Impact of dietary iron deficiency anemia on peripartum thyroid function in albino rats

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Abstract-The aim of the study was to observe whether the dietary iron deficiency anemia impairs the thyroid functioning during peripartum period. Iron deficiency anemia is widely prevalent in India. It is the most pervasive of all nutritional deficiencies, particularly affecting pregnant women. iron deficiency may lead to abnormal functioning of thyroid gland as it plays an important role in synthesis as well as metabolism of thyroid hormones. Iron is an important component of many enzymes including thyroid peroxidase (TPO). It is very important to note the changes in the thyroid activity by severe anemia during peripartum period. The female albino rats of Wister strain were fed on iron deficient diets (30, 15, 7, 2 mgFe/kg of diet) and control diets (50 mgFe/kg of diet). Prior to one month of mating the females were anaesthetized by ether and the tail blood was collected for the evaluation of blood hemoglobin, hematocrit and estimation of thyroid stimulating hormone (TSH) hormone. The rats were then kept for mating. The tail blood was again collected during 18th–20th day of gestation and after ten days of delivery by anaesthetizing the females by ether. They were kept on the same diet throughout gestation and also after the deliveries. The analysis of the thyroid stimulating hormone was done by RIA kits. The hemoglobin, hematocrit and TSH were compared in all the three stages (before pregnancy, during pregnancy and after delivery) with decreasing levels of dietary iron, within the groups and between the groups by one way ANOVA. Significant ($P<0.05$) differences in the levels of the three parameters were observed. The two-way ANOVA (between iron diets and stages i.e., before pregnancy, during pregnancy and after delivery) also showed a significant ($P<0.05$) rise in the levels of TSH. The severely iron deficient mothers showed postpartum hypothyroidism. The preterm delivery (between 12th to 15th days) was also observed in severely iron deficient mothers (7 and 2 mgFe/kg of diet). The females with severe iron deficiency anemia (7, 2 mgFe/kg of diet) could not lactate and failed to conceive months after their first premature deliveries. The possible reason of postpartum rise in the TSH and hypothyroidism in the severe iron deficiency anemic mothers may be the hypo function of pituitary and changes in the TSH secreting cells of the pituitary which led to the dysfunction of the thyroid gland after delivery. Due to severe iron deficiency low thyroid peroxidase activity possibly decreased the synthesis of thyroid hormones which in turn had affected the pituitary and resulted in hypothyroidism. No lactation and infertility also reflect the hypo function of pituitary.

Key words: gestation, hormones, hypothyroidism, peripartum, preterm

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 is widely prevalent especially amongst women in India (3, 4, 5). 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). Some studies have shown that ID can negatively impact thyroid function by interfering with oxygen transport or affecting thyroid peroxidase activity (8,9). IDA could impair thyroid metabolism through anemia and lowered oxygen transport (10). Malfunction of the thyroid gland is the second most common endocrine disorder encountered during pregnancy. It is well known that overt disease of the thyroid gland, either hyper or hypo can adversely affect pregnancy outcome (11). The present study was aimed to observe whether the dietary iron deficiency anemia impairs the thyroid functioning during peripartum period.

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 and hematocrit values. Serum was collected for the estimations of thyroid stimulating hormone. .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, hematocrit and estimation of TSH. After delivery the mothers were weighed and the activity of the mothers and 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%). Vitamin mixture (1%) and chemically pure inorganic salt mixture (4%) (Except iron) were added. Iron (crystalline ferrous sulfate, FeSO4.7H20) 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 contents. Rats were given free access of food and water. (Dietary ingredients were purchased from scientific and general agency, Jaipur).

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 TSH 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 hemoglobin and hematocrit decrease significantly (P< 0.05) in all the stages (before pregnancy, during pregnancy and after delivery) with decrease in the dietary iron contents (30, 15, 7 and 2 mg Fe/kg of diet). Decrease in the hemoglobin of the iron deficient groups (Groups C, D and E) were found significant (P< 0.05) in all the stages when compared with the iron sufficient control group (Group A) and this decline in the hemoglobin and hematocrit was again found significant in the groups D and E on comparing them with the group C. During pregnancy the significant (P< 0.05) decline in the hemoglobin contents in the groups A, B and C, and in all the groups after delivery, were found when were compared with the before pregnancy stage (Table 2).

Thyroid stimulating hormone (TSH)

TSH changes Significantly ($p<0.05$) in all the stages with the decreasing iron contents of the maternal diet. Before pregnancy and during pregnancy the hormone rises Significantly ($p<0.05$) in all the iron deficient groups as compared to the control group A and further in the severe iron deficient groups (D and E) the rise was observed significant when compared with the group C. After delivery the groups B and C showed a rise as compared to control group A and the severe iron deficient groups D and E showed a Significant ($p<0.05$) decline in the hormone when compared with the groups A and C.

The postpartum decline in the hormone of the severe iron deficient groups D and E was also found significant ($p<0.05$) as compared to pre-pregnancy stage (Table 3).

Body weight

In the control group A and group B, a significant (P< 0.05) rise in the body weight was observed during pregnancy and after delivery. Whereas maternal body weight was found significantly (P< 0.05) decreased after delivery as compared to before pregnancy stage, in the groups C, D and E (Table 3).

The decrease in the maternal body weight was significant (P< 0.05) in the iron deficient groups (group C, D and E) as compared to group A, in the stages during pregnancy and after delivery.

Neonates of severe iron deficient mothers were Very weak and feeble and the mothers were unable to lactate. The mothers showed no conception even after two months of deliveries.

4. DISCUSSION

Lacking appropriate nutritional elements in the pregnant women's diet, a number of maternal deficiencies including calcium, phosphates, vitamins and iron deficiency can often occur. Iron is intrinsic to the structure and proper functioning of hemoglobin, myoglobin, cytochromes, hemerythrins, and several enzymes active in porphyrin synthesis, oxygen regulation and immunity, thus it is the most abundant transition metal in the human body (12). Its central position in these metabolic systems makes iron deficiency one of the most debilitating of nutritional morbidities. According to the World Health Organization (WHO), iron deficiency anemia is the leading form of nutritional anemia throughout the world affecting at least 1.32 billion people (13). Young children from birth to 12 years of age, adolescents, and females of childbearing age are the most susceptible groups affected by iron deficiency anemia. Iron (Fe) deficiency in pregnancy has serious consequences for both the mother and her baby. In the immediate postnatal period, these include increased risk of low birth-weight, increased morbidity and mortality (14,15,16). In the neonatal period, there is an increased risk of impaired motor development and coordination. In children, language development and scholastic achievement can be affected and there are significant psychological and behavioral effects, decreased physical activity (17, 18) and impaired capacity for work (19).

In the present study it has been observed that dietary iron deficiency causes many hematological alterations and hormone imbalances in the female rats during the peripartum period.

The females of low iron groups (15, 7 and 2 mgFe/kg of diet) showed marked reduction in the body weight eight weeks after delivery. The degree of iron deficiency produced by our iron-restricted diets was severe enough to impair weight gain and to reduce final body weight. Iron deficiency results not only in reduced food intake but also in reduced feed efficiency (20,21,22).

When the deficiency of iron in the diet becomes severe (so that there are too few circulating red blood cells or the hemoglobin content of these cells is very low), the condition is diagnosed as iron- deficiency anemia. Anemia is a reduction in the number of red blood cells (RBCs), in the amount of hemoglobin in the blood and

in another related index called hematocrit (the volume of RBCs after they have been spun in a centrifuge). Iron deficiency anemia produces red blood cells (RBCs) that are smaller than usual, and hence, the term microcytic is used when referring to them. RBCs are not only reduced in size and number, but contain a subnormal amount of hemoglobin, which causes cells to become pale. The term used, when describing cells that are paler in color because they do not contain their full complement of hemoglobin, is hypochromic. Insufficient body stores of iron lead to a depleted RBC mass, which, in turn, leads to a decreased hemoglobin concentration (hypochromia) and decreased Oxygen carrying capacity of the blood (23).

Hemoglobin is an iron-containing protein that resides within red blood cells and accounts for about 95% of the protein in the red blood cell. Being the most important component of red blood cells, its protein called heme, binds oxygen that is exchanged for carbon dioxide in the lungs. When the body's supply of available iron is too low, a condition called iron deficiency results. RBC mass is depleted and it leads to a decrease in hemoglobin concentration. People with iron deficiency cannot produce an adequate amount of hemoglobin to meet their body's oxygen-transport needs (23, 24).

Hematocrit is the volume of RBCs after been spun in a centrifuge. As in anemia the number of red blood cells (RBCs) reduces this related index is also reduced. Pregnancy increases the risk for anemia in different ways. Pregnancy increases the body's demand for folic acid and therefore possess a risk for deficiencies and an increased risk for megaloblastic anemia. Maternal iron deficiency anemia is associated with increased weight or size of the placenta, a condition that may pose a risk for later high blood pressure in the offspring. Pregnancy is also associated with fluid retention, which in turn may produce high volumes of plasma (the fluid component of blood). This can dilute red blood cells, which may lead to anemia (25). Iron deficiency anemia could impair thyroid metabolism through anemia and lowered oxygen transport (26, 27). This may also alter central nervous system control of thyroid metabolism (28) and nuclear T3 binding (29). Another potential mechanism is impairment of thyroid peroxidase (TPO) activity. TPO is a 103-kDa Fe-dependent enzyme located at the apical membrane of the thyrocyte (30). TPO catalyzes the first two steps of thyroid hormone synthesis, iodination of thyroglobulin and coupling of the iodotyrosine residues. TPO activity requires a heme protein attached to ferriprotoporphyrin IX or a closely related porphyrin (31). IDA lowers the activities of other heme-containing enzymes, i.e., cytochrome oxidase, myeloperoxidase and succinate-ubiquinone oxidoreductases all are sensitive to depletion during Fe deficiency (32). Similarly, IDA lowers the TPO activity and thereby interferes with iodine metabolism in the thyroid (33) and as feedback the TSH increases. Pregnancy may have a considerable impact on thyroid homeostasis (34) and complicates the diagnosis of hypothyroidism.

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Table 1. diet

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	40.0 µg
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.

Table 2. Effect of different levels of dietary iron contents on the maternal Hemoglobin and Hematocrit values before pregnancy, during pregnancy and after delivery

	HEMOGLOBIN (g%)			HEMATOCRIT (%)		
Groups	Before Pregnancy (bp)	During Pregnancy (dp) (18-20 day of gestation)	After Delivery (ad) (Eight weeks postpartum)	Before Pregnancy (bp)	During Pregnancy (dp) (18-20 day of gestation)	After Delivery (ad) (Eight weeks postpartum
GROUP-A 50mg Fe/kg of diet (Control)	14.165±0.300	$12.22^{\#}$±0.245	11.72* ±0.439	42.05±0.213	$34.36^{\#}$ ±0.131	32.74*±0.084
GROUP-B 30 mg Fe/kg of diet	13.857±0.279	$11.51^{\#}$±0.335	11.39* ±0.420	40.20^{a}±0.145	$32.26^{a\#}$±0.116	32.59*±0.157
GROUP-C 15mg Fe/kg of diet	12.463^{a}±0.229	$10.43^{a\#}$±0.220	9.092^{a}*±1.072	29.95^{a}±0.331	$27.84^{a\#}$±0.106	26.18^{a}*± 0.186
GROUP-D 7mgFe/kg of diet	8.394^{ab}±0.254	7.723^{ab}±0.185	6.110^{ab}*±0.176	27.30^{ab}±0.154	$25.71^{ab\#}$±0.135	23.41^{ab}*±0.120
GROUP-E 2 mg Fe/kg of diet	6.285^{ab}±0.309	5.191^{ab}±0.439	3.840^{ab}*±0.307	24.03^{ab}±0.062	$20.10^{ab\#}$±0.060	17.85^{ab}*±0.184

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)

Table 3. Effect of different levels of dietary iron contents on the maternal body weights and the levels of maternal Thyroid Stimulating Hormone (TSH), values before pregnancy, during pregnancy and after delivery

	BODY WEIGHT(g)			TSH (mIU/L)		
Groups	Before Pregnancy (bp)	During Pregnancy (dp)	After Delivery (ad) (Eight weeks postpartum)	Before Pregnancy (bp)	During Pregnancy (dp) (18-20 day of gestation)	After Delivery (ad) (Eight weeks postpartum

		(18-20 day of gestation)				
GROUP-A 50mg Fe/kg of diet (Control)	208.5±4.318	230.26$^{\#}$±5.016	226.66*±2.108	1.89±0.208	1.12±0.084	1.88±0.220
GROUP-B 30 mg Fe/kg of diet	204.16±6.379	228.26$^{\#}$ ±4.231	225 *±1.825	2.418^{a} ±0.014	2.452^{a}±0.017	2.430^{a}±0.143
GROUP-C 15mg Fe/kg of diet	207.5±5.737	212.15 ^{a}±2.361	167.5^{a*} ±2.140	2.461^{a}±0.0210	2.462 ^{a}±0.019	2.313 ^{a}±0.123
GROUP-D 7mgFe/kg of diet	204.66±4.558	198.42^{ab}±5.434	162.5 ^{a*} ±2.140	3.367 ^{ab}±0.027	3.362^{ab}±0.019	0.180^{ab*}±0.061
GROUP-E 2 mg Fe/kg of diet	204.16 ±6.88	196.68^{ab}±4.234	148.33^{ab}*±2.108	3.440^{ab}±0.148	3.333^{ab}±0.155	0.125^{ab*}±0.042

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)