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ORIGINAL ARTICLE

Expression of iron transport protein Divalent metal transporter 1 (DMT1) increases in response to maternal iron deficiency anemia in near term to term placenta

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ABSTRACT

Introduction: Iron deficiency anemia (IDA) is the most prevalent nutritional deficiency disorder in pregnant women. During pregnancy, placental transport protein Divalent metal transporter1 (DMT1) plays a crucial role in transit of iron across placenta. The developing fetus is observed to be immune to anemia despite presence of anemia in the mother. Hence, we planned the present study to explore the effect of maternal IDA on the expression of DMT1 in the placenta.

Materials and methods: Two hundred pregnant women recruited, were divided into anemic and nonanemic groups based on their predelivery hemoglobin levels ($<$11 g/dL and $\geq$11 g/dL respectively). After delivery, placental expression of DMT1 was studied by immunohistochemistry and mRNA analysis and neonatal anthropometry was performed.

Results: Of the 200 women recruited, 58.8% were anemic with 60.35% having moderate anemia. Most of the red cell parameters were observed to be higher in cord blood than mothers. DMT1 protein immunohistochemical expression showed a statistically significant increase with increasing severity of anemia. Similarly, placental mRNA expression levels of DMT1 gene were observed to be higher in anemic mothers in comparison with nonanemic mothers.

Conclusion: Our study thus demonstrated a definite increase in expression of DMT1 at both protein and mRNA levels in term placenta, in maternal IDA.

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KEYWORDS
Cord blood; DMT1; iron deficiency anemia; m-RNA; pregnancy

Introduction

Iron deficiency anemia is the most prevalent nutritional deficiency disorder in the world [1]. During pregnancy, women are at high risk for the development of iron deficiency anemia [2]. Anemia in pregnancy leads to adverse birth outcomes like low birth weight and increased risk of maternal and perinatal mortality [3].

During pregnancy, the placenta transports nutrients from the mother to fetus *via* specialized nutrient transporter proteins localized to the villi of the placentas [4]. Divalent metal transporter1 (DMT1), is a metal-ion transporter in mammals, which mediates the transport of multiple divalent metal ions with a very high affinity for iron [5]. DMT1 is involved in the crucial step of iron transport in the placenta by releasing iron from endosomes into the cytoplasm of syncytiotrophoblasts.

Studies have found that the developing fetus is immune to anemia and can accumulate sufficient iron even in the face of iron deficiency in mothers [6]. Our earlier study showed interesting results in which newborns of anemic mothers had normal blood parameters despite the presence of anemia in mothers. Literature search performed by us revealed few to no studies on DMT1 expression in the placenta, in maternal iron deficiency [7,8].

We thus planned to study the protein and mRNA expression level of DMT1 in term placentas of women with anemia.

Materials and methods

In this cross-sectional study, 200 pregnant women between 36 and 42 weeks of gestation were enrolled. The institutional ethical committee report of National

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Institute of Nutrition (NIN) and Gandhi hospital was obtained before the start of the study.

The subjects, found to be anemic on admission, were asked to participate in the study after signing an informed consent form. Information on sociodemographic factors, dietary intake, anthropometry and clinical status was collected.

The study groups were as follows:

Pregnant women with hemoglobin (Hb) <11 g/dL as defined by the World Health Organization were included in the anemic group while healthy pregnant women with Hb ≥11 g/dL were included in the nonanaemic group.

Pregnant women 18–45 years of age, between 36 and 42 weeks of gestation and with singlepregnancy (Primiparous or multiparous) were included while those suffering from hemolytic anemia, hypertension, diabetes mellitus, thyroid disease and were HIV, HCV, HBsAg positive were excluded from the study.

Sociodemographic and anthropometric information

Using a well-designed questionnaire, information on age, family history, socioeconomic status and clinical history of the subjects were obtained. Weight and height of the mothers were recorded for calculating body mass index (BMI). After birth, anthropometric measurements of newborns were noted.

Sample collection and processing

Ten milliliters of blood was collected from the mothers, before delivery, in ethylenediaminetetraacetic acid (EDTA) and plain vacutainers (Beckton Dickinson). After delivery, 10 ml of cord blood was collected in similar vacutainers and immediately transported in ice to Pathology lab of NIN.Hemoglobin(Hb), red cell indices and serum ferritin were the parameters studied in maternal and cord blood.Hb estimation and complete blood count (CBP) was performed in an automated Coulter counter (Advia 120, Seimens). Hb, differential count, total leucocyte count (TLC), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) and hemoglobin distribution width (HDW) were analyzed. The serum was separated and stored at −80 °C until further analysis for ferritin using ferritin SA ELISA kit of Calbiotech, Inc (CBI) which uses solid-phase sandwich assay method, based on the streptavidin-biotin principle.

After delivery, placentas were collected and for m-RNA analysis, biopsies were taken from the maternal side of central cotyledons, omitting the membranous layer, about 1 cm below the surface and were kept in 5 ml of RNA *later* solution and stored at −80 °C until further analysis. The whole placentas were stored in containers filled with 10% neutral buffered formalin. After overnight fixation the placentas were weighed after removal of cords and membranes, their size, shape and gross findings were noted. Four sections were taken, about 2–4 cm from either side of the center, away from the margins and close to the maternal surface of the placentas. The tissues were processed in an automatic tissue processor (Shandon), embedded in paraffin and 5-μm thick sections taken.

The immunohistochemical expression for DMT1 protein was studied in formalin-fixed and paraffin-embedded placental tissues. The primary antibody used was SLC11A2 polyclonal antibody by Elabscience and the secondary antibody was Dako Real Flex Mini Envision Detection with peroxidase/Wash buffer/ Antigen retrieval buffer/DAB+, Rb/Mo one-Step Method. The stained sections were studied under a light microscope (Nikon Eclipse E800) by two histopathologists, and relevant images were captured in a digital camera attached to the microscope. Immunoreactivity was classified by estimating the percentage (*P*) of placental trophoblast cells showing the characteristic staining (from an undetectable level or 0% to homogeneous staining or 100%) and by estimating the intensity (I) of staining (1- weak staining, 2- moderate staining and 3-intense staining). Results were scored by multiplying the percentage of positive cells by the intensity, ie.by the so-called quick score (*Q*) ($Q = P \times I$; maximum = 300) [9].

After birth, the weight, crown-rump length, head, midarm circumference and skinfold thickness of the newborns were also recorded.

We analyzed the m-RNA expression of DMT1 gene by manual Trizol method. The snap-frozen placental tissue was initially subjected to the Trizol procedure and treated with DNase. About 20 mg of the placental tissue was treated with 1 ml of the TRI reagent in a 1.5-mL microcentrifuge tube before proceeding for total RNA isolation. The isolated RNA's quantity and purity were measured spectrophotometrically by measuring the OD at absorbance ratios of 260/280 and OD 260/230, respectively, using a Nanodrop 2000c spectrophotometer (Thermoscientific). RNA isolated (1 μg) per target was treated with DNase1, according to the manufacturer's instructions. The total RNA (200 ng) was reverse-transcribed into cDNA by using a transcriptor

cDNA synthesis kit (Bio-Rad). The reverse transcription reaction was carried out using a thermocycler (Applied Biosystems), under the following conditions; 25 °C for 5 min, 46 °C for 20 min, 95 °C for 1 min with a hold at 4 °C. RT-PCR primers for DMT1 have been previously described [10]. RT-PCR reactions were carried out using light cycler CFX 96 (Bio-Rad) and each reaction contained 0.5 μL of the primer (Bioartis), 10 μL 2× SYBR Green PCR Mastermix (Thermoscientific), 8 μL of nuclease-free water and 1 μL of 15 ng/μL of cDNA in 15-μL reaction. We set PCR reactions at 95 °C for 3 min, then 95 °C for 15 s and finally at 57 °C for 30 s (40 repeats). We obtained the results as cycle threshold, and single melt curves were obtained for all samples, indicating that a single PCR product is generated. We normalized the gene expression to β-actin, and relative expression of the gene of interest was expressed as $2^{-\Delta CT}$. We pipetted each sample into 96-well plates and ran the samples in duplicate. Negative control of PCR-grade water and positive control (human placental tissues) were used to correct for plate-to-plate variation. We included β-actin on each plate as the reference gene. The following primers were designed by using National Center for Biotechnology Information sequence ID and were purchased from Bio-Artis: DMT1 (divalent metal transporter): forward: 5′- CAC CGT CAGTATCCCAAGGT-3′, reverse: 5′- CATGTCTGAGCCGAT GAT AGC-3′ and β-actin: forward: 5′- CCAACCGCGAGAAGA TGA-3′, reverse: 5′- CCA GAG GCG TAC AGG GAT AG-3′. We controlled plate-to-plate variation by normalizing the gene expression to β-actin and a control placenta by using the ΔΔCT method.

Statistical analysis

We assumed a 95% confidence interval, a prevalence of 15% anemia in newborns and the margin of error being 5%, the required sample size calculated was 196.

We performed data processing and statistical analyses using SPSS version 19.0 (SPSS Inc, Chicago, IL). Continuous data were summarized as means ± SD and categorical data as numbers (%). We calculated descriptive statistics like mean, standard deviation (SD) and prevalence for all variables. We compared mean values for all variables by unpaired "t" test across both healthy and anemia groups. We calculated the relationships between Hb, MCV, MCH, MCHC, RBC, RDW, HDW, serum ferritin by correlation coefficients, and performed the chi-square test for associations. We performed a nonparametric test wherever required. We carried out Pearson's correlation analysis to evaluate the correlation between different variables. We considered the level of significance as 0.05.

Results

We recruited 200 pregnant women (total), 58.8% of them are anemic. Among the women with anemia, 60.35% have moderate anemia, 27.65% mild anemia and 12% severe anemia.

Clinical characteristics (Table 1)

71.6% of the women are in the age group of 18–23 years, among whom 38.5% are anemic.We observed a BMI > 23 in 75.25% of women of both groups. Nineteen women (9.6%) are college-educated while 89 (45.2%) each are illiterate and school educated. 59.6% of the illiterate women are anemic. Among the 86.8% unemployed women, 60.2% are anemic. The monthly family income of 68.7% is between Rs. 5000 and 10,000.

Table 1. Sociodemographic and economic characteristics of pregnant women.

Variables	Values	Anemic mothers (Hb < 11 g/dL) *n* (%)	Non-Anemic mothers (Hb ≥ 11 g/dL) *n* (%)	All mothers *n* (%)	*p*-Value
Age in years	18–23	37 (38.5%)	59 (61.5%)	96 (100%)	.52
	>23	9 (31%)	29 (69%)	29 (100%)	
BMI (kg/m^2)	Overall BMI	24.50 ± 2.51	24.71 ± 2.51	24.59 ± 2.43	.55
	<18.5	0 (0)	1 (100)	1 (100)	
	18.5–23	16 (33.3)	32 (66.7)	48 (100)	
	>23	66 (44.3)	83 (55.7)	149 (100)	
Education status	Illiterate	53 (59.6)	36 (40.4)	89 (100)	.13
	Schooling	48 (53.9)	41 (46.1)	89 (100)	
	College	15 (78.9)	4 (21.1)	19 (100)	
Occupation	Working	13 (50)	13 (50)	26 (100)	.39
	Not-working	103 (60.2)	68 (39.8)	171 (100)	
Monthly income of family in Rupees	<5000	9 (69.2)	4 (30.8)	13 (100)	.65
	5000−10,000	80 (58.8)	56 (41.2)	136 (100)	
	10,000−50,000	27 (55.1)	22 (44.9)	49 (100)	

p-Value was considered significant if $p < 0.05$.
BMI: body mass index; Hb: hemoglobin; g/dL: grams per deciliter; kg/m^2: kilograms per meter square.

Maternal red cell parameters are displayed in Table 2 where Hb, RBC count, PCV, MCV, MCH, MCHC, including serum ferritin are significantly lower in anemic mothers while RDW and HDW are significantly higher.Hb, PCV, RBC, MCVand MCH are higher while serum ferritin levels are significantly lower in cord blood of anemic mothers (Table 3). All neonatal anthropometric parameters are lower in newborns of anemic mothers (Table 4). Pearson's correlations highlight the negative correlation of maternal Hb with cord blood Hb but significant positive correlation with cord blood ferritin. Cord blood Hb shows a negative correlation with maternal ferritin (Table 5).

Serum ferritin values were significantly lower in anemic mothers (Graph 1). Among the different grades of anemia, serum ferritin is lowest in severe anemia (Graph 2). Higher levels of serum ferritin were observed in cord blood when compared to the mother's blood (Graph 3). A statistically significant increase in immunohistochemical staining for DMT1 was observed in placental cells with increasing severity of anemia (Graph 4). Figures 1–5 show immunohisto-

Table 2. Blood cell parameters in the anemic and nonanaemic pregnant women.

Variables	Anemic mothers (Hb < 11 g/dL) (n = 121) (59.02%)	Non-Anemic mothers (Hb ≥ 11 g/dL) (n = 84) (41%)	All mothers (n = 205)	p-Value
Hb g/dL	8.86 ± 1.54 (3.7–10.9)	12.44 ± 1.09 (11–15.4)	10.33 ± 2.23 (3.7–15.4)	.00***
RBCs/L	11.38 ± 4.97 (1.08–5.16)	13.33 ± 4.79 (2.84–0.09)	12.18 ± 4.97 (1.08–5.16)	.00***
PCV in %	25.48 ± 5.02 (9.30–2.60)	35.55 ± 31.91 (24.80–323.00)	29.6 ± 21.30 (9.30–323.00)	.00**
MCV in fl	71.61 ± 10.63 (44.79–118.00)	75.49 ± 7.90 (60.50–103.00)	73.20 ± 9.77 (44.79–118.00)	.00**
MCH in pg	25.11 ± 4.47 (15.00–42.60)	29.35 ± 3.17 (22.30–38.90)	26.85 ± 4.49 (15.00–42.60)	.00***
MCHC in g/dL	35.02 ± 4.00 (24.17–49.70)	39.00 ± 3.46 (31.70–45.30)	36.65 ± 4.26 (24.17–49.70)	.00***
RDW in %	16.85 ± 2.85 (11.70–24.80)	14.64 ± 2.53 (10.70–24.00)	15.94 ± 2.93 (10.70–24.80)	.00***
HDW in %	3.76 ± 1.49 (0.96–14.60)	3.72 ± 1.52 (1.06–13.40)	3.74 ± 1.50 (0.96–14.60)	.88
Ferritin ng/mL	16.70 ± 23.50 (1.04–147.30)	32.15 ± 28.87	23.07 ± 26.88 (1.04–147.3)	.00***

All values are in mean ± SD and those within brackets are in range.
*p < .05, **p < .01, ***p < .001.
Hb: hemoglobin; RBC: red blood cells; PCV: Packed cell volume/hematocrit MCV – Mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; fl: fentolitre; pg: pictogram; ng: nanogram; mL: millilitre; RDW: red cell distribution width; HDW: hemoglobin distribution width; SD: standard deviation.

Table 3. Comparison of blood cell parameters between cord blood of anemic and nonanaemic mothers.

Variables	Cord blood anemic mothers (Hb < 11 g/dL) (n = 113)	Cord blood non-anemic mothers (Hb ≥ 11 g/dL) (n = 78)	Total (n = 191)#	p-Value
Hb g/dL	15.83 ± 2.08 (11.10–23.30)	15.63 ± 2.25 (7.40–21.70)	15.75 ± 2.15 (7.40–23.30)	.51
RBCs/L	4.37 ± 0.52 (3.27–5.80)	4.31 ± 0.56 (2.12–5.49)	4.35 ± 0.54 (2.12–5.80)	.46
PCV in %	42.42 ± 7.85 (21–65.8)	39.47 ± 6.75 (18.8–58)	41.22 ± 7.54 (18.8–65.8)	.00**
MCV in fl	97.17 ± 11.54 (73.6–146.4)	91.6 ± 9.97 (65.1–116.4)	94.9 ± 11.24 (65.1–146.4)	.00**
MCH in pg	36.23 ± 2.29 (28.1–45.1)	36.23 ± 2.92 (25.1–45.4)	36.23 ± 2.56 (25,1–45.4)	.99
RDW %	15.45 ± 2.77 (9.9–25.8)	16.1 ± 3.34 (10.3–32)	15.73 ± 3.03 (9.9–32)	.12
Cord Ferritin ng/mL	96.94 ± 79.05 (5.57–352.7)	134.72 ± 87.87 (11.36–478.90)	112.46 ± 84.65 (5.57–478.9)	.00**

All values are in mean ± SD, and those within brackets are in the range.
*p < 0.05, **p < 0.01, ***p < 0.001.
The #Cord blood of the remaining nine newborns was clotted and hence could not be analyzed.
Hb: hemoglobin; RBC: red blood cells; PCV: packed cell volume/hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; fl: fentolitre; pg: pictogram; ng: nanogram; mL: millilitre; RDW: red cell distribution width; HDW: hemoglobin distribution width; SD: standard deviation.

chemical staining in trophoblasts, which is observed to be weak in mild anemia and strongly positive in severe anemia.mRNA expression of DMT1 gene is higher in anemic women in comparison to nonanaemic women (Graph 5).

Discussion

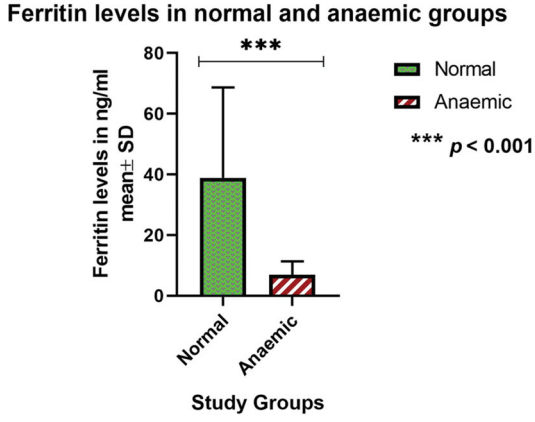
Iron transfer from mother to fetus takes place across the placenta with the help of iron transport proteins [11]. Our previous study demonstrated interesting results wherein newborns of anemic mothers had normal haematological values despite maternal anemia.

We enrolled 200 pregnant women, 58.8% of whom were anemic. The prevalence of anemia in our study was higher than the national prevalence of 50.3 and 49.8% state prevalence [12]. However, other studies have reported a lower prevalence of anemia [13,14]. The high prevalence of anemia is an important finding in our study indicating that maternal anemia is still a rampant problem in our country, which needs tackling at the community level at the earliest. The majority (60.35%) of the anemic women had moderate anemia followed by 27.65% with mild anemia and the least (12%) with severe anemia. Contrary to the present study, other studies, however, reported mild anemia as the commonest [12,15–17]. The reason for the higher incidence of moderate anemia (60.35%), in our study, could be either poor compliance of the women in taking iron and folic acid tablets supplied by the

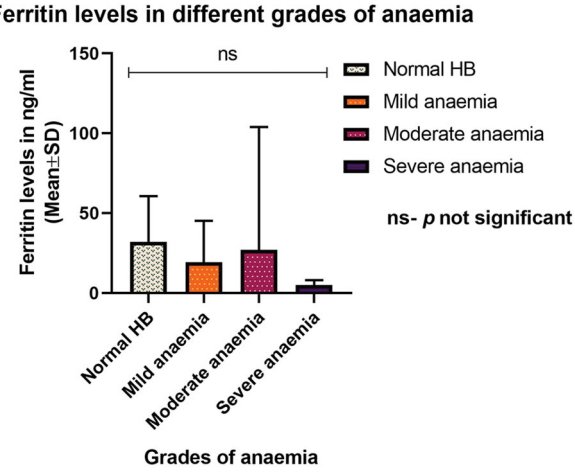
Table 4. Anthropometric data of newborns of anemic and nonanaemic mothers.

Variables	Anemic group	Non-Anemic group	*p*-Value
Placental weight in g	416.03 ± 90.65	422.82 ± 91.66	.609
BMI mothers in kg/m^2	24.50 ± 2.51	24.71 ± 2.51	.551
Birth weight in kg	2.80 ± 0.38	2.87 ± 0.49	.258
Crown-rump length in cm	30.48 ± 1.47	30.52 ± 1.68	.882
Skinfold thickness in cm	1.24 ± 0.19	1.27 ± 0.26	.300
Head circumference in cm	30.70 ± 1.48	30.78 ± 1.65	.702
Midarm circumference in cm	12.90 ± 1.09	12.91 ± 1.17	.937

All values are in mean ± SD.
BMI: body mass index; cm: centimeters; g: grams.



Graph 1. A comparison of serum ferritin values between anemic and non-anaemicnonanaemic pregnant women.

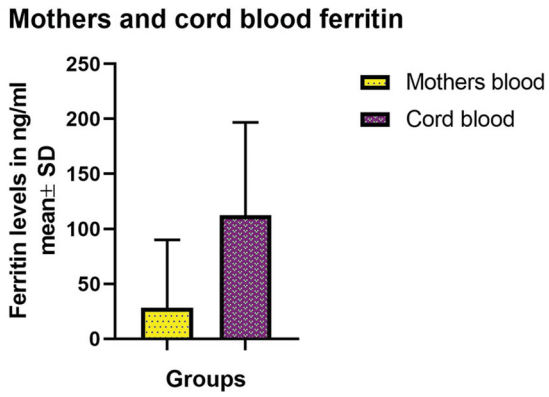


Graph 2. Serum ferritin values in pregnant mothers with different grades of anemia.

Table 5. Pearson's correlations between maternal and cord blood parameters.

Parameters	Hb (M)	RBC (M)	PCV (M)	MCV (M)	MCH (M)	MCHC (M)	RDW (M)	HDW (M)	S.Ferritinin (M)
Hb (C)	−0.023	0.854[a]	0.809[a]	0.314[a]	0.412[a]	−0.080	−0.139[b]	0.113	−0.133[b]
RBC (C)	−0.008	0.113	0.726[a]	0.051	−0.116	−0.154[c]	−0.068	0.102	−0.125
PCV (C)	−0.164[c]	0.726[a]	−0.019	0.684[a]	0.259[a]	−0.595[a]	−0.325[a]	0.067	−0.150[c]
MCV (C)	−0.241[c]	0.051	0.684[a]	0.266[c]	0.514[a]	−0.779[a]	−0.415[a]	−0.013	−0.110
MCH (C)	−0.025	−0.116	0.259[a]	0.514[a]	−0.07	0.118	−0.137[b]	0.048	−0.035
MCHC (C)	0.270[a]	−0.154[c]	−0.595[a]	−0.779[a]	0.118	0.473[c]	0.402[a]	0.074	0.117
RDW (C)	0.105	−0.068	−0.325[a]	−0.415[a]	−0.137[b]	0.402[a]	−0.09	0.840[a]	−0.037
HDW (C)	0.073	0.102	0.067	−0.013	0.048	0.074	0.840[a]	0.135	−0.065
S.Ferritinin (C)	0.201[c]	−0.125	−0.150[b]	−0.110	−0.035	0.117	−0.037	−0.065	0.053

[a]Correlation is significant at the 0.001 level (2-tailed).
[b]Correlation is significant at the 0.05 level (2-tailed).
[c]Correlation is significant at the 0.01 level (2-tailed).
(M): Mothers; (C): Cord blood.
Hb: Hemoglobin; RBC: Red blood cell; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width; HDW: Hemoglobin distribution width; S.Ferritin: Serum ferritin.



Graph 3. A comparison of serum ferritin values between the mothers and cord blood.

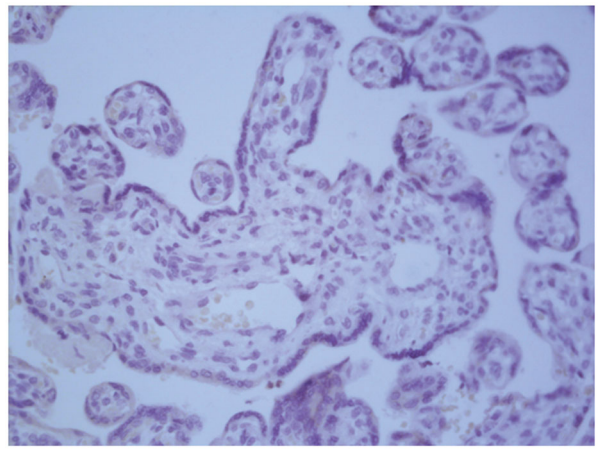
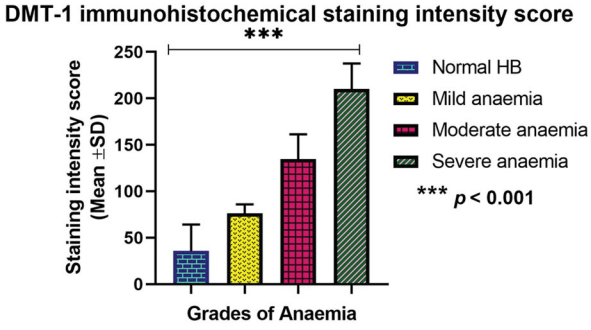


Figure 2. Microphotograph shows immunostaining for DMT1 in placentas from nonanaemic mothers. The trophoblastic cells show nil to faint immunostain. DMT1 immunostain; original magnification, ×40.



Graph 4. The immunohistochemistry staining intensity score for DMT1 in the placenta, in different grades of anemia in the mothers.

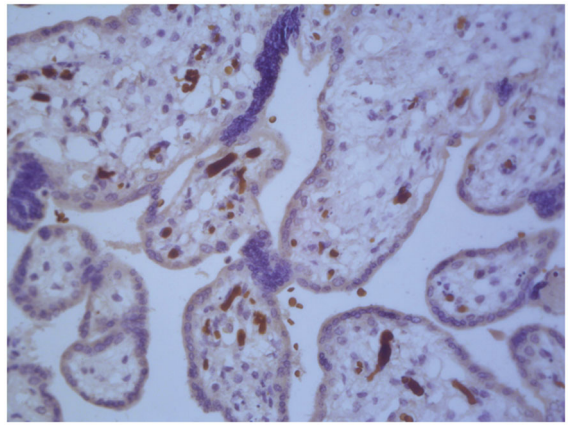


Figure 3. Microphotograph shows immunostaining for DMT1 in placentas from mothers with mild anemia. A mild degree of immunostaining is observed in the cytoplasm of the trophoblastic cells. DMT1 immunostain; original magnification, ×40.

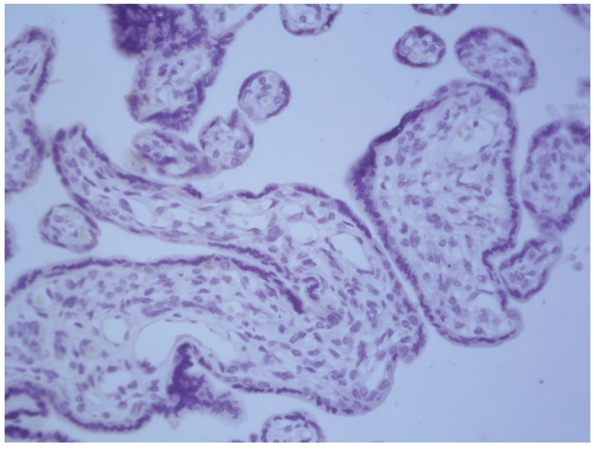


Figure 1. Microphotograph is of negative control in which the trophoblastic cells show no immunostain as the only secondary antibody is added. Original magnification, ×40.

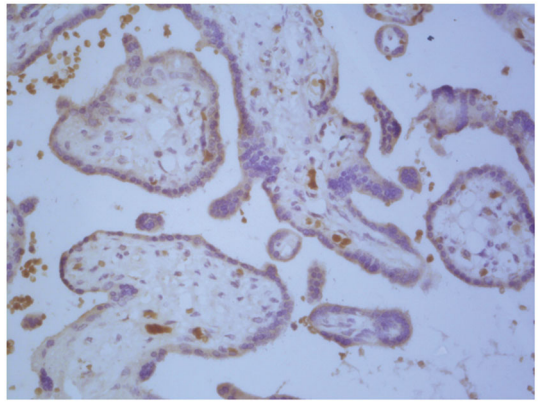


Figure 4. Microphotograph shows immunostaining for DMT1 in placentas from mothers with moderate anemia. A moderate degree of immunostaining is observed in the cytoplasm of the trophoblastic cells. DMT1 immunostain; original magnification, ×40.

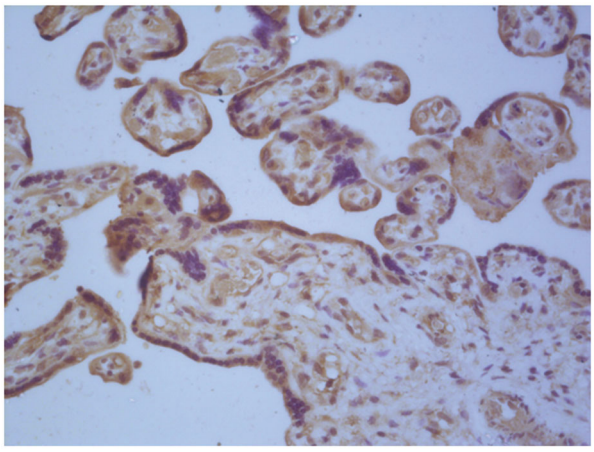
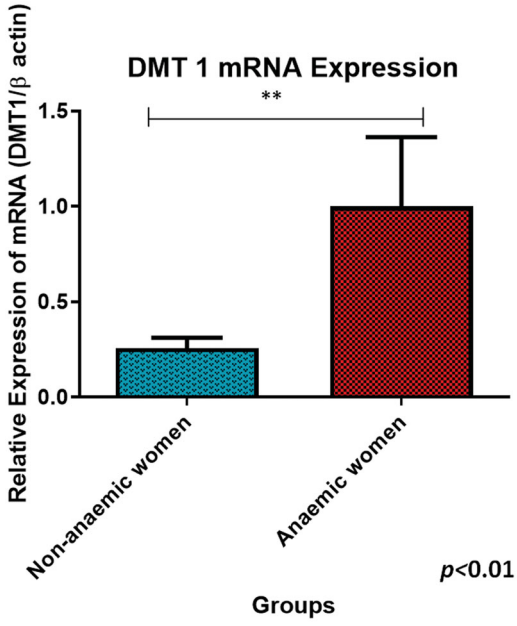


Figure 5. Microphotograph shows immunostaining for DMT1 in placentas from mothers with severe anemia. Intense staining is observed in the cytoplasm of the trophoblastic cells. DMT1 immunostain; original magnification, ×40.



Graph 5. mRNA expression levels of DMT1 in the placentas of anemic and non-anaemicnonanaemic mothers.

government or associated vitamin B12 and folate deficiencies. Moreover, it also a disturbing finding which calls for more in-depth analysis of the cause and also measures to tackle it, as maternal anemia has numerous long term adverse effects on the fetus.

Majority of the women (75.25%) had an association between anemia and a high BMI > 23, which is similar to other studies [18,19]. Although obesity and iron deficiency usually represent opposite ends of the spectrum of malnutrition, the association between high BMI and anemia in our study proves to be a significant finding and thus needs to be addressed as a measure of nutritional and health status.

We observed that most of the anemic women were illiterate and unemployed in our study. This observation is in agreement with the study [19], in which a significant number of women with anemia were illiterate and unemployed. These findings highlight the fact that illiteracy, low family income and lower levels of education are risk factors for the development of anemia either due to inaccessibility of the women to food or due to lack of their knowledge on the intake of iron-rich food which thus leads to the development of anemia.

Maternal red cell parameters like Hb, RBC count, PCV, MCV, MCH, MCHC, including serum ferritin, were significantly and predictably low in anemic mothers, consistent with other studies [17]. Another parameter useful for the diagnosis of iron deficiency is RDW, which is a quantitative measure of anisocytosis. In iron deficiency, RDW levels are always found to be increased [18]. In our study, too, RDW of anemic pregnant women was observed to be higher when compared to nonanaemic ones, thus corroborating the literature [20,21].

Many studies have been carried out in maternal and cord blood.While some studies have reported a negative impact of maternal iron deficiency anemia on the iron stores of the newborns [22], others could not find any relationship [23]. However, Jaime-Pérez et al. and other investigators [14,17,24] had reported normal to above normal Hb levels in the cord blood thus corroborating with our mean Hb value of15.75 ± 2.15 g/dL, which is above the average cord blood Hb value of 13 g/dL. This finding shows that the fetus maintains normal Hb levels irrespective of maternal Hb levels.

A negative correlation was found between cord blood Hb and mother's Hb, RBC, PCV, MCV and MCH. This finding thus indicates that cord blood Hb values are independent of the mother's values, unlike Sameer T et al. who found a positive correlation [25].

Placental weight, the weight of newborns, crown-rump length, midarm and head circumference, all were observed to be lower in the newborns of anemic mothers when compared to the nonanaemic women, consistent with other studies [26,27]. This highlights the crucial role played by iron as an essential nutrient for the development and growth of the fetus.

DMT-1 is a protein which is critically involved in the transfer of iron from the syncytiotrophoblastic endosome into the cytoplasm.It has variably been reported to have a punctate cytoplasmic distribution and to be localized predominantly to the apical [28,29] or basal membranes [29,30] of syncytiotrophoblasts [31]. Few animal studies show that, in the presence of a low

supply of micronutrients in the maternal diet, signals from the fetus can upregulate the expression of micronutrient transporters in the placenta for its nutrition . Li et al. treated BeWo cells with desferrioxamine (DFO) and found that both DMT1 mRNA and protein increased significantly with DFO treatment [31]. However, there are very few studies on the behavior of DMT-1 in human subjects, especially in the context of anemia in pregnancy.

Moreover, the interesting results of our previous study in which the newborns presented with higher Hb levels prompted us to undertake the present study in which we hypothesized that maternal iron deficiency increases placental DMT-1 expression by upregulating the DMT-1 gene.In our study, the immunohistochemical staining for the DMT1protein was localized to the cytoplasm of the trophoblastic cells and was also observed to significantly increase with increasing severity of anemia.In mild anemia, the trophoblastic cells showed a weak staining intensity while in severe anemia; the cells showed intense positive staining.

In order to further strengthen our findings and test our hypothesis, we studied the expression of DMT-1 at the genetic level by performing the m-RNA analysis of the DMT1 gene in placental tissue. Consistent with the protein expression, mRNA expression, too, was observed to be higher in anemic women when compared to the nonanaemic women.Thus, our study for the first time showed that in maternal iron deficiency anemia, there is upregulation of DMT-1 gene leading to increased expression of DMT1 protein in the placenta, thus confirming our hypothesis.

Our study shows that apart from a high prevalence of moderate anemia in pregnant women of our city, there is increased expression of DMT1 in the placenta at both protein and mRNA level which probably explains the immunity of fetus to development of anemia despite the presence of maternal anemia.

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Disclosure statement

All the authors declare that they have no conflicts of interest related to this study, and the results of this manuscript have not been distorted by research funding or conflicts of interest.

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