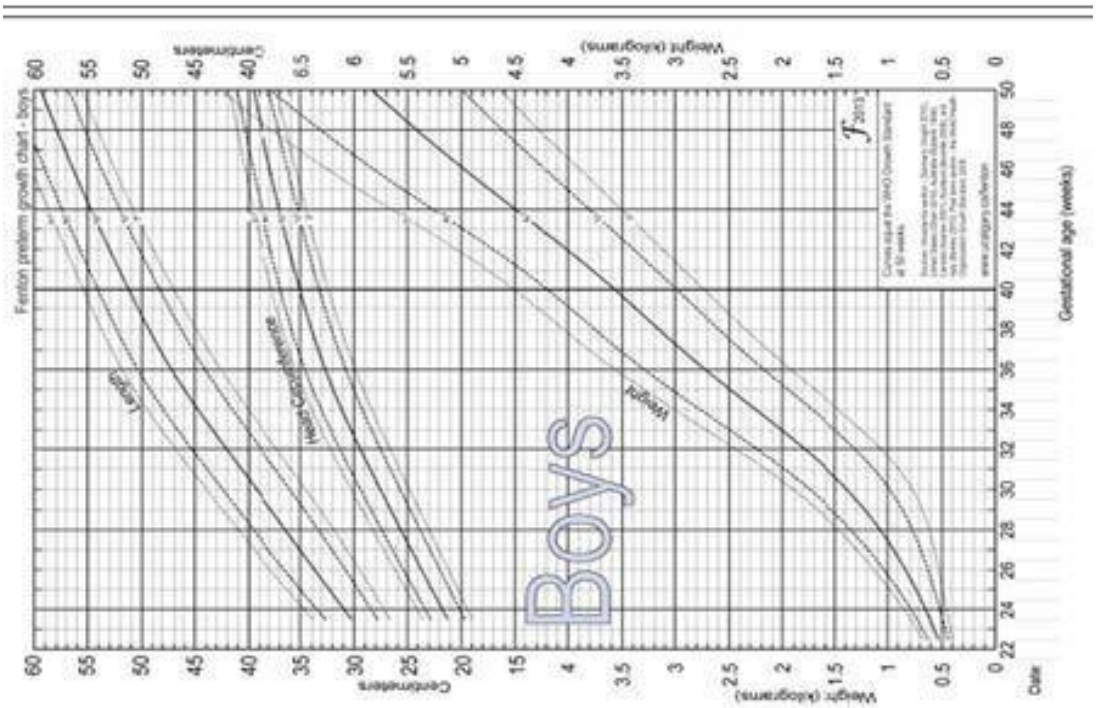
score	weeks
-10	20
-5	22
0	24
5	26
10	28
15	30
20	32
25	34
30	36
35	38
40	40
45	42
50	44

Annexures

ANNEXURE III

FENTON'S CHART





Annexures

ANNEXURE IV

INFORMED CONSENT FORM

Subject identification number for this trial _____

Title of the Project: _____

Name of the Principal Investigator _____ Tel.No._____

I have received the information sheet on the above study and have read and / or understood the written information.

I have been given the chance to discuss the study and ask questions.

I consent to take part in the study and I am aware that my participation is voluntary.

I understand that I may withdraw at any time without this affecting my future care.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible persons (ethics committee members / regulatory authorities). I give access to these individuals to have access to my records.

I understand I will receive a copy of the patient information sheet and the informed consent form.

_____ _____

Signature / Thumb Impression of subject Date of signature

_____

Printed name of the subject in capitals

_____ _____

Signature / Thumb Impression of legally accepted representative Date of signature

<<The legally acceptable representative signature should be added if the subject is a minor or is unable to sign for themselves. The relationship between the subject and the legally acceptable representative should be stated. The impartial witness signature should be added if the subject / legally acceptable representative is unable to read or write and consent should be obtained in his presence.>>

Annexures

_____

Printed name of legally acceptable representative in capitals

_____

Relationship of legally accepted representative to subject in capitals

_____ _____

Signature of the person conducting the informed consent discussion

Date of Signature

_____

Printed name of the person conducting the Informed consent discussion in capitals

_____ _____

Signature of impartial witness

Date of signature

_____

Printed name of the impartial witness in capitals.

Annexures

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Annexures

ANNEXURE V

PATIENT INFORMATION SHEET

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ANNEXURE VI

ABBREVIATIONS USED

AAP	:	American Academy of Pediatrics
ABG	:	Arterial Blood Gas
ACOG	:	American College of Obstetrics & Gynecology
AGA	:	Appropriate for Gestational Age
HIE	:	Hypoxic Ischemic Encephalopathy
LSCS	:	Lower Segment Cesarian Section
NICU	:	Neonatal Intensive Care Unit
NNPD	:	National Neonatal Perinatal Database
NRBC	:	Nucleated Red Blood Cell
NVD	:	Normal Vaginal Delivery
WHO	:	World Health Organization
EPO	:	Erythropoietin
RBC	:	Red Blood Cell
SGA	:	Small for Gestational Age
LGA	:	Large for Gestational Age
ROC	:	Receiver Operating Characteristic
GIT	:	Gastro Intestinal Tract
CNS	:	Central Nerve System
pH	:	Power of Hydrogen
MSAF	:	Meconium Stained Amniotic Fluid
IDH	:	Intra Dural Haemorrhage
SDH	:	Sub Dural Haemorrhage

Annexures

PIH	:	Pregnancy Induced Hypertension
MSL	:	Meconium Stained Liquor
TRBC	:	Total Red Blood Cell Count
APH	:	Antepartum Haemorrhage
CRT	:	Capillary Refilling Time
DLC	:	Differential Leucocyte Count
CRP	:	C- Reactive Protein.
EDTA	:	Ethylene Diamine Tetraacetic Acid
PPV	:	Positive Predictive Value
NPV	:	Negative Predictive Value

s/no	Sex	Birth weight(kg)	Mother age	GPLA	Mother's Hb	MBG	Leaking History	Amniotic fluid type	presentation	Mode of delivery	Gestation(wk)	APGAR score 1 min	APGAR Score at 5 min	Baby Hb (g/dl)	
1	F	3.81	22	G1P1	9.8	A B +	NO	MSL	Vertex	LSCS	38	3	4	17.3	1
2	M	3.31	30	G2P1	10.3	AB+	NO	Clear	Vertex	NVD	39	5	7	17.1	1
3	F	2.75	22	G1P1	12.1	A+	YES	Clear	Vertex	NVD	40	5	7	14.1	
4	M	2.61	25	G2P1	12.1	A+	NO	Clear	breech	LSCS	38	2	3	16.7	
5	F	3.21	25	G1P1	12.1	A+	NO	MSL	breech	LSCS	40	2	3	17.1	1
6	F	2.75	26	G2P1	12.3	A+	NO	MSL	Vertex	LSCS	40	1	0	17.2	1
7	F	2.91	21	G1P1	11.1	O+	NO	Clear	Vertex	NVD	38	4	5	17.4	1
8	F	2.71	21	G1P1	12.1	B+	NO	Clear	Vertex	NVD	40	4	5	16.3	1
9	M	3.21	26	G2P1	12.1	A+	NO	MSL	Vertex	LSCS	40	2	3	14.5	
10	F	2.51	22	G2P1	11.6	B+	NO	Clear	Vertex	LSCS	38	3	5	16.6	3
11	M	3.21	22	G1P1	9.61	A+	NO	MSL	breech	LSCS	40	2	3	17.1	
12	F	2.81	23	G2P1	8.12	B+	NO	MSL	Vertex	LSCS	38	4	5	14.6	1
13	F	2.56	30	G1P1	9.41	O+	NO	Clear	Vertex	LSCS	40	4	4	17.6	
14	M	3.42	24	G2P1	10.2	O+	NO	Clear	Vertex	NVD	39	6	8	15.1	1
15	M	2.85	22	G1P1	10.2	B+	NO	MSL	Vertex	LSCS	39	3	4	15.9	1
16	M	2.67	24	G2P1	12.1	B+	NO	MSL	Vertex	LSCS	37	6	8	15.4	3
17	F	2.81	25	G1P0	11.1	B+	NO	MSL	breech	LSCS	39	2	4	21.5	1
18	F	3.81	22	G1P0	9.8	B+	NO	MSL	Vertex	LSCS	40	2	4	17.3	1
19	M	3.21	28	G2P1	11.5	O+	NO	MSL	Vertex	NVD	39	3	5	14.7	2
20	M	2.61	20	G1P0	10.1	A+	YES	MSL	Vertex	LSCS	38	3	4	21.8	2
21	M	2.92	25	G2P1	10.8	AB+	NO	MSL	breech	LSCS	40	6	8	16.8	
22	M	2.71	20	G1P1	12.6	O+	NO	Clear	breech	LSCS	37	2	3	18.1	2
23	f	2.53	27	G1P1	11.6	A+	NO	Clear	Vertex	LSCS	38	4	4	17.3	2
24	M	2.46	30	G2P1	10.1	A+	NO	MSL	Vertex	NVD	38	2	4	16.9	1

s/no	Sex	Birth weight(kg)	Mother age	GPLA	Mother's Hb	MBG	Leaking History	Amniotic fluid type	presentation	Mode of delivery	Gestation(wk)	APGAR score 1 min	APGAR Score at 5 min	Baby Hb (g/dl)	
40	M	2.62	21	G1P1	10.2	AB+	NO	MSL	Vertex	LSCS	40	4	4	22.1	2
41	M	2.71	24	G1P1	10.2	A+	NO	MSL	Vertex	LSCS	40	6	8	17.1	2
42	F	3.41	24	G1P1	10.8	AB+	NO	MSL	breech	LSCS	40	1	0	17.1	1
43	F	3.02	23	G2P2	11.1	B+	NO	Clear	Vertex	LSCS	39	3	6	16.4	1
44	M	2.71	24	G2P2	10.3	B+	NO	Clear	Vertex	NVD	38	5	8	17.1	1
45	F	2.91	20	G1P1	9.9	O+	NO	MSL	breech	LSCS	40	4	8	16.1	1
46	F	3.11	21	G1P1	11.2	B+	NO	Clear	Vertex	NVD	40	5	9	17.5	1
47	M	2.78	22	G2P2	10.2	A+	YES	Clear	Vertex	NVD	39	6	8	17.5	1
48	M	2.91	25	G2P2	9.7	A+	NO	Clear	Vertex	LSCS	40	3	4	15.9	1
49	F	3.31	24	G3P2	11.3	B+	NO	Clear	Vertex	NVD	38	6	8	15.1	1
50	M	3.21	22	G1P1	11.8	AB+	NO	MSL	Vertex	LSCS	40	4	4	16.1	1
51	M	2.52	25	G3P2	9.8	A+	YES	MSL	breech	LSCS	39	2	3	15.6	1
52	F	2.91	24	G1P1	10.2	B+	NO	MSL	breech	LSCS	40	4	4	16.9	1
53	M	2.72	22	G2P1	12.1	AB+	NO	Clear	Vertex	NVD	40	4	5	16.5	1
54	F	2.72	23	G1P1	11.3	O+	NO	Clear	Vertex	NVD	40	5	8	17.4	2
55	M	2.51	26	G2P1	9.5	B+	YES	MSL	Vertex	LSCS	38	2	3	20.6	2
56	F	2.62	27	G3P2	11.5	B+	YES	MSL	Vertex	LSCS	40	2	3	15.5	1
57	M	2.62	25	G2P1	10.8	A+	NO	Clear	Vertex	NVD	40	5	8	18.6	1
58	M	2.72	26	G1P1	9.8	AB+	NO	MSL	breech	LSCS	40	3	4	19.7	1
59	F	2.62	29	G3P2	12.5	A+	NO	Clear	Vertex	LSCS	40	2	3	15.2	1
60	M	2.52	24	G1P1	12.8	B+	NO	MSL	Vertex	NVD	40	4	4	16.8	2
61	F	2.68	26	G2P2	9.9	O+	YES	Clear	Vertex	NVD	38	5	9	16.5	1
62	M	2.71	28	G3P1	11.8	A+	NO	Clear	Vertex	NVD	40	5	8	21.9	1
63	F	2.61	24	G2P1	11.6	O+	NO	MSL	Vertex	LSCS	40	3	4	20.7	1

s/n	sex	Birthweight(kg)	Age of mother	GPLA	Mother's Hb(gm/dl)	MBG	Amniotic fluidtype	presentation	Type ofdelivery	Gestation(week)	APGAR score1min	APGAR ScoreAt5min	
1	M	2.61	23	G2P2	10.1	AB+	Clear	Vertex	NVD	37	8	9	16.1
2	F	3.71	23	G2P2	12.1	O+	Clear	Vertex	NVD	38	9	10	14.4
3	M	2.51	23	G2P2	14.1	AB+	Clear	Vertex	NVD	39	8	10	15.1
4	F	3.11	22	G2P2	10.6	A+	Clear	Vertex	NVD	38	9	10	17.1
5	M	3.21	25	G1P1	11.2	B+	Clear	Vertex	NVD	37	8	9	20.1
6	F	2.71	25	G2P2	11.1	O+	MSL	Breech	LSCS	38	8	9	14.5
7	M	2.81	27	G3P2	11.1	A+	Clear	Vertex	LSCS	38	8	9	16.4
8	F	3.11	28	G1P1	10.8	AB+	Clear	Vertex	LSCS	39	8	9	17.2
9	M	2.71	24	G2P2	12.1	B+	Clear	Vertex	NVD	38	8	9	16.4
10	F	2.68	24	G2P1	12.1	B+	Clear	Vertex	NVD	38	9	9	17.2
11	F	2.61	26	G1P1	12.2	O+	Clear	Vertex	NVD	37	8	9	17.8
12	M	3.11	25	G3P3	11.5	O+	Clear	Vertex	LSCS	37	8	9	15.6
13	F	2.61	22	G2P2	11.1	A+	MSL	Vertex	NVD	38	7	9	15.6
14	M	3.11	22	G2P2	12.8	B+	Clear	Vertex	NVD	38	8	9	14.8
15	F	2.44	25	G3P3	11.3	B+	Clear	Vertex	NVD	39	8	9	15.5
16	M	2.76	26	G2P2	11.1	AB+	Clear	Vertex	NVD	38	8	9	16.2
17	M	2.81	25	G3P2	10.4	B+	Clear	Breech	NVD	38	9	9	16.3
18	F	2.71	24	G3P3	10.4	O+	Clear	Vertex	NVD	37	8	9	16.6
19	M	2.85	26	G2P2	13.1	B+	MSL	Vertex	NVD	38	8	9	15.2
20	M	3.11	26	G2P2	11.6	B+	Clear	Vertex	NVD	37	8	9	17.4
21	M	3.21	25	G1P1	12.3	0+	Clear	Vertex	NVD	38	7	9	17.5
22	F	2.48	24	G1P1	11.6	O+	Clear	Vertex	NVD	38	9	10	16.5
23	M	3.21	26	G2P2	11.3	B+	Clear	Breech	NVD	39	9	9	16.7
24	F	2.61	22	G2P1	10.6	AB+	Clear	Vertex	NVD	38	8	9	16.4
25	M	2.81	21	G1P1	10.9	A+	Clear	Breech	NVD	37	7	9	15.6

s/n	sex	Birthweight(kg)	Age of mother	GPLA	Mother's Hb(gm/dl)	MBG	Amniotic fluidtype	presentation	Type ofdelivery	Gestation(week)	APGAR score1min	APGAR ScoreAt5min	
39	M	3.02	22	G2P2	11.1	B+	Clear	Vertex	NVD	38	8	9	17.9
40	M	3.61	19	G1P1	10.7	O+	Clear	Vertex	NVD	39	8	9	17.2
41	F	2.51	26	G2P2	10.8	A	Clear	Vertex	NVD	37	8	9	16.8
42	M	3.41	24	G2P2	11.8	B+	Clear	Vertex	NVD	37	9	10	16.4
43	F	2.76	25	G2P2	11.7	AB+	Clear	Vertex	NVD	38	9	10	16.9
44	M	2.89	24	G1P1	12.2	B+	Clear	Vertex	NVD	37	9	10	17.4
45	F	2.63	26	G2P2	11.6	A+	Clear	Vertex	NVD	38	8	9	19.1
46	M	3.21	24	G1P1	12.5	B+	Clear	Vertex	NVD	37	8	10	15.4
47	M	2.91	28	G3P3	12.1	AB+	Clear	Vertex	NVD	38	9	9	15.1
48	F	3.23	26	G2P2	11.5	A+	MSL	Vertex	LSCS	37	7	9	16.7
49	M	2.81	23	G1P1	12.1	AB+	Clear	Vertex	NVD	39	8	10	16.8
50	F	2.71	26	G2P2	10.6	B+	Clear	Vertex	NVD	37	9	9	16.1
51	M	2.21	25	G2P2	9.1	B+	MSL	Vertex	LSCS	38	7	9	17.2
52	F	2.41	23	G1P1	10.2	A+	Clear	Vertex	NVD	37	8	9	17.3
53	M	2.61	27	G3P2	11.1	AB+	Clear	Vertex	NVD	38	9	10	15.1
54	F	2.81	22	G1P1	12.2	B+	Clear	Vertex	NVD	37	9	10	16.1
55	F	3.01	23	G2P2	13.2	A+	Clear	Vertex	NVD	37	8	9	16.3
56	M	3.11	22	G1P1	12.8	O+	Clear	Vertex	LSCS	38	7	9	18.3
57	M	2.91	24	G1P1	12.4	A+	MSL	Breech	NVD	38	7	9	18.1
58	F	2.45	21	G1P1	11.2	B+	Clear	Vertex	NVD	40	8	9	19.2
59	M	2.51	27	G3P2	10.1	B+	Clear	Vertex	NVD	39	9	9	15.4
60	F	2.61	24	G2P2	9.8	AB+	Clear	Vertex	NVD	40	8	9	16.1
61	F	2.31	23	G1P1	9.7	O+	Clear	Vertex	NVD	40	9	10	15.3
62	M	2.91	21	G1P1	10.4	A+	MSL	Breech	LSCS	37	8	9	16.2
63	F	2.81	22	G1P1	11.7	B+	Clear	Vertex	NVD	40	9	9	18.4