

Original Research Article

Association of Cord Blood Nucleated Red Blood Cells with Socio-Demographic Factors in Term Pregnancy

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Abstract: Increased number of nucleated red blood cells (NRBC) are seen in newborns in long standing erythropoietin induced erythropoiesis, acute stress, hypoxia or sometimes idiopathic release. In general NRBC increases with blood pressure and decreases as pregnancy advances. Keeping this in mind the present study was done to find any association of cord blood NRBC with socio-demographic factors in term pregnancy. 240 pregnant women with singleton term pregnancy were included in the study after obtaining written informed consent. Cord blood NRBC count per 100 leucocytes was counted and results were analysed to find association of NRBC with socio-demographic factors. A weak negative association was seen with maternal age, gravidity and gestational age. No significant difference in mean cord blood NRBC count was seen in women according to religion, residence or literacy status. A moderate positive association was seen with systolic and diastolic blood pressure. NRBC count has a positive correlation with systolic and diastolic blood pressure. Maternal socio-demographic status except BMI and economic status had no significant association with NRBC. A negative correlation was seen with gravidity and gestational age.

Keywords: NRBC, socio-demographic factors, BMI, maternal age.

INTRODUCTION

Nucleated red blood cells (NRBC) are primarily produced in the fetal bone marrow in response to erythropoietin and are stored in the marrow as precursors to reticulocytes and mature erythrocytes. Circulating NRBC are commonly seen in newborn children and rarely found in older children [1]. 0.1% of the circulating RBC or about 500 NRBCs/mm³ is represented by NRBC in the first few hours of life in healthy term newborns and they are rapidly cleared from circulation after birth [2-4]. About 50% of the circulating NRBC are cleared by 12 hours and only 20–30 NRBCs/mm³ are seen after 48 hours. After third or fourth day no NRBC are found in healthy term newborns but in preterm newborns a small number of NRBC may persist up to 1 week [5, 6].

Increased numbers of circulating NRBCs are seen in association with long standing erythropoietin induced erythropoiesis, acute stress mediated release of normoblasts from the marrow, postnatal hypoxia and idiopathic release. Tissue hypoxia results in increased

levels of erythropoietin, which in turn leads to stimulation of erythropoiesis and increased numbers of circulating NRBCs. Various conditions associated with increase erythropoietin levels and NRBC counts are intrauterine growth restriction [7, 8], maternal hypertension [9], pre-eclampsia [7, 10, 11] maternal smoking [12] Rh isoimmunisation [13], and maternal diabetes [9, 11, 14, 15]. Very few studies have been done to find any association of NRBC with socio-demographic variables of mother. Keeping this in mind the present study was done to find any association of NRBC count with socio-demographic factors of pregnant women.

MATERIAL AND METHODS

It was a hospital based observational study, done in the Department of Obstetrics & Gynaecology, S.M.S. Medical College, Jaipur from Feb 2013 to July 2013. 240 pregnant women with singleton term pregnancy were included in the study after obtaining written informed consent. Women with multifetal pregnancy, IUFD, congenital malformed fetus, with pre

existing hypertensive, cardiovascular or renal disease, diabetes mellitus or chronic disease, history of difficult delivery and who were smokers were excluded from the study. A detailed history, examination and investigations were done. Immediately after delivery of the fetus the umbilical cord was double-clamped and 1 ml umbilical cord blood was collected in a tube containing 1.5 mg ethylene diamine tetra-acetic acid (EDTA). Blood films were prepared and stained using the leishman stain for 20 min. The number of NRBCs per 100 leukocytes was counted. All data were entered on MS excel sheet and the data were analyzed. Quantitative data were summarized in form of mean and S.D. (Standard Deviation) and the difference in means were analyzed in using student 't' test, anova or Pearson correlation coefficient. The level of significance for all statistical analysis was kept at <0.05.

OBSERVATIONS

Majority of women were in the age group 20 – 30 years (66.7%), Hindu (75.4%), from urban area (59.6%), literate (56.2%) and belonged to middle socio-economic status (53.8%). Mean cord blood NRBC Count increased as age of the women increased except in age group 30-35 years but the difference in mean cord blood NRBC count was not significant (p value 0.32). Mean cord blood NRBC count was slightly more in Muslim women, belonging to urban area and who were literate but no significant difference in mean cord blood NRBC count was seen in women according to religion, residence or literacy status. Mean cord blood NRBC count was lower for women belonging to low socio-economic status than for women belonging to middle or upper socio-economic status and the difference was statistically significant (p value 0.01). (Table 1).

Table 1: Association of socio-demographic profile of the women with cord blood NRBC count

| Variable | Number of women | | Mean cord NRBC count |
|-----------------------|-----------------|------|----------------------|
| | No. | % | |
| Age (years) | | | |
| 15 – 20 | 49 | 20.4 | 10.06 ± 7.76 |
| 20 – 25 | 84 | 35.0 | 11.12 ± 8.29 |
| 25 – 30 | 76 | 31.7 | 11.89 ± 7.70 |
| 30 – 35 | 31 | 12.9 | 9.10 ± 6.86 |
| P value | | | 0.32 not significant |
| Religion | | | |
| Hindu | 181 | 75.4 | 10.41 ± 7.49 |
| Muslim | 59 | 24.6 | 12.00 ± 8.81 |
| P value | | | 0.29 not significant |
| Residence | | | |
| Urban | 143 | 59.6 | 11.13 ± 8.23 |
| Rural | 97 | 40.4 | 10.31 ± 7.25 |
| P value | | | 0.4 not significant |
| Literacy | | | |
| Illiterate | 105 | 43.8 | 10.41 ± 7.30 |
| Literate | 135 | 56.2 | 11.10 ± 8.26 |
| P value | | | 0.5 not significant |
| Socio-economic status | | | |
| Low | 56 | 23.3 | 8.14 ± 6.87 |
| Middle | 129 | 53.8 | 11.73 ± 8.04 |
| Upper | 55 | 22.9 | 11.33 ± 7.84 |
| P value | | | 0.01 significant |

Table 2 shows correlation of nucleated RBC count with various maternal variables. Mean cord blood NRBC count was 10.76 ± 7.87. Mean maternal age was 23.9 ± 4.54. The value of Pearson coefficient correlation (R) for maternal age and cord blood NRBC count is -0.0028 which shows a negative correlation although weak. The value of coefficient of determination (R²) is 0. Correlation of maternal age and cord blood NRBC count was observed not to be

significant (p-value .96). Mean BMI was 26.68 ± 2.78. A weak positive correlation was observed between BMI and NRBC count (R = 0.1706). The R² was 0.029, it means only 3% of the total variation in BMI was explained by the linear relation with cord blood NRBC count. Correlation of BMI with cord blood NRBC count was observed to be significant (p-value .008). Mean systolic BP was 129.83 ± 23.52 mm of Hg. A moderate positive correlation was observed with systolic BP and

cord blood NRBC count (R=0.6593) i.e. as systolic BP increases there is increase in cord blood NRBC count and vice versa. The R² was 0.4347 it means about 43 % of the total variation in systolic BP was explained by the linear relation with cord blood NRBC count. Correlation of systolic BP with cord blood NRBC count was observed to be extremely significant (p-value .00001). Mean diastolic BP was 86.29 ± 12.81 mm of Hg. A moderate positive correlation was observed with diastolic BP and cord blood NRBC count (R=0.6736) i.e. as diastolic BP increases there is increase in cord blood NRBC count and vice versa. The R² was 0.4537 it means about 45 % of the total variation in diastolic BP was explained by the linear relation with cord blood NRBC count. Correlation of diastolic BP with cord blood NRBC count was observed to be extremely significant (p-value .00001). A weak negative correlation was seen with gravidity and cord blood NRBC count (R = -0.1393) i.e. as gravidity increases there is decrease in NRBC count. Only 1% of the total variation in gravidity was explained by the linear

relation with cord blood NRBC count. A significant correlation was seen between gravidity and cord blood NRBC (p value .03). Mean gestational age was 38.47 ± 2.36 weeks. A weak negative correlation was seen with gestational age and cord blood NRBC count (R = -0.3247) i.e. as gestational age increases there is decrease in cord blood NRBC count or vice versa. The R² was 0.1054 it means about 10 % of the total variation in gestational age was explained by the linear relation with cord blood NRBC count.

15.4% women had Intra Uterine Growth Retardation (IUGR) with cord blood NRBC count of 17.00 ± 7.78. Level of mean cord blood NRBC count was significantly more in women with IUGR than in women without IUGR (p value .001). Mean NRBC count was 9.25 ± 7.29 for women who had normal delivery which was significantly lower than 14.25 ± 6.51 for women who had caesarean delivery (p value 0.0001) (Table 3)

Table 2: correlation of nucleated RBC count with various maternal variables

| N = 240 | Mean ± SD | R | R square | P-value |
|------------------------------|----------------|----------|----------|-------------------------------|
| Cord blood NRBC count | 10.76 ± 7.87 | | | |
| Maternal Age | 23.9 ± 4.54 | - 0.0028 | 0 | .96 not significant |
| Maternal BMI | 26.68 ± 2.78 | 0.1706 | 0.0291 | .008 highly significant |
| Systolic Blood Pressure | 129.83 ± 23.52 | 0.6593 | 0.4347 | .00001 extremely significant |
| Diastolic Blood Pressure | 86.29 ± 12.86 | 0.6736 | 0.4537 | 0.00001 extremely significant |
| Gravidity | 1.42 ± 0.67 | - 0.1393 | 0.0194 | 0.03 significant |
| Gestational Age | 38.47 ± 2.36 | - 0.3247 | 0.1054 | .00001 highly significant |

Table 3: correlation of nucleated RBC count with IUGR and mode of delivery

| Variable | Number of women | | Mean cord blood NRBC count |
|------------------|-----------------|------|----------------------------|
| | No. | % | |
| IUGR | | | |
| Present | 37 | 15.4 | 17.00 ± 7.78 |
| Absent | 203 | 84.6 | 9.63 ± 7.36 |
| P value | | | <0.001 highly significant |
| Mode of delivery | | | |
| Normal delivery | 148 | 61.7 | 9.25 ± 7.29 |
| LSCS | 92 | 38.3 | 14.25 ± 6.51 |
| P value | | | 0.0001 highly significant |

DISCUSSION

In our study mean maternal age of the women and gestational age were 23.9 ± 4.54 years and 38.47 ± 2.36 weeks respectively which were lower than reported by Darkhaneh RF *et al* [16] who reported mean age of the women as 27.17 ± 6.04 years and mean gestational age as 38.47 ± 2.36 weeks. A higher maternal age was also reported by Habber S *et al* [17] their study. A lower age of the mother in our study could be a

reflection of the culture of the society where early girl marriage is still prevalent. Our results were comparable with the study done by Mohanty AK *et al* [18] and S Sivakumar *et al* [19]. A weak negative correlation of maternal age and cord blood NRBC count was seen though the correlation of maternal NRBC and cord blood NRBC count with maternal age was statistically not significant.

No association was found among literacy status, religion and residence of the mother on cord blood NRBC count. Our results were similar to the observation made by Amritanshu *et al* [20] in their study they also found no association among various educational and occupational status of the mother and outcome. As socio-economic status of the women improved there was increase in maternal and cord blood NRBC count and the difference was statistically significant.

A positive correlation was seen between maternal BMI and NRBC count which was statistically highly significant (p value .008). Our findings were similar to that observed by Sheffer-Mimouni G *et al* [21] who reported that infants of overweight and obese mothers have increased circulating absolute nucleated red blood cells counts at birth compared with infants of non-obese mothers. S Barak *et al* [22] confirmed that elevation of circulating absolute nucleated red blood cells counts in infants of overweight and obese mothers was due to increased production of fetal erythropoietin and they found that there was a significant correlation between erythropoietin concentrations and maternal BMI (R=0.427, P=0.007). They speculated that increasing maternal BMI may represent a relative hypoxic burden on the fetus.

There was a significant correlation between Systolic BP and NRBC (R=0.6593, P =m0.00001) and Diastolic BP and NRBC (R=0.6736, P =0.00001). Mean cord blood NRBC count was 16.19 ± 7.05 in women with pre-eclampsia and 5.41 ± 3.91 for normotensive women. Our findings are in concurrence with earlier studies conducted at different places and reported by different investigators. In the study of BS Aali *et al* [23] 26% of the pre-eclamptic cases compared with 12.7% of normal pregnant women had a cord blood NRBC count greater than 14/100 WBC which was statistically significant. The mean cord blood NRBC count in the control group in the study was 6.2 ± 8.1 compared to 18.2 ± 31.8 in pre-eclamptic groups. In the study of S Sivakumar *et al* [19] the mean cord blood NRBC count in pre-eclamptic group was 7.38 compared to 1.72 in normotensive group, which was statistically significant. Shripad Hebbar *et al* [24] concluded that the mean cord blood NRBC count in pre-eclamptic group was 40.0 ± 85.1 and in normotensive group was 5.9 ± 6.3 which was statistically significant. Roya Faraji Darkhaneh *et al* [25] found that the mean cord blood NRBCs was 11.12 ± 5.5 for newborns in the pre-eclampsia group and 2.74 ± 2.9 for newborns in the normotensive group (P < 0.05), which was statistically significant. F Bayram *et al* [26] found in their study that mean cord blood NRBCs count was 17.1 ± 6.8 in the pre eclamptic group without IUGR, and 26.3 ± 7.5 in the pre-eclampsia group with IUGR and 9.9 ± 2.7 in normotensive group which was statistically significant. A study by Akercan *et al* (2005) also revealed NRBC count elevation in the newborn of pre-eclamptic

patients [27]. It has been stated that the inability of cytotrophoblasts to differentiate correctly and subsequent failure to invade the uterus and its arterioles efficiently in pre-eclampsia lead to a relatively hypoxic placenta. So compensatory mechanism like enhanced production of nucleated RBCs is activated to counteract this imbalance. Thus increased NRBCs count is seen in cord blood of pre-eclamptic mothers.

59.6% women were primigravida in our study. Our results were comparable to that observed by Sylvia M Colaco *et al* [28] and Maha Al-Bayati, Basma *et al* [29]. There was a weak but significant negative correlation of gravidity and cord blood NRBC (R= -0.1393, p = 0.01)

A moderate but extremely significant negative correlation was observed between gestational age and NRBC count (R= -0.3247, p = .00001). Our observation was similar to Hermansen M C [6] and Maha Al-Bayati, Basma *et al* [29] who also observed that NRBCs have an inverse relation with the gestational age. The coefficient of regression in our study was 0.1054 indicating that only 10% of variation in NRBC is explained by gestational age. S Habber *et al* [17] in their study observed that the coefficient of regression was 0.1413, indicating that only 14% of variation in NRBC count was explained by gestational age

15.4% women had IUGR and mean NRBC count was significantly higher than that in women without IUGR. Maha Al-Bayati, Basma *et al* [29]. In their study observed IUGR in 12% women. And the difference was highly significant between IUGR and without IUGR. A study by Ghosh *et al* indicated a positive correlation between the neonatal nucleated red blood cells level, evidence of chronic ante partum asphyxia, and the presence of pregnancy induced hypertension and intrauterine growth restriction [30]. 38.3% women had LSCS for various reasons and the difference in NRBC was highly significant between women delivered vaginally and by LSCS. Similar results were observed by G, Sikarwar S, Gupta S [31] where 35% women had LSCS and by Amritanshu, *et al* [20] where 32.16% women had LSCS. Our results were quite different from that of Lode N *et al* [32] who in their study reported that 53% mother had caesarean delivery. Similarly Itoo *et al* [33] also found that deliveries by caesarean section were significantly higher in their study group.

CONCLUSION

In our study a positive correlation of NRBC count with maternal age, BMI, blood pressure was observed. No association was found among literacy status, religion and residence of the mother on cord blood NRBC count. As socio-economic status of the women improved there was increase in maternal and cord blood NRBC count. A negative correlation of NRBC count with gravidity and gestational age was

observed. Mean NRBC count was significantly higher in women with IUGR and who had LSCS.

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