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# "EVALUATION OF CORD BLOOD TO SCREEN FOR NEONATAL ANEMIA AND ITS CORRELATION WITH MATERNAL ANEMIA IN A TERTIARY MEDICAL COLLEGE IN EASTERN INDIA"

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**ABSTRACT** An estimated 87% of pregnant women in India suffers from iron deficiency anemia in some point in their pregnancy. Maternal anemia has been found to be associated with low birth weight babies, asphyxia, preterm delivery and neurological deficits & the neonatal iron stores are solely dependent on the transplacental iron transport, about 1gm in term newborns. The transfer of iron from the mother to the foetus is tightly regulated by factors such as transferrin, coelomic fluid and hepcidin. This study aims to find whether maternal anemia has a correlation with neonatal anemia. Also, it aims to screen for neonatal anemia based on cord blood evaluation.The parameters used for comparison from cord blood are serum iron, ferritin and total iron binding capacity (TIBC) and blood hemoglobin concentration. This study was conducted over a period of two months, from July 22 nd 2021 to September 21 , 2021, in Medical College & Hospital , Kolkata and a total of 124 mother-infant pairs were taken out of which 62 were anemic mothers (Hb<11g/dl) and 62 non anemic. The cord Hb, iron and ferritin levels were significantly ($p<0.05$) lower in the anemic mothers than in the non anemic ones. The cord TIBC in the anemic group was higher but it was not significant ($p=0.083$). There was a moderate positive correlation between mother's Hb and cord iron levels, $r= 0.46$, $p< 0.00001$, a slight negative correlation between mother's Hb and cord TIBC was observed but the values were not significant, $r= -0.13$, $p=0.31$, serum ferritin in cord blood was found to have moderate positive correlation with maternal Hb, $r= 0.40$, $p < 0.00001$. This study was able to establish a correlation between maternal Hb and cord Hb, iron and ferritin thus showing that maternal iron status affects the iron profile of the neonate. This emphasises the need for proper iron and folic acid supplementation during the course of the pregnancy.

**KEYWORDS :**

## INTRODUCTION

Anemia during pregnancy is a serious health problem faced in developing countries such as India. An estimated 87% of pregnant women in India are anemic at some point in their pregnancy [1] and a major portion of this is due to iron deficiency anemia. Prophylactic doses of iron and folic acid tablets are given depending on the term of pregnancy and the severity of anemia, however, almost 39% of the mothers coming to a tertiary healthcare centre are found to be anemic throughout the pregnancy. [2]

Maternal anemia has been found to be associated with low birth weight babies, asphyxia, preterm delivery and neurological deficits. [3] The neonatal iron stores are solely dependent on the transplacental iron transport, about 1gm in term newborns. [4] After birth, breast milk is the only source of iron but its iron content gradually decreases over the course of lactation. [5] Along with this gradual decrease in intake, the iron requirement of the growing infant increases after 6 months. Reduced iron stores affect the mental and motor development of the child including reduced myelination and neurotransmitter actions even if anemia is not clinically significant. [6]

Thus, there is a need to ascertain a correlation between maternal iron reserves and its effect on the neonate.

The World Health Organisation recommends universal screening for anemia at 1 year of age, [7] but there have been very few measures to implement a uniform screening method. Iron deficiency anemia is very common among young children 6 to 24 months of age. [8] Awareness regarding anemia in infants is low but slowly growing.

The transfer of iron from the mother to the foetus is tightly regulated by factors such as transferrin, coelomic fluid and hepcidin. [9] A significant rise in the foetal iron stores is found after the 36th week of gestation. In preterm infants the iron stores are lower and the expected postnatal decline in Hb is more severe than in term infants. [10] There have been many studies focussing on anemia of prematurity and the effects of gestational age on neonatal iron stores.

However, the focus on term infants has been lower due to the anemia not being clinically significant. This study is designed to evaluate the status of iron stores in term neonates and compare it with maternal iron status. An initial assessment of the maternal anemia status was done from the blood hemoglobin (Hb) concentration. The cohort was then divided into two equal groups, 62 each, one with maternal anemia present (Hb< 11g/dl) and the other without maternal anemia.

The neonatal iron stores were evaluated based on the data gathered from cord blood sample analyses. Phlebotomy was not chosen as it is an invasive procedure and neonates are often burdened with laboratory phlebotomies for other illnesses. The parameters used for comparison from cord blood are serum iron, ferritin and total iron binding capacity (TIBC) and blood hemoglobin concentration.

The data from this study may have importance in understanding the impact of anemia during pregnancy on the neonatal iron stores. It also provides an insight into anemia in neonates and emphasises the importance of screening vulnerable infants.

### Review of Literature

Neonatal anemia has been an important issue of study for a long duration given the negative consequences associated with it. The relevance of iron deficiency anemia during pregnancy hasbeen stressed upon by numerous authors. However, given the limitations associated with such studies, a large portion of information about maternal anemia and its consequences is not available.

In a notable study, Mihaila et al. used rat models to identify a period of vulnerability of the fetus if iron was restricted in the mother. [11] They were able to establish a correlation between

iron deficiency and central nervous system development that could have an effect on language and cognitive functions. However, reviews of several randomized control trials were unable to prove beneficial effects of iron supplementation on cognitive development. [12] Whether this is due to a permanent effect of iron deficiency or lack of proper trails still remains unknown. What remains certain is that prophylactic measures, that is iron supplementation, would have beneficial effects on the child.

The ongoing research in this field has led to the evolving methods for the management of anemia during pregnancy. Lee's study on adolescent mothers, a group at high risk for iron deficiency anemia, identified mid gestational serum ferritin as the most predictive marker for maternal iron reserves. [13] Infants born to mothers with low reserves could then be screened further but it is not practically possible to get serum ferritin tests throughout pregnancy particularly in low resource areas.

A study assessing whether a screening program could be implemented for anemic children found that pallor had a low sensitivity for mild and moderate anemia and a finger prick is more effective. [8] The results of our study could help in identifying infants vulnerable to iron deficiency at the time of birth, creating a target group for a screening program later.

### Aims and Objectives

This study aims to find whether maternal anemia has a correlation with neonatal anemia. Also, it aims to screen for neonatal anemia based on cord blood evaluation.

## MATERIALS AND METHODS

### Study Type and Site

This is a cross-sectional study conducted in a tertiary healthcare center ,ie – Medical College & Hospital, Kolkata.

### Study Duration

This study was conducted over a period of two months, from July 22 nd 2021 to September 21 , 2021.

### Number of Samples

A total of 124 mother-infant pairs were taken out of which 62 were anemic mothers (Hb < 11g/dl) and 62 non anemic.

### Selection Criteria

Only apparently healthy neonates were selected for the study. Neonates with any major illness which affects the normal hematological and biochemical parameters for anemia and iron stores such as thalassemia, sickle cell anemia, severe jaundice, etc. were excluded. Preterm deliveries were excluded as they have comparatively low iron stores and would interfere with the comparative analysis with maternal anemia. Mothers having diabetes mellitus, hypertension or any other illness were excluded from the study.

### Data Collection

Proper history of the mother was taken to rule out major illnesses. Blood hemoglobin levels of the mother were obtained from the hospital records.

### Sample Collection

Immediately after delivery, 5ml of venous umbilical cord blood was collected by a sterile syringe and divided into two parts. One part was transferred to an EDTA vial for hemoglobin measurement. The other part, transferred to a serum separator tube for the measurement of serum iron, TIBC and ferritin levels.

### Laboratory Analysis

Quantitative determination of blood hemoglobin is done by colorimetric method in an automated cell counter. Hemoglobin is oxidized by potassium ferricyanide into methaemoglobin. It is converted into cyanmethemoglobin by potassium cyanide. The intensity of the color formed is proportional to the hemoglobin concentration in the sample and is measured at 540 nm wavelength in a colorimeter.

Serum ferritin is measured by electrochemiluminescence immunoassay method. First incubation in ferritin-specific monoclonal antibody and a labeled ferritin-specific antibody create a sandwich complex. Second incubation- After streptavidin-covered micro-particles are added, by way of interaction between biotin and streptavidin, the complex is linked to a solid phase. Reaction mixture is aspired into a measurement cell where micro-particles are magnetically caught by electrode surface. Voltage application on electrode causes chemiluminescence emission, and this is measured by a photomultiplier. Total Iron Binding Capacity (TIBC) is measured by colorimetric method from the serum. Two reagents are used sequentially. The first is an acid buffer with iron-binding due and ferric chloride. It releases iron from transferrin which forms a coloured complex with the dye. The second reagent is a neutral buffer. Shift in pH increases the affinity of transferrin for iron. The decrease in absorbance of the dye-iron complex is measured and is directly proportional to the TIBC. Serum iron is measured by making the medium acidic, releasing ferric iron from transferrin and reduced to ferrous form. It is complexed with a chromogen, producing coloured chromophore that absorbs maximally at 546 nm. Intensity is directly proportional to iron concentration.

### Statistical Analysis

The means of normally distributed data and their standard deviation (SD) were calculated.

Differences in group means of normally distributed variables were compared using independent *t*-tests. Correlations between two variables were done using scatter plots and Pearson's coefficient of correlation ('r') was calculated. The confidence interval was 95%. Only values with $p < 0.05$ were considered significant.

### Confidentiality

All data collected and test results obtained are anonymous.

### Consent

Only those who have read and signed the Informed Consent Form were taken into account. All procedures were duly explained before taking consent.

## OBSERVATIONS AND RESULTS

A total of 124 mother-infant pairs were taken out of which 62 were anemic mothers (Group A) and 62 non anemic mothers (Group B). The average values and median of mother's Hb, cord Hb, iron, TIBC and ferritin in the entire group, anemic and non anemic groups along with the independent t test results (with p-value) of comparison between group A and B are shown in Table 1.

*Table 1: Mean ± SD and median of maternal Hb and neonatal iron parameters with t-test between anemic and non anemic group*

| Parameters | Median | Mean ±SD | Group A mean (Anemic) | Group B mean (Non Anemic) | T-Test | p-value |
|---|---|---|---|---|---|---|
| Mother's Hb (g/dl) | 10.95 | 10.91±1.07 | 10.05±0.73 | 11.78±0.50 | -15.43 | < 0.00001 |
| Cord Hb (g/dl) | 16 | 15.92±2.25 | 14.80±1.85 | 17.28±1.95 | -6.00 | <0.00001 |
| Cord Iron (µg/dl) | 132.2 | 133.56±28.60 | 121.31±26.03 | 146.46±25.62 | -4.30 | 0.00005 |
| Cord TIBC (µg/dl) | 289.5 | 301.73±80.92 | 314.39±84.83 | 288.87±75.29 | 1.75 | 0.083 |
| Cord Ferritin (ng/ml) | 167.65 | 161.34±50.34 | 141.54±56.29 | 181.14±33.99 | -4.74 | <0.00001 |

The cord Hb, iron and ferritin levels were significantly ($p<0.05$) lower in the anemic mothers than in the non anemic ones. The cord TIBC in the anemic group was higher but it was not significant ($p=0.083$).

A correlative analysis between mother's Hb and cord Hb, iron, TIBC and ferritin was done by using scatter plots. Mother's Hb and cord Hb are found to be strongly positively correlated, $r= 0.65$, $p< 0.00001$ (Fig 1).

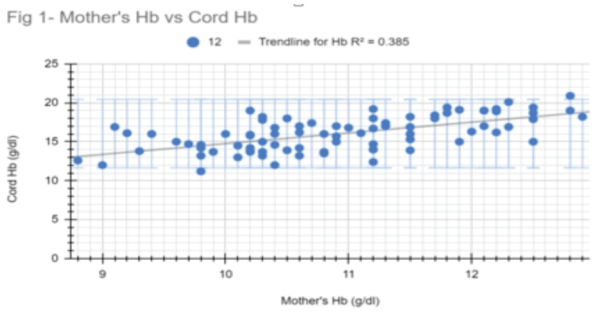


*Fig 1- Scatter plot showing correlation between Mother's Hb and Cord Hb*

There was a moderate positive correlation between mother's Hb and cord iron levels, $r= 0.46$, $p< 0.00001$ (Fig 2).

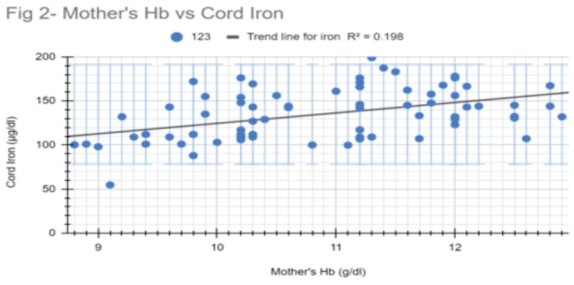


*Fig 2- Scatter plot showing correlation between Mother's Hb and Cord Iron*

A slight negative correlation between mother's Hb and cord TIBC was observed but the values were not significant, $r= -0.13$, $p=0.31$ (Fig 3).

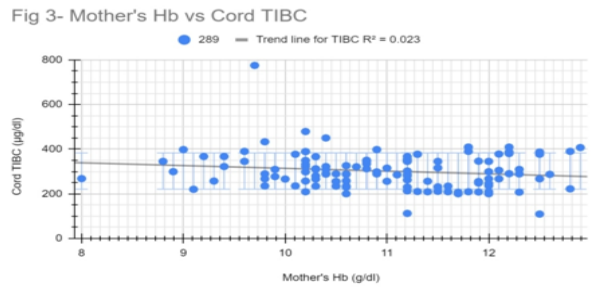


*Fig 3- Scatter plot showing correlation between Mother's Hb and Cord TIBC*

Serum ferritin in cord blood was found to have moderate positive correlation with maternal Hb, $r= 0.40$, $p < 0.00001$ (Fig 4).

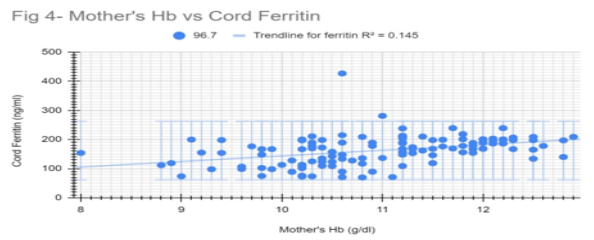


*Fig 4- Scatter plot showing correlation between Mother's Hb and Cord Ferritin*

Amongst the neonates under study, 79 (63.7%) of them developed physiological jaundice during their period of stay in the hospital. 41 of them were in group A and 38 in group B. Mean ± SD values were obtained for mother's Hb, cord Hb, iron, TIBC and ferritin for both the groups with jaundice and without and t-test was performed (Table 2).

No significant variation in the hemoglobin and iron parameters were found amongst the jaundiced and healthy group. No other clinical signs of anemia were observed.

## DISCUSSION

Anemia in young children is a matter of concern because it interferes with normal growth and development. There is a high iron requirement for growth and the amount of bioavailable iron in a child's diet is low. [8] It was observed that even after the implementation of programs for iron and folic acid supplementation during pregnancy, a large number of pregnant women coming to a tertiary healthcare centre are anemic.

This study found that neonatal iron stores were significantly affected by maternal iron status. The mean values of cord Hb, iron and ferritin were much lower in neonates born to anemic mothers.

Other studies have also had similar results thus indicating that maternal iron status is a crucial factor in identifying neonates with low iron reserves. [13,14,15]

A strong linear positive correlation was found between maternal Hb and cord Hb ($r=0.65$, $p<0.00001$). Hb deficit is a late sign of iron deficiency, indicating that neonatal iron stores must have been adversely affected at birth.

Serum ferritin is a well known indicator of iron status in the body. Cord ferritin values were positively correlated with maternal Hb showing that it is significantly ($r=0.40$, $p < 0.00001$) affected by iron status of the mother. The median cord ferritin (167.65ng/dl, $n=125$) is 35% higher than that observed in the US (124ng/dl) [13] and 1.3% lower than China (170ng/dl). [16]

The values of cord serum iron had a significant positive correlation with mother's Hb ($r=0.46$, $p< 0.00001$), in contrast to other studies which have found no association between the two. [13,14] This could be due to a smaller sample size compared to the other studies.

Cord TIBC did not have a significant relationship with the mother's Hb. This can be due to a multifactorial, complex mechanism of transport of iron to the fetus. Hepcidin has been shown to be an important regulator for the absorption of iron and its fetal distribution. [9] It could be one of the reasons why TIBC was not found to be a marker of low iron stores.

Amongst the neonates in the study, 63.7% developed physiological jaundice. However, this was not significantly ($p>0.05$) related to any of the parameters under evaluation. No other clinical signs of anemia were present in the neonates during the period of this study. Studies have been undertaken to find a proper method of screening for anemia in young children including cost effective parameters such as conjunctival pallor and pica along with peripheral blood smear, Hb estimation and serum ferritin. [8] Due to the time constraint and difficulty in compliance of the subjects in this study, the neonates could not be followed up for further assessment of iron reserves. Therefore, an assessment of whether low iron profile in the cord blood manifested clinically as anemia could not be made. To prevent the lack of diagnosis of anemia in infants and children, screening of infants born with low cord iron stores for anemia at the time of immunization could be implemented.

## CONCLUSION

This study was able to establish a correlation between maternal Hb and cord Hb, iron and ferritin thus showing that

maternal iron status affects the iron profile of the neonate. This emphasises the need for proper iron and folic acid supplementation during the course of the pregnancy. Neonates born to anemic mothers could be screened at regular intervals and diet changes or medical intervention could be undertaken to prevent iron deficiency and retardation of growth and development. Since placental iron transport is a complex mechanism, association of other parameters responsible for neonatal anemia remains a matter for further study.

**Summary**

Anemia among pregnant women is highly prevalent in India however its effect on the neonate is still unclear. The aim of this study was to find whether cord iron profile could be used to screen neonates and if maternal anemia had an effect on the same. The study included 124 pairs of mothers and neonates, 62 anemic mothers (Hb<11g/dl) and 62 non-anemic. Cord blood was collected after delivery and estimation of cord Hb, iron, TIBC and ferritin levels were done.

Maternal Hb showed a significant positive correlation with cord Hb (r= 0.65, p< 0.00001), cord serum iron (r=0.46, p< 0.00001), and cord serum ferritin(r=0.40, p < 0.00001). However, TIBC did not show a significant correlation with maternal Hb. Development of physiological jaundice was unrelated to the iron stores of the mother and in the cord blood. Maternal anemia could have an effect on the neonatal iron stores but it has a complex mechanism. Neonates born to anemic mothers showed reduced iron status and should have regular follow ups.

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