EFFECTS OF DAILY ORAL IRON SUPPLEMENTATION ON HISTOMORPHOLOGY OF RAT PLACENTA

Umbreen Noor, Afeera Afsheen*, Humaira Arshad, Khadija Qamar*****

Riyadh Elm University, Riyadh Kingdom of Saudi Arabia, *Pakistan Naval Ship Shifa Hospital Karachi Pakistan, **Rawal Institute of Health Sciences Islamabad Pakistan, ***Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To assess the effects of daily oral iron supplementation during pregnancy on the histomorphology of rat placenta.

Study Design: Randomized controlled trial.

Place and Duration of Study: NIH and Army Medical College Rawalpindi, from Oct 2010 to Jan 2011.

Material and Methods: Thirty two adult female and ten adult male Sprague Dawley rats were taken. After pregnancy was confirmed they were divided randomly into two equal groups and Ferrous Sulphate (FeSO4) was administered at the dose of 0 mg/Kg body weight (control) and 10 mg/Kg body weight (Experimental), starting from gestation day 0 (GD 0). Half of the animals from each group were sacrificed at GD 17 and half at GD 20. The placentae were separated; sections were taken and processed for light microscopic examination. In H&E and PAS stained slides, thickness of labyrinthine zone, trophospongium zone and fetomaternal barrier was measured and number of giant cells were counted.

Results: The results were assessed at GD 17 and GD 20. The results showed no significant difference in the histomorphology of placenta at GD 17. At GD 20, significant decrease in thickness of labyrinthine zone and trophospongium zone and significant reduction in the number of giant cells in experimental group as compared to the control group was noted. There is no significant difference in the thickness of fetomaternal barrier.

Conclusion: Adequate iron supplementation is crucial for maintaining healthy pregnancy. Blood volume increases during pregnancy, so extra iron is needed to make more hemoglobin that is critical for the normal development of placenta especially duing the last trimester. There should be increased awareness regarding the need of healthy diet and iron supplementation at the beginning of the pregnancy.

Keywords: Fetomaternal barrier, Ferrous sulphate, Gestation day, Giant cells, Labyrinthine zone, Trophospongium zone.

INTRODUCTION

Iron plays essential role in the metabolic processes involved in tissue oxygenation[1]. The requirement for iron increases during pregnancy due to the expansion of maternal blood volume and increasing demand of the growing fetus. The placenta acts as a conduit to transfer iron from maternal circulation to the fetus[2]. With increasing size of the fetus and placenta, the rate of maternal fetal transfer also increases and is maximal just prior to parturition[3]. Iron deficiency during pregnancy affect placental vascularization[4], growth and development of fetus and in many cases leads to fetal anemia and organ specific hypoxia[5,6].

As dietary iron alone is insufficient to meet the increasing requirements during pregnancy, maternal oral iron supplementation is highly recommended[7], of which the most commonly prescribed form is ferrous sulphate[8]. Daily oral iron supplementation during pregnancy improves iron stores, reduces the risk of maternal anemia at term and helps to prevent the deleterious effects on the baby[9,10]. Excessive maternal iron intake can be associated with premature delivery, low birthweight[11], local iron excess and iron mediated oxidative stress in intestinal mucosa, liver, spleen, bone marrow and placenta[12]. Iron overdose during pregnancy can

Correspondence: Dr Khadija Qamar, Prof & HOD of Anatomy Department, Army Medical College Rawalpindi Pakistan
Email: colkhadijaqamar@gmail.com
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result in multiple organ failure leading to maternal death and fetal demise[13].

The placenta is an amazing organ that is essential for intrauterine development. Placental dysfunction can seriously affect fetal growth, development and maintenance of pregnancy[14]. The most reproductive toxicity studies conducted in rats used placental weight as a parameter to assess placental toxicity and histopathological examination is not performed. The placenta originates from the proliferation and differ-entiation of trophoectoderm of embryo and endo-metrium of dam[15]. Thus, histopathological examination of placenta forms an essential tool to assess placental toxicity and its subsequent effects on fetus. The major goal of this study is to determine the effects of oral administration of Ferrous sulphate (at therapeutic dose 200mg/ day) on histomorphology of rat placenta. As there exists a good correlation between human and rodent placentas, this study seems to be useful in order to translate our findings to human scenario.

MATERIAL AND METHODS

This randomized control trial was performed at Anatomy department, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad, from Oct 2010 to Jan 2011. All experimental procedures were approved by the institutional animal ethical committee. Thirty two adult female and ten adult male Sprague Dawley rats weighing 200-250 grams were selected by simple random sampling. The female rats were caged with mature male breeders (4:1) overnight. On the following morning, the presence of Vaginal plug was regarded as gestation day 0 (GD 0). By using simple randomization at this point pregnant rats were allocated into two groups (group A and B) of sixteen rats each according to Ferrous sulphate content given. Group A (control) was given 5 ml distilled water by oral gavage tube at 9 am daily and maintained throughout the experiment, half of the animals from this group were sacrificed at GD 17 (group A-I) and half were sacrificed at GD 20 (group A-II). Group B (Experimental) was given 5 ml distilled water by oral gavage tube containing ferrous sulphate 10 mg/kg of body weight/day (Appendix-I) at 9 am daily, and half of the animals from this group were sacrificed at GD 17 (group B-I) and half were sacrificed at GD 20 (group B-II). Throughout the experiment the

Appendix – I

Preparation of Dosage of Drug

Iron (crystalline ferrous sulphate) was finely ground by mortar and pestle. Dose was adjusted on the basis of human dose which is 10 mg/Kg body weight/ day[23]. As the mean weight of animal was 250 mg, so 2.5mg of ferrous sulphate was weighed by means of digital electrical balance and mixed in 5ml of distilled water to make single dose of one day for each animal.

Appendix – II

Nih Laboratory Rat Pellet Diet Composition

Ingredients	Quantity
Wheat	28.5 percent
Wheat bran	28.5 percent
Dry skimmed milk powder	20.0 percent
Fish meal	15.0 percent
Soybean	5.0 percent
Molasses	1.5 percent
Common salt powder	0.5 percent
Vitamins Premix and Minerals	In traces

Note: A rat takes an average of 15-20 grams of diet per day. Daily water intake is about 15-20 ml water per rat per day.

animals were kept in cages at standard room temperature with 12 hour light/dark cycle at NIH. Each animal was given standard NIH diet and water was available ad libitum (Appendix-II). The animals were sacrificed by an overdose of ether anaesthesia. The gravid uterine horns were exposed by lower midline abdominal incision. The placenta and fetuses were examined in situ. One placenta was taken from each uterine midhorn, (to avoid biasing selection of embryos by weight) and preserved in 10% buffered neutral formalin. Tissue was further processed for light microscopic examination. H&E stain for routine histological study, and PAS stain for staining the PAS positive cells of the placenta were used. At X4 objective lens, PAS stained slides were focused in the microscope. The thickness of labyrinthine zone (LZ) close to the central portion was measured from its junction with chorioallantoic connective tissue to its junction with trophospongium zone. The thickness of trophospongium zone (TZ) close to the central portion was measured from its junction with the labyrinthine zone to the outer layer of giant cells. For measuring the barrier, H&E stained slides were studied in the microscope under the X100, oil immersion objective lens. The maternal sinusoids were identified as those vascular spaces which had mononuclear cells projecting into their lumen. The other vessels were regarded as fetal capillaries which were lined by flat endothelial cells. The barrier between these two vessels was considered as the feto-maternal barrier (FMB). This was measured at three different sites where it was relatively thin. The measurement was taken with the help of an ocular micrometer and average value was calculated. The number of giant cells was counted in three different fields under X40 objective and then their average was taken as a final reading for that animal.

The data were entered into computer software SPSS version 19. Descriptive statistics were used to calculate means and standard error of the quantitative data. The means were compared for significance using Mann-Whitney U-test. The "*p*" values were considered to be significant at a *p*-value less than or eual to 0.05, and highly significant at a *p*-value less than or equal to 0.001.

Table: Effect of antenatal Iron; Comparison between control and experimental group (Number of animals in each group 16).

Day of Gestation	Control	Experimental	Statistical significance of difference (*p*-value)
	Mean ± SE	Mean ± SE	
Mean thickness (mm) of labyrinthine zone			
17th	1.6 ± 0.043	1.6 ± 0.045	1
20th	2.66 ± 0.032	2.39 ± 0.035	<0.001*
Mean thickness (mm) of trophospongium zone			
17th	0.5906 ± 0.033	0.5906 ± 0.025	1
20th	0.524 ± 0.051	0.339 ± 0.034	0.011*
Mean thickness (μM) of fetomaternal barrier			
17th	1.706 ± 0.12	1.746 ± 0.071	0.778
20th	1.746 ± 0.063	1.746 ± 0.044	1
Mean number of giant cells			
17th	10.38 ± 0.518	10.5 ± 0.19	0.831
20th	8.25 ± 0.37	9.25 ± 0.25	0.04*

* Statistical difference between the two groups is significant

RESULTS

All rats showed no abnormal clinical signs during the experimental period. Histological examination of placenta showed the norma arrangements (fig-1) and was divided into following layers (proceeding from the fetal surface). (i) Labyrinthine zone formed the major portion of placental disc. It was composed of communicated network of thin fetal capillaries

lined by flat endothelial cells that were surrounded by trophoblast cells which separated them from the maternal sinusoids (Fetomaternal barrier). The maternal sinusoids were characterized by the presence of large mononuclear cells projecting into the lumen of the sinusoids. (ii) Trophospongium zone seen immediately

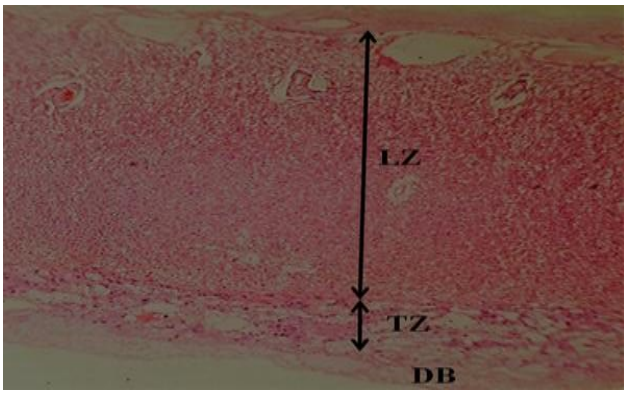

Figure-1: Photomicrograph of a cross section of a placenta of control group at GD 20. Showing Labzrinthine zone (LZ) and trophospongium zone (TZ). Decidua basalis (DB) can also be visualized. H & E stain. Approx. X120.

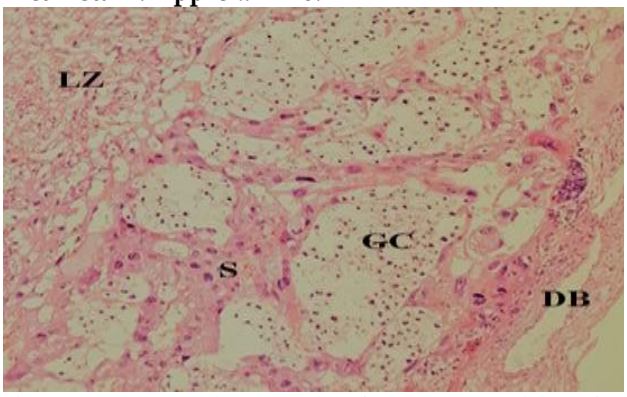

Figure-2: Photomicrograph of a cross section of a placenta of control group at GD 20. Showing Glycogen cells (GC) and Spongiotrophoblasts (S) within the trophospongium zone. Labyrinthine zone (LZ) and decidua basalis (DB) can also be visualized. H & E stain. Approx. X300.

beneath the labyrinthine layer and consisted of variable sized highly eosiniphilic spongiotrophoblasts and clusters of small masses of H&E negative and PAS positive glycogen cells (fig-2). (iii) Giant cell layer: An indefinite and sparse single layer of giant basophilic cells were seen invading the deciduas.

Histopathologically, no significant difference could be seen in the thickness of labyrinthine zone, trophospongium zone and feto-maternal barrier between the group A-I and B-I. Also no significant difference could be observed in the mean number of giant cells between group A-I and B-I. There was significant decreased in the thickness of labyrinthine zone and trophospongium zone in group B-II when compared to A-II (fig-3). No significant difference could be seen in the thickness of feto-maternal barrier.

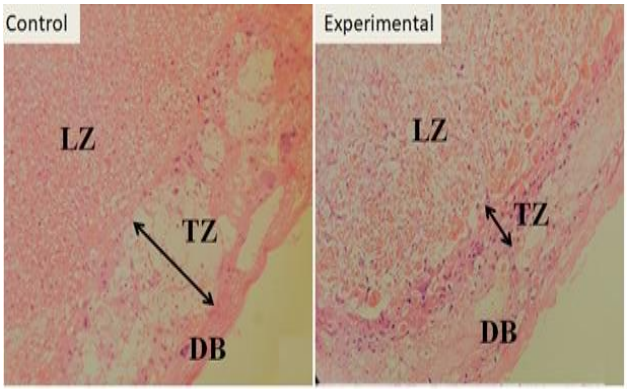

Figure-3: Photomicrograph showing thickened trophospongium zone (TZ) in the control group as compared to experimental group at GD 20. H & E stain. Approx. X300.

Giant cells were normal but reduced in number in the experimental group B-II (table).

In the trophospongium zone, the number of glycogen cells is reduced at GD 20 when compared to GD 17. As a result the thickness of trophospongium also decreases from GD 17 onwards. The loss of glycogen cells was accompanied by spaces filled with PAS-positive colloidal material. In these spaces the cells seemed to be broken with partial disappearance of their cytoplasm leading to a lace like or net like pattern. A little remaining cytoplasm appeared close to the cell membrane, sometime along with a small nucleus. However, the few cells remaining at such sites appeared to be normal and healthy.

DISCUSSION

Oral iron replacement considered as standard first line therapy for treating iron-deficiency anemia. We have previously shown that blood hemoglobin and serum ferritin values decreased during pregnancy in the iron deficient rats as compared to the rats supplemented with ferrous sulphate[16]. The cytoarchitecture, size, development and function of placenta can be affected by nutritional status of the mother, oxygen delivery and maternal diseases[17,18].

The present study showed no significant differences in the histomorphology of placenta in iron deficient group when compared to iron supplemented group at GD 17. At GD 20, significant increased in the thickness of labyrinthine zone and trophospongium zone in group A-II when compared to B-II, suggests that placenta is highly sensitive to oxygen (or iron) deficiencies. In the iron restricted goup, maternal hypoxia stimulates the total cross-sectional area of the placenta, as a compensatory mechanism to increase oxygen delivery to fetus. As labyrinthine is the main exchange region between the fetus and the mother so any adverse intrauterine environment would lead to increase in the thickness of this zone as an adaptative pheno-menon. The study conducted by Lilac also showed significant increase in volume density and total volume of blood vessels of terminal villi in human placenta in iron deficiency anemia[18]. This view is further supported by Woodman, who reported increase in placental size in rats in moderate and severe iron deficiency anemia[6], and by Lewis *et al*., who demonstrated that volume of placental labyrinth was increased in the iron restricted group[19].

The thickness of trophospongium zone was significantly increased and the number of giant cells was significantly decreased in A-II as compared to B-II. The oxygen tension profoundly influenced proliferation and differentiation of human placental cells. The cytotrophoblasts proliferate in vitro under low oxygen conditions but differentiate at higher oxygen levels. The spongiotrophoblasts are homologues with cyto-trophoblast in human placenta[20]. So in the present study, the spongiotrophoblast will proliferate leading to increase in the thickness of trophospongium zone. This view can be further explained by the findings presented by David *et al*., which state that hypoxia promotes in vitro differentiation of trophoblast stem cells into spongiotrophoblasts as opposed to giant cells[21]. Our results also showed that under low oxygen tension, there was increased in proliferation of trophoblast; which would lead to increased in thickness of trophospongium zone, and decreased in differentiation of trophoblast; which would lead to decreased in the number of giant cells. These changes were not evident in the placenta of GD17 group but were more significant in the placenta of GD20 group. This was due to the reason that there is rapid growth of fetus during this late gestational period in rats[22]. As there was significant fetal weight gain from GD17 to GD20[16], so this would lead to significant adaptative changes in the histo-morphology of placentae at GD20 as compared to GD17.

There was no significant difference in the thickness of FMB among the control and experimental groups at GD 17 and GD 20. Our data coincides with the one presented by Lewis *et al.*, which showed that surface area of the maternal fetal interface in the placental labyrinth was not significantly different between the control and iron restricted group[21].

CONCLUSION

There was reduction in Hb value of control group, leading to significant hypoxic changes in the histomorphology of placenta. Further research is indicated to compare the effectiveness of daily supplementation of iron with intermittent supplementation during pregnancy. There should be increased awareness regarding the need of healthy diet and iron supplementation at the beginning of the pregnancy.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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