Indian J Pediatr
DOI 10.1007/s12098-015-1775-6

ORIGINAL ARTICLE

Effect of Severe Maternal Iron Deficiency Anemia on Neonatal Platelet Indices

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Received: 3 February 2015 / Accepted: 15 April 2015

Abstract
Objective To evaluate the effect of maternal iron deficiency anemia (IDA) on fetal thrombopoiesis.
Methods In this prospective observational study, maternal and cord blood iron status parameters (serum iron, serum ferritin, total iron-binding capacity, and transferrin saturation), and platelet indices, such as, absolute platelet count (APC), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit, were estimated in a convenient sample of 142 mothers with IDA (hemoglobin <11 g/dl and serum ferritin <12 ng/ml) and an equal number of healthy non-anemic (hemoglobin ≥11 g/dl) mothers, who delivered singleton live neonates at term gestation. Mothers with antenatal thrombocytopenia, infections, inflammatory conditions, pregnancy-induced hypertension and neonates with perinatal asphyxia, sepsis and congenital malformations were excluded.
Results For statistical analysis, the IDA group was further subdivided into mild-to-moderate (hemoglobin 7–10.9 g/dl) and severe (hemoglobin <7 g/dl) anemia. Cord blood APC and PDW were comparable between non-anemic and mild-to-moderate anemic mothers (242,550±54,320/μL *vs*. 235,260 ±34,620/μL for APC and 16.2±1.4 *vs*. 16.4±1.8 fl for PDW, respectively), but in severe IDA group, cord blood APC and PDW were significantly lower (74,520±12,380/μL and 17.8± 2.1 fl, respectively, $p<0.001$). MPV and plateletcrit were comparable. None of the study neonates had a platelet count <30,000/μL or showed any evidence of clinical bleeding.
Conclusions Neonates born to mothers with severe IDA had moderate thrombocytopenia with increased PDW, though no change was observed in MPV and plateletcrit. Further studies should be carried out to identify the cause and consequences of this observation.

Keywords Absolute platelet count · Iron deficiency anemia · Maternal · Mean platelet volume · Neonatal · Platelet distribution width

Introduction

Iron deficiency is a leading micronutrient deficiency and a primary cause of anemia worldwide, affecting almost one-quarter of the world's population [1]. Though all age groups are affected, prevalence is higher in adolescent girls, pregnant mothers, infants and preschool children. Prevalence of anemia in women of childbearing age is approximately 40% in developing countries and 20% in industrialized countries [1], and the rate increases further during pregnancy, reaching to 59 and 24% in developing and developed countries, respectively [2].

It is well known that fetal iron store is compromised in maternal anemia. The common belief that developing fetus is a "perfect parasite," and is able to accrete sufficient iron from the mother even when she is deficient in iron [3], has been challenged and a number of studies on fetal iron homeostasis have documented low serum ferritin and other parameters of iron accretion in maternal anemia [4–6]. However, there is paucity of literature regarding the effect of maternal iron deficiency and low fetal iron store on fetal thrombopoiesis. Several studies in children

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Published online: 19 May 2015

or older age group have found a relationship between iron metabolism and thrombopoiesis with iron deficiency anemia (IDA), either in the form of abnormal platelet counts [7, 8], or abnormal platelet function [9, 10].

The present study was conducted with a hypothesis that maternal IDA may affect fetal thrombopoiesis. Maternal and cord blood iron status parameters, such as, serum iron, serum ferritin, total iron-binding capacity (TIBC), and transferrin saturation (Tfsat) and platelet indices, such as absolute platelet count (APC), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit were estimated to measure the effect of maternal iron deficiency on neonatal platelet indices.

Material and Methods

This prospective observational study was carried out in the Neonatal Unit, Department of Pediatrics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India after obtaining approval from the Institute Ethics Committee.

The study group comprised of a convenient sample of consecutively admitted 142 mothers with IDA (hemoglobin <11 g/dl and serum ferritin <12 ng/ml) and an equal number of healthy non-anemic (hemoglobin ≥11 g/dl) mothers, who delivered singleton live neonates at term gestation (37–41wk). Mothers with antenatal thrombocytopenia, congenital infections, chorioamnionitis, chronic diseases, inflammatory conditions and obstetric complications, such as, pregnancy-induced hypertension (PIH), antepartum hemorrhage, diabetes *etc*. and neonates with perinatal asphyxia, isoimmune hemolytic anemias and congenital malformations were excluded. A written informed consent in local language was taken from all parents before inclusion in the study.

Demographic details, antenatal and perinatal history including nutritional supplementation (iron, folic acid and other micro and micronutrients) received by the mother were noted. Neonates were examined thoroughly after birth to exclude any congenital malformation or other systemic diseases. Birth weight was taken soon after birth. Gestational age was determined by maternal history of last menstrual period and corroborated with antenatal ultrasonography and postnatal

Table 1 Comparison of the maternal and neonatal characteristics of the study population

Parameter	Controls (Hb ≥ 11 g/dl) (*n*=142) Group II	Mild-to-moderate anemia (Hb 7 – 10.9 g/dl) (*n*=86) Group IA	Severe anemia (Hb < 7 g/dl) (*n*=56) Group IB	ANOVA/ Chi square test (*p* value)
Maternal characteristics				
Age (years) (Mean±SD)	25.2±4.4	24.8±4.7	24.2±4.2	0.357 (NS)
BMI (kg/m^2) (Mean±SD)	24.3±3.2	21.8±2.6	20.5±2.2	<0.001
Gravida, Median (IQR)	2 (2 – 4)	2 (2–4)	2 (2–4)	1.000 (NS)
Parity, Median (IQR)	0 (0 – 1)	0 (0 – 1)	0 (0 – 1)	1.000 (NS)
Antenatal care taken, n (%)	136 (95.8)	28 (32.6)	7 (12.5)	<0.001
Iron and folate supplementation, n (%)	142 (100.0)	42 (48.9)	4 (7.1)	<0.001
Details of delivery				
Mode of delivery				
SVD, n (%)	109 (76.8)	74 (86.0)	48 (85.7)	0.140 (NS)
Cesarean section, n (%)	33 (23.2)	12 (14.0)	8 (14.3)	
Presentation				
Vertex, n (%)	131 (92.3)	82 (95.3)	51 (91.1)	0.559 (NS)
Breech, n (%)	11 (7.7)	4 (4.7)	5 (8.9)	
Neonatal characteristics				
Gestational age (wk) (Mean±SD)	38.4±1.3	38.7±1.6	38.8±1.2	0.108 (NS)
Birth weight (g) (Mean±SD)	2965±298	2680±432	2340±346	<0.001
Apgar score, Median (IQR)	9 (7–10)	9 (7–10)	9 (7–10)	1.000 (NS)

SD Standard deviation; *SVD* Spontaneous vaginal delivery; *IQR* Inter quartile range; *NS* Not significant

assessment of New Ballard Score [11]. Neonates were observed for development of any bleeding or other complications during the hospital stay and managed as per authors' unit protocol.

After complete delivery of the neonate, 5 ml of maternal venous blood was collected by aseptic venepuncture of a peripheral vein. Ten milliliter of free flowing cord blood was collected from the placental end of the umbilical cord without milking. Quantitative estimation of complete blood count (CBC) including APC, MPV, PDW and plateletcrit was done by auto analyzer (COULTER® LH 750 Hematology Analyzer, Beckman Coulter, Inc. USA). For the measurement of serum iron, ferritin, TIBC and Tfsat, serum was separated immediately by centrifugation (2000–2500 rpm/min for 15–20 min) and was measured by auto analyzer (R_X Suzuka Clinical Chemistry Analyzer RANDOX, Inc. UK).

The statistical program SPSS version 16.0 was used for data entry and analysis. Independent samples *T* test, Chi square test and analysis of variance (ANOVA) were calculated to compare the variables among different groups. After ANOVA showed significant difference, *post-hoc* Tukey-Kramer multiple comparison test was used to find out intra-group difference. A *p*-value of <0.05 was considered statistically significant.

Results

For statistical analysis, the mothers with IDA were further subdivided into mild-to-moderate (hemoglobin 7 – 10.9 g/dl; Group IA) and severe (hemoglobin <7 g/dl) anemia groups. The baseline characteristics of the study groups are shown in Table 1. Significant differences were observed in maternal BMI, receipt of antenatal care and iron and folate supplementation favoring non-anemic group ($p<0.001$). The mean birth weight of the neonates born to non-anemic mothers were significantly higher ($p<0.001$). In severe anemia group, 23 (41%) neonates were low birth weight (LBW; birth weight <2500 g).

A comparison of maternal and cord blood iron status parameters and platelet indices is shown in Tables 2 and 3. Significant differences were observed in iron status parameters among anemic and non-anemic groups. Maternal APC and platelet indices were comparable among non-anemic and anemic groups. All mothers had a platelet count >150,000/μL. Cord blood APC and PDW were comparable between non-anemic mothers and those with mild-to-moderate anemia (242,550±54,320/μL *vs.* 235,260±34,620/μL for APC and 16.2±1.4 *vs.* 16.4±1.8 fl for PDW, respectively), but APC and PDW were significantly lower in severe anemia group (74,520±12,380/μL and 17.8±2.1 fl, respectively; $p<0.001$). MPV and plateletcrit were comparable among the three groups. *Post-hoc* Tukey-Kramer multiple comparison test showed significant difference among the subgroups (Table 3).

No correlation was observed between maternal APC and cord blood APC or platelet indices. Erythrocyte count and leucocyte counts remained within the normal range in all study groups. Within the severe anemia group, there

Table 2 Comparison of maternal and cord blood iron status parameters and platelet indices between anemic (hemoglobin < 11 g/dl) and non-anemic (hemoglobin ≥ 11 g/dl) groups

Parameter	Maternal blood/ Cord blood	Controls (Hb≥11 g/dl) (*n*=142) Group I	Mild-to-moderate anemia (Hb 7 – 10.9 g/dl) (*n*=86) Group IIA	Severe anemia (Hb<7 g/dl) (*n*=56) Group IIB	ANOVA (*p* value)
Hemoglobin (g/dl) Mean±SD	Maternal	12.8±0.6	9.4±1.3	4.3±1.3	<0.001
	Cord	16.3±1.5	15.8±1.8	12.4±1.0	<0.001
Serum iron (μg/dl) Mean±SD	Maternal	124.8±18.3	54.2±8.6	32.4±7.8	<0.001
	Cord	148.4±34.6	114.8±22.4	54.6±18.2	<0.001
Serum ferritin (ng/ml) Mean±SD	Maternal	22.8±4.2	10.4±1.7	4.2±1.4	<0.001
	Cord	138.4±32.5	98.7±25.8	40.2±11.7	<0.001
Absolute platelet count (/μL) Mean±SD	Maternal	262,400±62,350	276,840±71,430	267,740±65,870	0.565(NS)
	Cord	242,550±54,320	235,260±34,620	74,520±12,380	<0.001
Mean platelet volume (fl) Mean±SD	Maternal	10.2±1.7	10.14±1.52	10.22±1.67	0.951 (NS)
	Cord	10.7±2.4	10.6±2.1	10.1±1.9	0.225 (NS)
Platelet distribution width (fl) Mean±SD	Maternal	15.2±2.1	15.8±1.92	15.4±2.24	0.108 (NS)
	Cord	16.2±1.4	16.4±1.8	17.8±2.1	<0.001
Plateletcrit (%) Mean±SD	Maternal	0.26±0.09	0.28±0.1	0.24±0.11	0.055 (NS)
	Cord	0.26±0.08	0.25±0.11	0.07±0.01	<0.001

SD Standard deviation; *TIBC* Total serum iron binding capacity; *NS* Not significant

Table 3 *Post-hoc* Tukey-Kramer multiple comparison test for intra-group differences in iron status parameters and platelet indices

Parameter	Maternal blood/ Cord blood	Group I *vs.* Group IIA	Group I *vs.* Group IIB	Group IIA *vs.* Group IIB
Hemoglobin (g/dl)	Maternal	33.933	74.322	41.297
Mean±SD		($p<0.001$)	($p<0.001$)	($p<0.001$)
	Cord	3.409	23.023	18.445
		($p<0.05$)	($p<0.001$)	($p<0.001$)
Serum iron (μg/dl)	Maternal	51.372	58.220	12.623
Mean±SD		($p<0.001$)	($p<0.001$)	($p<0.001$)
	Cord	12.164	29.405	17.343
		($p<0.001$)	($p<0.001$)	($p<0.001$)
Serum ferritin (ng/ml)	Maternal	40.365	52.429	16.060
Mean±SD		($p<0.001$)	($p<0.001$)	($p<0.001$)
	Cord	14.923	31.964	58.500
		($p<0.001$)	($p<0.001$)	($p<0.001$)
Absolute platelet count (/μL)	Maternal	–	–	–
Mean±SD	Cord	1.833 (NS)	36.590	32.165
			($p<0.001$)	($p<0.001$)
Mean platelet volume (fl)	Maternal	–	–	–
Mean±SD	Cord	–	–	–
Platelet distribution width (fl)	Maternal	–	–	–
Mean±SD	Cord	1.231 (NS)	8.529	6.858
			($p<0.001$)	($p<0.001$)
Plateletcrit (%)	Maternal	–	–	–
Mean±SD	Cord	1.247 (NS)	20.513	17.858
			($p<0.001$)	($p<0.001$)

SD Standard deviation

was no significant difference in platelet indices between LBW and non-LBW neonates (Table 4). There was no difference in parameters between neonates of two genders in any group. None of the study neonates had a platelet count <30,000/μL or showed any bleeding manifestation. All of them were discharged from the hospital without any medication or transfusion.

Discussion

In the present study moderate thrombocytopenia and increased PDW in neonates born to mothers with severe IDA was found, whereas no change was observed in MPV and plateletcrit. Platelet indices in mild-to-moderate anemia group remained comparable with the non-anemic controls.

Table 4 Comparison of cord blood platelet indices between low birth weight (birth weight < 2500 g) and normal birth weight (birth weight ≥ 2500 g) neonates born to mothers with severe anemia (hemoglobin < 7 g/dl)

Parameter	Low birth weight neonates ($n=23$)	Normal birth weight neonates ($n=33$)	Independent samples *T* Test (p value)
Absolute platelet count (/μL) Mean±SD	73,280±12,464	75,384±12,320	0.534 (NS)
Mean platelet volume (fl) Mean±SD	10.14±1.78	10.07±1.98	0.893 (NS)
Platelet distribution width (fl) Mean±SD	18.4±2.18	17.4±2.04	0.085 (NS)
Plateletcrit (%) Mean±SD	0.068±0.013	0.071±0.008	0.289 (NS)

SD Standard deviation; *NS* Not significant

There are limited studies on the effect of IDA on platelet parameters, though an association between iron metabolism and thrombopoiesis is postulated. The effect of severe maternal IDA on neonatal platelet indices has not been studied so far. Several authors have demonstrated platelet aggregation and adhesion dysfunction and its reversal after iron therapy in children with IDA [9, 10]. Perlman et al. have demonstrated an association between severe IDA and thrombocytopenia in children of age 14 mo to 17 y. The platelet count varied from 11,000 to 102,000/μL. Bone marrow examinations showed increased numbers of megakaryocytes. After treatment with oral iron, a rapid increase in platelet counts is found in all the patients. The authors postulated an essential role of iron in late stage of thrombopoiesis [8]. In adult women with IDA, Park, et al. demonstrated thrombocytosis, higher PCT, and MPV [12], whereas, others have reported a diphasic pattern of platelet response (both thrombocytosis and thrombocytopenia), in patients with IDA. Moderate IDA was found to be associated with reactive thrombocytosis, whereas severe IDA was found to decrease thrombopoiesis leading to thrombocytopenia [7, 12]. Kadikoylu et al. have shown an inverse relationship between platelet counts and MPV and a linear relationship between PDW and MPV [13].

Thrombocytopenia (APC <150,000/μL) is a common hematological abnormality in newborns, particularly documented in preterm and sick neonates. Common causes of thrombocytopenia at birth are disorders associated with placental insufficiency (*e.g.,* maternal hypertension), intrauterine growth restriction (IUGR), sepsis, perinatal asphyxia and neonatal alloimmune thrombocytopenia [14–17]. Except IUGR, the authors excluded all other conditions in index study. They did not include preterm neonates as platelet counts may be low even in normal healthy preterm newborns [18]. Though there were cases with IUGR and LBW, no difference in platelet indices was observed between IUGR and their age-appropriate counterparts, concluding that it was not growth restriction but severe maternal IDA which had led to the changes in platelet indices.

It is difficult to explain how severe maternal IDA reduced platelet count in neonates at birth. Neonatal platelet production is a complex process involving four major steps; production of thrombopoietin, proliferation of megakaryocyte progenitors, differentiation and maturation of these progenitor cells and finally the production and release of platelets into systemic circulation [18]. Since iron is an essential micronutrient for the growth of placenta [19], hypoferremia-induced placental dysfunction might have led to the suppression of fetal megakaryopoiesis. The authors also observed that the mean MPV was comparable among the groups, though a trend was seen towards smaller platelet size in severe IDA. Large-sized platelets denote high metabolic activity and often have been reported in association with neonatal sepsis [20, 21]. Several authors documented organism-specific changes in thrombocytes [20, 22, 23]. Larger platelet size has also been associated with poor prognosis in neonatal sepsis [21, 24], or septic shock in older age group [25].

The clinical implication of moderate thrombocytopenia in neonates born to mothers with severe IDA is not clear. None of the neonates had a platelet count less than 30,000/μL or any bleeding manifestation or required any therapy. The major limitation of the index study is that authors did not measure platelet function or megakaryocytopoiesis by measuring thrombopoietin. Secondly, they did not follow the platelet indices in these infants to document the outcome of moderate thrombocytopenia.

To conclude, it is observed that severe maternal IDA, but not mild-to-moderate anemia, leads to moderate thrombocytopenia and increase in PDW in cord blood of term neonates. Further studies may be carried out to identify the cause and consequences of this observation.

Acknowledgments The authors would like to thank all the residents who managed the neonates and all the mother-infant pairs who were included in the study.

Contributions SB: Conceptualization and design of the study, collection of data, patient management, critical literature review and drafting of the manuscript; NK: Concept and study design, collection of samples, patient management, literature review and drafting of the manuscript; RS: Concept and study design, biochemical analysis of samples, literature review and drafting of the manuscript; AK: Concept and study design, patient management, critical literature review and drafting of the manuscript. All authors have approved the final version of the manuscript. SB will act as guarantor for this paper.

Conflict of Interest None.

Source of Funding None.

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