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Pathophysiological changes in cord blood and placenta in anaemic pregnant women

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Abstract
Background: *Infant and maternal mortality has raised serious concerns since ages. Ongoing researches in this field aim to develop insights in successful maternal and infant health care programmes.*
Objective: *A comparative study of pathophysiological changes in cord blood and placenta in anaemic pregnant women with normal pregnant women.*
Method: *This study was conducted in the department of Physiology in collaboration with the department of Obstetrics and Gynaecology and department of Pathology, Govt. Medical College, Kannauj after taking permission from the institutional Ethics Committee. Sample size included 100 third trimester pregnant women of 20-40 yrs. Group I included subjects (n=50) with haemoglobin concentration more than 11gm/dl and group II included subjects (n=50) with haemoglobin concentration less than 11gm/dl. Haematological parameters (haemoglobin concentration, total RBC count, Blood Indices, total platelets count, total WBC count, differential leucocyte count in cord blood) and gross changes in placental morphology (weight, shape, surface area, thickness, no of cotyledons) and histopathological changes (syncytial knot formation, fibrinoid necrosis, syncytial trophoblastic proliferation, hyalinised villi, calcification in placenta) were duly studied.*
Results: *Hb concentration, RBC count, MCV, MCH, MCHC, Absolute neutrophil count, Absolute lymphocyte count were significantly lower in anaemic mothers, but no statistical significant differences were noted between mother's and her cord blood investigations.The placental weight, thickness, surface area were significantly higher in anaemic group. In anaemic group, more mean number of cotyledons were observed. Calcified areas, Syncytial knot formation, Fibrinoid necrosis, hyalinised villi, perivillous fibrin deposition, intervillous space, hypovascular villi were seen significantly more in anaemic group as compared to normal group.*
Conclusion: *Anaemia in pregnancy influence haematological changes in cord blood and morphology of placenta which in turn adversely affect the perinatal outcome.*
Keywords: *Anaemia, Placenta, Cord blood, Third Trimester.*

Introduction

Anaemia in pregnancy is very common, but severe anaemia in pregnancy may have adverse effects on pregnancy, delivery and neonatal infants. Anaemia is the nutritional deficiency disorder and 56% of all women living in

developing countries are anaemic (World Health Organisation)[1]. It is the most common haematological disorder during pregnancy. Prevalence of anaemia in South Asian countries is among the highest in the world and India has the highest prevalence of anaemia (87%)[2].

According to the report of World Health Organisation (WHO), in developing countries, 35% to 75% (56% on average) of pregnant women and in industrialised countries, 18% of women are anaemic[2].

According to the World Health Organization (WHO), anaemia contributes to 40% of maternal deaths in third world countries. In India, anaemia contributes to 10-15% of direct maternal deaths. According to the WHO, a haemoglobin level less than 11g/dl is considered as anaemia during pregnancy. The centres for Disease Control and Prevention has define anaemia as less than 11g/dl in the first and third trimester and less than 10.5 g/dl in the second trimester. Indian Council of Medical Research classifies anaemia in pregnancy as Mild: 10.1 to 10.9 g/dl, Moderate: 7.1 to 10.0 g/dl, Severe: 4.1 to 7.0 g/dl, Very severe: 4.0 g/dl and below[4].

Anaemia is known to be associated with multiple factors such as poor socioeconomic status, high parity, short birth interval, poor diet both in quantity and quality, lack of health and nutrition awareness, and a high rate of infectious diseases and parasitic infestation[2].

In developing countries, underprivileged people have often limited access to medical care and preventive measures, increasing their risk of becoming anaemic and contributing to high maternal mortality[2]. Sideropenic anaemia is a common pregnancy disorder, with the incidence among pregnant women from 20 to 40%.

Depending on severity, maternal anaemia can significantly influence morphometric characteristics of placental tissue, pregnancy course and outcome[3]. So, in this study we tried to study the haematological parameters in maternal and cord blood along with morphological and histological changes in placenta of anaemic mothers, which may affect the status of the mothers and their foetus. Thus an early diagnosis may allow prompt treatment or facilitate proper planning to improve quality of life in these mothers and their newborns.

Materials and Methods

This study was conducted in the department of Physiology in collaboration with the department of Obstetrics and Gynaecology and department of Pathology, Govt. Medical College, Kannauj after taking permission from the institutional Ethics Committee.

Inclusion and exclusion criteria in the study-

Inclusion

- Pregnant women aged 20-40 years.
- Pregnant women in third trimester from the outpatient department (OPD) or on the day of delivery admitted in labour room or operation theatre.
- Women with primary and multiple pregnancies.
- Pregnant women with normal haemoglobin concentration (>11gm/dl) in group I and haemoglobin concentration (<11gm/dl) in group II were included.
- Non-smokers, non-alcoholic, non-diabetic mothers having perfect sense of physical, mental and psychological well being.

Exclusion Criteria

- Elderly women aged more than forty years.
- Pregnant women in 1st and 2nd trimester.
- Pregnant women with a known case of coronary artery disease/ischemic heart disease or congenital heart disease.
- Subjects having a history of neurological disorder, diabetes, with features of hypo- or hyperthyroidism, patients on any drug that alters the sinus node impulse generation and AV conduction, patients with fever and features suggestive of infections, patients with chronic obstructive pulmonary disease and other chronic lung disorders were excluded.

Sample size included in the study was 100 pregnant women aged 20-40 years.

Patients were divided into two groups-

The subjects that satisfied the inclusion and exclusion criteria were divided into:

1. Group I- *Normal healthy group I (n=50)*

2.Group II- *Anaemic group II (n=50)*

Eligible participants were recruited from the outpatient department (OPD) and labour room in the third trimester based on the inclusion and exclusion criterias. The demographic data of the mothers (age, chronic disease, drug usage, parity, blood group, gestational week, history of past illness, history of previous childbirth, problems during pregnancy, mode of delivery, administration of anaesthesia, presence of meconium, foetel decleration and presence of a nuchal cord), birth weight of the newborn, length of the newborn, gender, heart rate, body temperature, maturity of the newborn were recorded, apgar scores at the first and fifth minutes were recorded. The blood samples from mother and her cord blood were collected and sent to central lab of institute for investigations. Placentas with cord and membrane were collected from labour room, operation theatre after delivery for gross and histopathological studies and sent to pathology lab.

Observations and Results

In the above study, there is no significant difference was observed in gestational age, parity, apgar score, gender, shape of placenta, mode of delivery between the groups. The weight of newborned baby was significantly higher in anaemic group (3364± 347). Hb concentration (8±1.2), RBC count(3±0.5), MCV(81±6.8), MCH(26±5.4), MCHC(27±4.0), Absolute neutrophil count (2415±482.5), Absolute lymphocyte count (2126±449.4) were significantly lower in anaemic group, but no significant differences were noted between mother's and her cord blood investigations. The higher placental weight (549.08±8.60) was observed in anaemic group as compared to normal group (482.34±21.50). In anaemic group, the significant higher thickness (3.82±0.35) and surface area (566.89±81.36) were noted. The more mean number of cotyledons (28.04±2.39) were observed in anaemic group. Calcified areas (27.18±2.14), Syncytial knot formation (24.44±1.38), Fibrinoid necrosis (10.26±1.71), hyalinised villi (11.12±1.23), perivillous fibrin deposition (9.92±1.36), intervillous space (10.24±1.39), hypovascular villi (11.48±0.97) were seen significantly more in anaemic group as compared to normal group.

Table 1 Basal Parameters in Pregnant Women

Parameter	Control group(n=50)	Anaemic group (n=50)	Statistical significance P <.05
1. Maternal age (years)	27±4.7	29 ±4.5	Not significant
2.BMI(Kg/m^2)	22±1.7	21±1.2	Not significant
3. Blood Pressure (mmHg)	SBP-118±4.3 DBP-81±5.0	SBP-119±4.5 DBP-81±4.2	Not significant
4. Parity	Primi-12 Multi-38	Primi-16 Multi-44	Not significant
5. Diet	Veg-40 Non-veg-10	Veg-42 Non-veg-8	Not significant
6. Education	Edu-35 Unedu-15	Edu-27 Unedu-13	Not significant

Student's t-test applied. Variables are presented as mean ± SD. The * represents significant at p < .05.

Table 2 Basal Parameters in newborn

Parameter	Control group(n=50)	Anaemic group (n=50)	Statistical significance P <.05
1. Gestational age (weeks)	40± 0.4	40± 0.7	**Not Significant**
2. Premature (≤ 37 weeks)	6	7	**Not Significant**
3. Mature(>37weeks)	44	43	**Not Significant**
4. Mode of delivery (vaginal/cesarean)	NVD-44 LSCS-6	NVD-43 LSCS-7	**Not Significant**
5. Apgar score at 1st minute	7± 0.9	6± 1.1	**Not Significant**
6. Apgar score at 5th minute	8± 0.8	7 ±0.8	**Not Significant**
7. Birth weight (gm)	3175±210	3364±413**	**Significant**
8. Gender	Male-33 Female-17	Male-28 Female-22	**Not Significant**

Student's t-test applied. Variables are presented as mean ± SD. **=p<0.01

Table 3: Haematological Parameters in maternal Blood

Parameter	Control group (n=50)	Anaemic group (n=50)	Statistical significance P <.05
Hb (gm%)	12±0.5	8±1.2**	**Significant**
Total RBC count (million/mm^3)	4± 0.2	3±0.5*	**Significant**
Haematocrit (%)	35± 1.7	31±1.2**	**Significant**
MCV (fl)	96±2.4	81±6.8**	**Significant**
MCH (pg)	29 ±2.1	26±5.4**	**Significant**
MCHC (%)	35± 2.5	27±4.0**	**Significant**
Total WBC count (thousands/ mm^3)	7696 ± 1173.4	6215±1222.8	**Not significant**
ANC (thousands/ mm^3)	4415 ± 723.6	2415±482.5**	**Significant**
ALC (thousands/ mm^3)	2928 ±569.9	2126±449.4**	**Significant**
Platelets count (lacs/cc)	2 ±0.4	2±0.3	**Not Significant**

Student's t-test applied. Variables are presented as mean ± SD. *=p < .05 **=p<.01

Table 4: Haematological Parameters in cord blood

Parameter	**Control group (n=50)**	**Anaemic group (n=50)**	Statistical significance P <.05
Hb (gm%)	12.6±0.7	8±1.2**	Significant
Total RBC count (million/mm^3)	4±0.3	3.4±0.6*	Significant
Haematocrit (%)	37±1.6	32±1.2**	Significant
MCV (fl)	97±6.6	88±5.5**	Significant
MCH (pg)	29±2.3	25±5.0**	Significant
MCHC (%)	33±1.9	29±3.5**	Significant
Total WBC count (thousands/ mm^3)	7556±1127.5	6230±1232.0	Not Significant
ANC ((thousands/ mm^3)	4409±719.0	2409±490.0**	Significant
ALC ((thousands/ mm^3)	2833±502.8	2255±456.2**	Significant
Platelets count (lacs/cc)	3±0.3	2.8±0.5	Not Significant

Student's t-test applied. Variables are presented as mean ± SD. *=p<0.05 **=p<0.01

Table 5: Morphometric and Microscopic Findings of the placenta in pregnant women

Parameter	**Control group (n=50)**	**Anaemic group (n=50)**	Statistical significance P <.05
Placental weight(gm)	482.34±21.50	549.08±8.60**	**significant**
Thickness(cm)	2.60±0.37	3.82±0.35**	**significant**
Shape	Circular-38 Oval-12	Circular-40 Oval- 10	**Not significant**
Surface area(cm^2)	225.40±32.04	566.89±81.36***	**significant**
Mean no of cotyledons per placenta	16.6±1.66	28.04±2.39***	**Significant**
Mean no of calcified areas per placenta per lpf	1.4±1.07	27.18±2.14***	**Significant**
Mean no of areas of Syncytial knot formation per lpf	5.04±1.16	24.44±1.38***	**Significant**
Mean no of areas of Fibrinoid necrosis per lpf	1.82±1.49	10.26±1.71***	**Significant**
Mean no of areas of hyalinised villi per lpf	1.77±1.17	11.12±1.23***	**Significant**
Mean no of areas of Perivillous fibrin deposition per lpf	1.31±1.18	9.92±1.36***	**Significant**
Mean no of areas of intervillous space per lpf	1.25±1.15	10.24±1.39***	**Significant**
Mean no of areas of Hypovascular villi per lpf	3.62±1.09	11.48±0.97***	**Significant**

Student's t-test applied. Variables are presented as mean ± SD. * = p < 0.05 **=p<0.01 ***=p<0.001

Fig 1: Microphotograph showing Placental Villi (H& E stain,100 x)

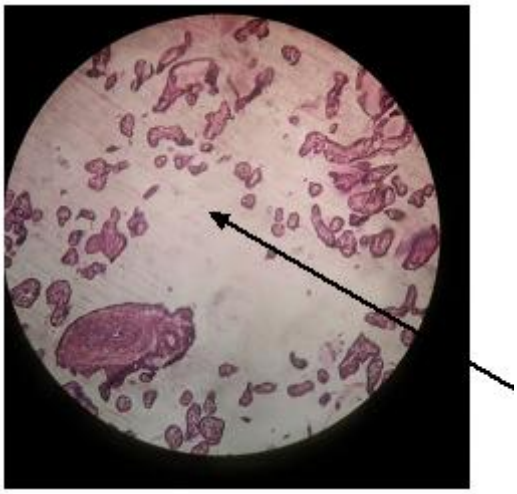

Fig 2: Microphotograph showing Increased Intervillous space (H& E stain,100 x)

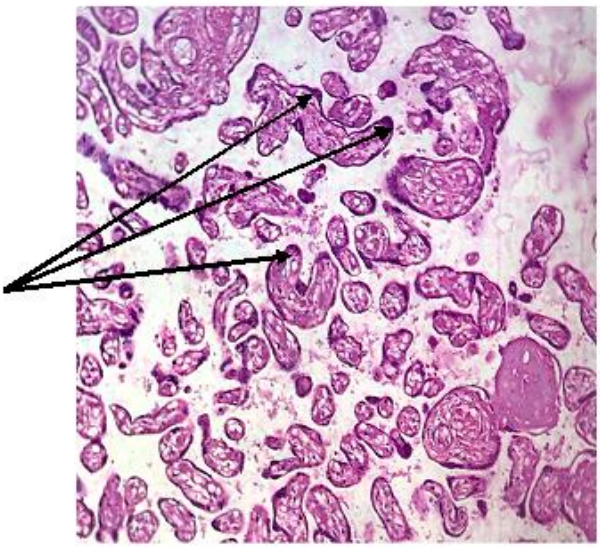

Fig 3: Microphotograph showing Syncytial Trophoblastic proliferation (H& E stain,100 x)

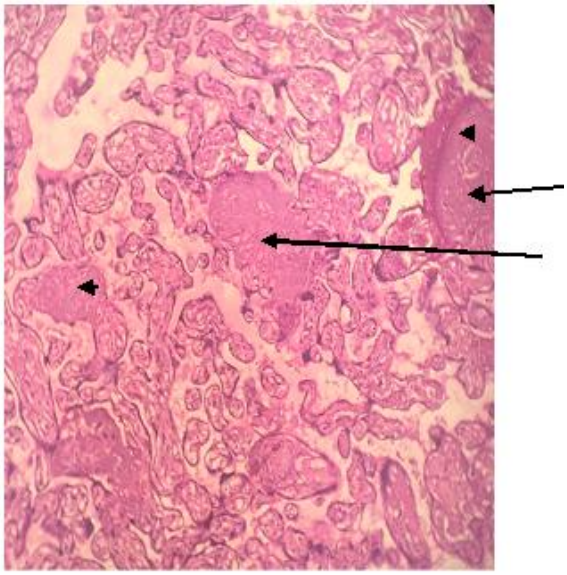

Fig 4: Microphotograph showing Fibrinoid necrosis (H& E stain,100 x)

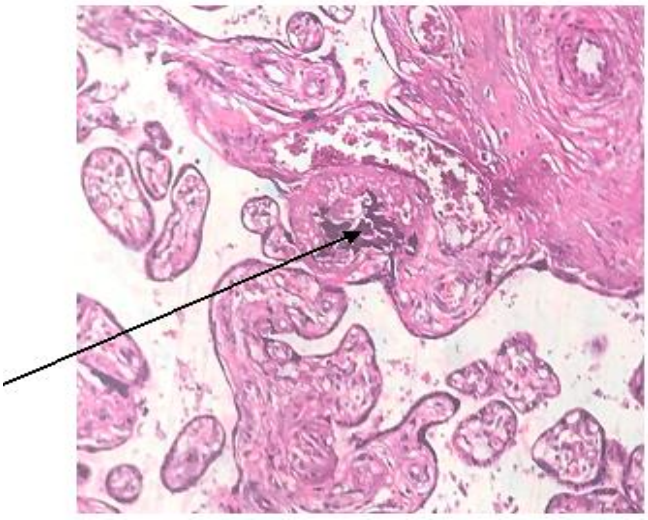

Fig 5: Microphotograph showing Calcification (H& E stain,100 x)

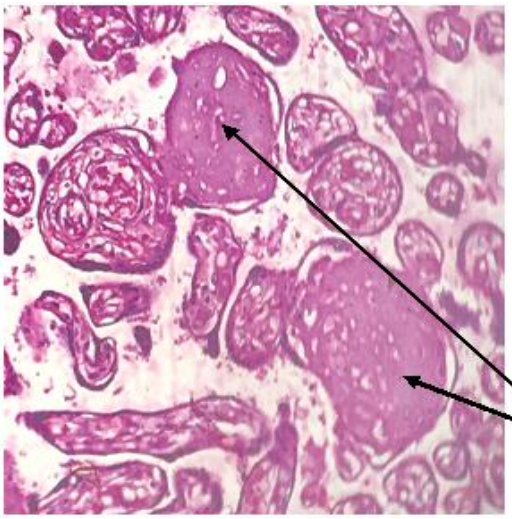

Fig 6: Microphotograph showing Hyalinized villi (H& E stain,100 x)

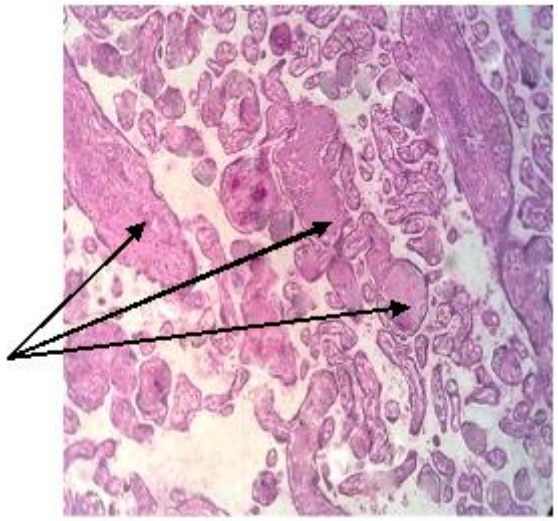

Fig 7: Microphotograph showing Hypovascular Villi (H& E stain,100 x)

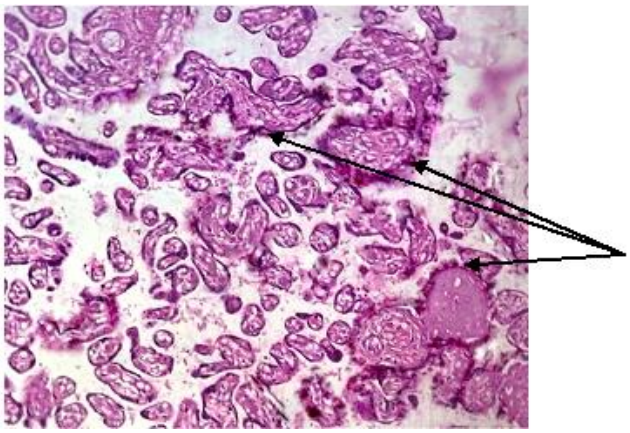

Fig 8: Microphotograph showing Perivillous fibrin deposition (H& E stain,100 x)

Discussion

These changes are supposed to be due to anaemia in female during pregnancy which leads to maternal hypoxia and it might produces changes on the maternal circulatory system and affects both mother and fetus. As placental growth and functions are precisely regulated and coordinated to ensure the exchange of nutrients and waste products between maternal and fetal circulatory systems and it shares same stress and strain to which the fetus is exposed. Thus, any disease affecting mother and fetus also has great impact on placenta.

Some previous studies suggested that neonatal haematological parameters are influenced by genetic, socio-economic status as well as various environmental factors experienced by the pregnant mother during her pregnancy. Some studies explained that maternal anaemia represents an independent risk factor for abnormal placental growth and hypertrophy. It has been reported that hypoxia can cause morphological changes in placental weight, diameter, thickness and surface area Placental anomalies therefore can be an early warning signs about fetal problem. The haematocrit value in non-pregnant women ranges from 38% to 45%. However, in pregnant women because of hemodilution normal values can be much lower than this percentage. It can be said that the haematological parameters are easy to perform and when properly interpreted along with their normal range and cut off values, as done in this study, it can aid in early recognition of type of anaemia during pregnancy and a combination of different other parameters can improve their usefulness[5]. Besides this, cord blood haematology is a reflection of neonatal genetic effect and maternal factors. The possible explanation for increased weight, diameter, surface area of placenta is that the maternal anemia results in fetal hypoxemia and stimulates placental growth.

Conclusion

The possible explanation for increased weight, diameter, surface area of placenta is that the maternal anemia results in fetal hypoxemia and stimulates placental growth.

Cytotrophoblastic proliferation was found more in anaemic group. It might be for replacement of damaged cytotrophoblast caused by ischemia. Increased fibrosis and formation of Syncytial knots indicates ischemic response under hypoxic condition like maternal anemia or functional inactivity.

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