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Original Article

Dietary iron deficiency anemia and its consequences on pregnancy outcome and duration of gestation in rats

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ABSTRACT

To study the effect of dietary iron deficiency anemia on duration of gestation and pregnancy outcome the female albino rats of Wistar strain, with similar body weight (180-200g) were fed on iron deficient diets (30, 15, 7, 2mgFe/kg of diet) and one group on iron sufficient control diet (50 mgFe/kg of diet) for about one month. The females were then kept for mating which was confirmed by detection of a vaginal plug, and this day was denoted as day 0. The females were kept on the same diets through out the pregnancy and the blood samples were collected at the starting of the experiment, during pregnancy, at the time of delivery and after one month of delivery for estimation of adrenocorticotropic hormone (ACTH), prolactin and blood hemoglobin. The hormone analyses were performed on fully automated Chemiluminescent Immuno Assay based instrument (ADVIA centaur, immunoassay system, USA). After delivery weight of mother and neonates and the number of neonates was noted. Student's t-test showed no significant difference in the number and survival of the fetuses, with decrease in the maternal dietary iron. A significant ($p<0.001$) reduction in the gestation period was observed in the anemic mothers. The weights of the neonates of the iron deficient females were decreased significantly ($p<0.001$). Two-way analysis of variance showed a very significant ($p<0.05$) rise in the level of ACTH when analyzed with two grouping factors (iron status and stages viz. before pregnancy, during pregnancy, after delivery). After delivery significant low levels of prolactin was observed in the severe anemic mothers, resulting in the failure of lactation. High ACTH in anemic mothers caused the stimulation of corticotropin releasing hormones in the fetuses and resulted into stress due to which the blocking effect of progesterone diminishes and leads to the preterm delivery. Insufficient supply of nutrients to the developing fetuses causes low neonatal weight.

1. Introduction

The deficiency of iron continues to be a widespread condition affecting millions of people throughout the world. Although poor populations suffer from it most, lack of iron is one of the few nutrition pathologies present in affluent societies with pre-school age children and women of childbearing ages being the most vulnerable groups. Iron deficiency is the most common nutritional deficiency encountered in surveys of diverse populations in industrialized countries [1] and it is said to be the most common cause of anemia in the world. The world health organization estimates that 66-80% of the population is suffering from Fe deficiency [2]. A UN report in 1980 on world nutrition quotes a figure of 43% of children between birth and four years, as being anemic, 20% of adult men and 35% of adult women are anemic with iron deficiency accounting for more than half of the cases. Iron deficiency anemia (IDA) is widely prevalent especially amongst women in India [3, 4, 5]. Being the most pervasive of all nutritional deficiencies Iron deficiency particularly affects women, especially pregnant women. Iron deficiency anemia is considered very serious during pregnancy, with deleterious consequences for both the mother and developing fetus. In most species, maternal blood volume increases and hematocrit and hemoglobin concentration fall during pregnancy; this is known as the anemia of pregnancy. However, in a high percentage of women, the fall in hemoglobin levels is greater than that which is regarded as both physiological and safe [6]. A significant proportion of these anemias arise as a result of iron (Fe) deficiency [7]. A specific association between iron-deficiency anemia (low hemoglobin and serum ferritin values) early in pregnancy and premature delivery

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has been found, in contrast to anemia from other causes, following the WHO [7] and CDC [8] anemia cut-off points. Perinatal fetal and maternal complications have been found to increase exponentially under extreme conditions once hemoglobin values decreases further below 90g/l. These values are observed almost exclusively in populations where chronic blood losses, malaria or other hemolytic conditions are common.

The maternal iron-deficiency anemia may result in the poor pregnancy outcomes with shorter gestation period. The purpose of the present study is to find out the association between iron-deficiency anemia and pregnancy outcome and gestation period and also to explore the possible mechanism through which iron-deficiency anemia might cause poor outcomes of pregnancy with reduced time of gestation.

2. Materials and Methods

2.1. Experimental animals

Female rats of Wister strain were bred under laboratory conditions from the stock colonies for the constant availability throughout the period of study. The experimental animals were of similar body weight (170-200gms), size and age and were group-housed in cages under constant temperature and humidity. Controlled illumination with a 12hrs light and 12hrs dark photoperiod was maintained to ensure regular estrous cycles. All animals were fed ad libitum and provided with distill water. Thirty female rats were fed control diet for two weeks to adapt to the new conditions, before being randomized into five groups. The first group of rats (n=6) remained on the control diet (50mg/kg), whereas the remaining four groups (n=6each) were placed on experimental diets of reduced Fe content (30, 15, 7, 2mg/kg). All diets were freely available, and body weights were recorded three times per week throughout the experiment. All groups were fed these diets for four weeks before mating. To prepare the rats and reduce the stress response at the time of blood collection, all were picked up and handled daily.

The tail blood from the rats (in proesterus) of each group was collected into heparinized collection tubes to determine baseline hemoglobin values. Serum was collected for the estimations of adrenocorticotropic hormone and Prolactin hormones. The rats were then mated and the mating was confirmed by detection of a vaginal plug, and this day was denoted as day 0. The female rats were maintained on the same experimental diet throughout the pregnancy and one week after that. The day of delivery was noted down to find out the duration of gestation. Maternal blood was collected at the starting of the experiment, during pregnancy, at the time of delivery and after one month for the measurement of Hemoglobin and estimation of ACTH and Prolactin hormones. After delivery the mother and the neonate were weighed the number of the neonates was noted.

2.2. Diet

The experimental diets used were based on casein (as a source of protein 20%), starch (as a source of carbohydrate 70%) and vegetable oil (as a source of fat 5%). Vitamins mixture (1%) and chemically pure inorganic salt mixture (4%) (Except iron) were added. Iron (crystalline ferrous sulfate, $FeSO_4 \cdot 7H_2O$) was finely ground by mortar and pestle and then added to achieve dietary levels of added Fe of 50 (control diet), 30, 15, 7 and 2mg/kg. (National Institute of Nutrition, ICMR, Hyderabad). Non-nutritive cellulose was deleted from diets because of its variable iron content. Rats were given free access of food and water. (Dietary ingredients were purchased from scientific and general agency, jaipur

Composition of mineral mixture (g/100g of salt mixture)	
Calcium carbonates	38.1400
Cobalt chloride	0.0023
Cupric sulfate	0.0477
Magnesium sulfate	5.7300
Potassium iodide	0.0790
Potassium phosphate monobasic	38.9000
Sodium chloride	13.93
Zinc sulfate	0.0548
Composition of vitamin mixture	
Vitamin A+	2000 IU
Vitamin D+	200 IU
Vitamin E	10 IU
Vitamin K (Menadione)	0.5mg
Thiamine	0.5mg
Riboflavin	0.8mg
Pyridoxin	0.5mg
Calcium pantothenate	4.0mg
Niacin	4.0mg
Inositol	10.0mg
Para aminobenzoic acid	10.0mg
Biotin	40.0µg
Folic acid	0.2mg
Vitamin B12	3.0µg
Ccholin chloride	200.0mg

All the above ingredients were mixed and sufficient amount of starch was added to make up to one gram.

2.3. Hematological measurements and hormone assay

Maternal blood was collected at the starting of the experiment, during pregnancy, at the time of delivery and after one month. Hemoglobin was measured in hemoglobinometer. Estimation of ACTH and Prolactin hormones were done. The hormone analyses were performed on fully automated Chemiluminescent Immuno Assay based instrument (ADVIA centaur, immunoassay system, USA).

2.4. Statistical analysis

All the values are expressed as means ± SEM. To find out the significance of difference between maternal hemoglobin, number of neonates, neonatal body weight and duration of gestation, the mean values were calculated and compared with that of controls by the student's t-test with accepted level of significance of 0.001. The values of ACTH and Prolactin were analyzed by two-way ANOVA (by Analyse-it+general 1.73), with two grouping factors (iron status and stages viz. before pregnancy, during pregnancy and after delivery). If interactions were found between grouping factors, data were reanalyzed by one-way ANOVA with accepted level of significance of 0.05.

3. Result

The present study revealed that maternal dietary iron deficiency (15, 7 and 2mgFe/kg of diet) reduces the maternal hemoglobin, neonatal weights and duration of gestation significantly ($P<0.05$) (Table.1). Significant ($P<0.05$) hormonal changes, with

Table 1. showing the effect of different levels of dietary iron on hemoglobin, number of neonates, neonatal body weight and duration of gestation.

Groups	Maternal hemoglobin (g %) (During 18th-20th day of gestation)	Number of neonates	Neonatal body weight (g)	Duration of gestation (days)
50mgFe/kg of diet (control)	12.26±0.025	12.00±0.333	5.4±0.0527	20.8±0.2805
30mgFe/kg of diet	12.25±0.031	11.83±0.435	5.1±0.0333	20.5±0.2041
15mgFe/kg of diet	9.51±0.160[a]	12.16±0.280	4.8±0.0111[a]	17.8±0.2805[a]
7mgFe/kg of diet	8.06±0.132[a]	12.33±0.304	3.6±0.0410[a]	15.5±0.2041[a]
2mgFe/kg of diet	6.51±0.064[a]	12.66±0.390	3.3±0.0666[a]	14.8±0.2805[a]

Data are presented as mean ± SEM (n=6). Statistical analysis was carried out by student's t-test. Here 'a' represents significance p<0.001.

Table 2. Effect of different levels of dietary iron contents, on the levels of ACTH, before pregnancy, during pregnancy and after delivery.

	ACTH (mμ/ml)		
Groups	Before Pregnancy (bp)	During Pregnancy (dp) (18th-20th day of gestation)	After Delivery (Ad) (Eight weeks Postpartum)
GROUP- A 50mg Fe/kg of diet (Control)	0.3752 ±0.050	0.706±0.0127[†]	1.017 ±0.0527*
GROUP- B 30 mg Fe/kg of diet	0.774±0.0456[a]	0.9558 ±0.0347[a†]	1.100±0.05247*
GROUP- C 15mg Fe/kg of diet	0.638 ±0.0227[a]	1.452a† ±0.0213[a†]	1.278 ±0.02701[a]*
GROUP- D 7mgFe/kg of diet	0.908 ±0.0195[ab]	1.853 ±0.0512[ab†]	0.560 ±0.0225[ab]*
GROUP- E 2 mg Fe/kg of diet	1.077 ±0.0410[ab]	1.988 ±0.0383[ab†]	0.520 ±0.0186[ab]*

Values Mean ± S.E.M. (n=6)

[a] P<0.05 groups B, C, D and E compared with group A

[b] P<0.05 groups D and E compared with group C

[*]P<0.05 stage after delivery compared with before pregnancy (bp),

† P<0.05 stage during pregnancy compared with before pregnancy (bp)

decreasing dietary iron, in all the three stages (before pregnancy, during pregnancy and after delivery) were observed. Significant (p<0.05) changes in the hormone were observed in all the stages (before pregnancy, during pregnancy and after delivery) of iron deficient groups. The increase in ACTH before pregnancy and during pregnancy in the iron deficient groups (B, C, D and E) was found Significant (p<0.05) on comparing them with the group A. Further the rise in groups D and E was compared with the group C before pregnancy and during pregnancy and found Significant (p<0.05), whereas after delivery the hormone declines in the iron deficient groups D and E as compared to groups A and C. The hormone rises significantly (p<0.05) during pregnancy as compared to before pregnancy stage and significantly (p<0.05) declines after delivery in the severe iron deficient groups D and E (Table 2). Significant (p<0.05) changes in PRL, in all the stages and iron deficient groups were observed. Significant (p<0.05) decrease in the Prolactin levels in the groups D and E as compared to control group A and iron deficient group C was observed in all the three stages. The hormone rises in all the groups during pregnancy and then declines significantly (p<0.05) after delivery (Table 3). Further the severe anemic mothers failed to lactate and showed no conception even after two months of deliveries.

4. Discussion

Our findings suggest that the maternal hemoglobin contents lowers down with the decreasing levels of iron in the diet. The neonates born to iron deficient mothers were low in weight. Like Lewis et al. [9] we observed fetal growth retardation in litters of iron deficient mothers. The results of the present study confirm the previous findings [10] that neonatal body weight decreases with the severity of maternal iron deficiency. Maternal iron deficiency anemia diagnosed by Scholl and Hediger [11] at entry to prenatal care, was associated with low dietary energy and iron, inadequate gestational sgain and two fold or greater increase in the risk of preterm delivery and low birth weight. During pregnancy, the fetal demand for iron increases maternal daily iron requirements from ≈1 to 25 mg/d in early pregnacy and 6.5 mg/d in the third trimester. The average daily diet in the developed world contains ≈10-14 mg nonheme iron but not all of this can be absorbed. Percentage of nonheme iron absorbed from food during normal pregnancy increases from 7% at 12 wk of gestation to 36% at 24 wk and 66% at 36 wk. These dramatic changes enable the healthy pregnant woman to cope with the extra demands of pregnancy without becoming anemic, but only if there is adequate iron in her diet. If the woman's diet is deficient in iron, as is the case in many developing countries, fetal requirements can be met only

Table 3. Effect of different levels of dietary iron contents on the levels of maternal Prolactin (Prl) before pregnancy, during pregnancy and after delivery

	PRL (ng/ml)		
Groups	**Before Pregnancy (bp)**	**During Pregnancy (dp) (18th-20th day of gestation)**	**After Delivery (Ad) (Eight weeks Postpartum)**
GROUP- A 50mg Fe/kg of diet (Control)	34.145±0.100	38.015±0.054†	33.948 ±0.084*
GROUP- B 30 mg Fe/kg of diet	34.442 ±0.156	37.615±0.248†	33.008±0.112*
GROUP- C 15mg Fe/kg of diet	34.652 ±0.118	1.452 ±0.0213[a]†	21.371±0.285*
GROUP- D 7mgFe/kg of diet	33.128±0.082[ab]	1.853 ±0.0512[ab]†	10.101 ±0.139[ab]*
GROUP- E 2 mg Fe/kg of diet	28.123±0.105[ab]	1.988 ±0.0383[ab]†	2.656 ±0.056[ab]*

Values Mean ± S.E.M. (n=6)
[a] P<0.05 groups B, C, D and E compared with group A
[b] P<0.05 groups D and E compared with group C
*P<0.05 stage after delivery compared with before pregnancy (bp)
† P<0.05 stage during pregnancy compared with before pregnancy (bp)

by additional contributions of iron from maternal stores. This demand by the developing fetus may cause the mother to develop iron deficiency anemia, if she had inadequate iron stores at the beginning of pregnancy [12]. Changes in placental vascularization might contribute to the fetal growth retardation as also suggested by Lewis et al. [9] the body temperature rises by the heat produced by the fetoplacental unit. The heat loss is increased by peripheral vasodilation and the blood pressure drops down, aldosterone is released from the adrenal gland and this causes the retension of salts and water. The drop in osmolality reduces blood viscosity and enhances blood flow in the low-pressure system of the intervillous space [13]. This enhanced blood flow improves fetal growth. The hemoglobin concentrations changes due to the changes in plasma volume. The plasma volume fails to expand adequately and can lead to restricted fetal growth. Our findings assume that the iron deficient mothers fail to meet the increasing requirements of the fetus. Being a critical mineral when iron supply does not meet fetal demand, the non-haem containing tissue such as skeletal muscle, heart and brain becomes iron deficient [14]. Along with the risk of low birth weight the preterm delivery also exists with decreasing levels of dietary iron in the mothers. Severe anemia, particularly in the first trimester is significantly associated with adverse pregnancy outcome. Maternal anemia influences birth weight and preterm delivery, but in their population, is not associated with adverse perinatal outcome [9]. Similarly infants born to women with low hemoglobin level in Korea showed a lower birth weight and height [15]. Although we did not observe perinatal mortality as was observed by Geelhoed et al. [16] yet lowering in birth weight was increased with decreasing levels of iron in the diet. We disagree the findings of Golub et al. [3] which suggests that fetuses and new born of the iron deprived rhesus monkey were not growth retarded relative to the controls and the gestation length did not differ significantly by diet group. But we agree with their findings with the fact that the number of neonates did not differ significantly in the iron deficient groups from the controls. Our results showed no significant difference in the number of neonates with difference in iron status of mother as was observed by Gambling et al. [17] that maternal iron deficiency does not affect viability and the number of fetuses. Observations showed the preterm delivery in iron deficient mothers. The results agree with Scanlon and colleagues who confirmed the relation between early anemia (based on hemoglobin alone), and preterm delivery. Zhou et al. [18] suggested an effect of maternal anemia on preterm delivery that was most detectable during the 1st trimester, before maternal plasma volume expanded. The relationship between anemia or iron deficiency anemia and increased risk of preterm delivery has been supported by several studies.

Oxidative stress may be the major cause but infection and hypoxia due to iron deficiency can also contribute in the process of preterm delivery. The oxygen demands are high during pregnancy because of the metabolism process taking place in the fetus also.

Oxygen transportation to the fetus is reduced in iron deficient mothers [19]. Iron deficiency increases norepinephrine concentrations [20], as does hypoxia [21]. Norepinephrine is a strong stimulus for the release of CRH [22] and cotrisol. In our studies the levels of adrenocorticotropic hormone was found high peripartum. There was a significant difference between the levels of ACTH in all the three stages i.e. before pregnancy, during pregnancy and after delivery. These increases in the level of

ACTH in iron deficient mothers may be the major cause of preterm delivery. The placenta also secretes CRH and is added into the fetal circulation in amounts high enough to stimulate the production of ACTH by the adenohypophysis of the fetus. The fetal cortisol level increases. The fetal adrenal produces dehydroepiendrosterone sulphate that is converted to estrogen by the placenta. This inhibits the blocking activity of progesterone and helps the uterus to contract synchronously. Estrogen along with the help of increasing oxytosin and prostaglandins onsets the process of delivery and the labor begins. Women in preterm labour were reported to have high plasma concentrations of CRH compared with control women at the same stage gestation [23]. Earlier it has being showed that women having abnormally high CRH concentration early in pregnancy had preterm delivery [24]. Higher concentration of CRH during labour also predicts shorter labour duration.

Low levels of prolactin in severe anemia are due to poor pituitary function. Lactotrophic secretion of prolactin increases during the first trimester, because of estrogen and progesterone stimulation of the lactotrophic cells. In the second and third

trimesters, the decidua is the source of much increased prolactin production. Failure of lactation in the severe anemic mothers after delivery is due to low circulating prolactin. This lack of postpartum milk production is the first manifestation of the Sheehan's syndrome [10]. After delivery, rapid hypophyseal involution occurs with sharp curtailment of its vascularity. Added to this, postpartum hemorrhage and vasomotor collapse may cause an extreme reduction in pituitary circulation very importantly in the severe anemic mothers.

5. Conclusion

From the present study it can be concluded that iron deficiency anemia (IDA) has adverse health consequences for both the mother and her neonates Deficiency of iron affects the work performance, hormone functions and metabolic processes. Preterm deliveries and very weak neonates are the results of poor iron in the maternal diets. Very low iron in the maternal diet may lead to hypopituitism and causes post-partum pituitary hormone deficiencies, as low circulating levels of prolactin was observed, due to which lactation was ceased and the neonates of the severe anemic mothers couldn't survive. Therefore by increasing the dietary iron contents to the level that meet the requirements of the mother and the foetus properly, not only the pregnancy outcome can be improved but the mothers can also be saved from the complicated situations of endocrine disorders, biological and physiological disturbances after delivery.

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