IRON DEPOSITION IN THE GROWTH PLATE OF LONG BONES OF THE OFFSPRING WHEN GIVEN DURING PREGNANCY IN RAT MODEL

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ABSTRACT

Objective: To evaluate histologically the deposition of iron in the epiphyseal cartilage of offspring’s of dams given iron supplementation during pregnancy and lactation in rat model.

Study Design: Laboratory based experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi and National Institute of Health (NIH) Islamabad, from Mar to Nov 2016.

Methodology: In this study, 16 female and 4 male adult rats were chosen for breeding. After confirmation of pregnancy, pregnant rats were separated in two groups. One group was given oral iron supplementation for four weeks till delivery and half of the pups fed by mothers who were given iron during lactation. The other group was kept on normal lab diet. Deposition of iron in the epiphyseal cartilage of newborn rats after four weeks was evaluated histologically in pups.

Results: Iron deposition was maximum in group C i.e. 1.30 ± 0.48; in group B it was 0.20 ± 0.44. Statistically significant iron deposition (p<0.001) was observed in the growth plate of off springs when mothers were given iron supplements during pregnancy and lactation.

Conclusion: Present study proves that injudicious iron supplementation through pregnancy results in deposition of iron in epiphyseal growth plate of the fetus and it can have damaging effects on bones of fetus.

Keywords: Epiphyseal cartilage, Iron deposition, Pregnancy.

INTRODUCTION

Iron supplementation during pregnancy is recommended in third world countries as iron deficiency anemia is common. Iron supplementation has some side effects as it gets accumulated in different tissues in the body e.g. liver, heart, and endocrine glands, leading to cirrhosis, cardiomyopathy, diabetes mellitus, and other endocrinopathies1.

There is a proof of the adverse secondary effects caused by excess hemoglobin and increased serum ferritin levels in pregnancy. Women with raised serum ferritin levels in the last trimester of pregnancy, perhaps related with overload of iron, have a significantly increased chance of preterm delivery.

Iron supplementation during pregnancy is routinely done2. According to World Health Organization, about 2 billion of the world’s population is anemic, though occurrence rates are variable because of differences in socioeconomic conditions, routines, food consumption, and rates of different diseases. Iron deficiency is the most common cause of anemia and is the most prevalent especially in pregnant women. According to recent recommendations of the Center of Disease Control, anemia in pregnancy is labelled by a hemoglobin level <10.5g/dl. Another indication of the unwanted effects caused by excess hemoglobin is the high ferritin levels due to the failure of maternal plasma volume to expand3. Iron deficiency (ID) is the most common micronutrient deficiency in children. Universal problem of ID assessed to be 43% worldwide in 20114.

Iron is measured the second most ample metal in nature and is crucial for many biological processes. It catalyzes the formation of free radicals through the Haber-Weiss reaction, triggering oxidative stress in different cells5. Iron is vital for hemoglobin production, and metabolic activities. It is found in foods from animal as well as plant sources, as well as fortified cereal foods6. Normal iron levels are strongly controlled, with increased intestinal absorption and cellular uptake transferrin receptor in reduced states. Iron supplementation is suggested to pregnant women at danger of anemia, lasting after delivery. However, debate exists about iron supplementation to all pregnant women due to unfavorable effects of iron excess. Oral ferrous preparations leading to elevated non-transferrin-bound iron, aggravate inflammation and oxidative stress. Free iron is extremely reactive and can lead to free radical damage; There is some clue that co-treatment of non-pregnant rats with anti-oxidants, e.g. vitamin E
Iron Deposition in The Growth Plate

It was laboratory based experimental study conducted at department of Anatomy, Army Medical College, Rawalpindi, from March to November 2016. The protocol of study was approved by Ethics Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi (Letter no. ERC/SA-16).

Sixteen adult female and four male sprague dawley rats, weighing on average 250gm and four weeks of age were selected from the animal house of National Institute of Health (NIH), Islamabad. They were maintained on breeding with one male and water ad libitum. They were given standard lab diet, group B comprising of ten pups of mothers given iron supplementation during pregnancy only and standard laboratory diet during weaning and group C ten pups of mothers given iron supplementation during pregnancy and lactation. After four weeks’ time the male and female rats from group A, B and C (pups) of 4 weeks age were euthanized. Ether anesthesia was used for this. The right leg of each animal was dissected and right femur was detached after dividing it from hip and knee joints. Femurs were then placed in 10% formaldehyde solution. Longitudinal bone sections of 5 micrometer thickness were obtained using rotary microtome. Sections were then stained with Perl’s stain14, for the detection of iron in the cartilage matrix. Iron deposition in chondrocytes and in matrix. Iron deposition inside and outside the chondrocyte lacunae was measured on Perl’s staining. Scoring of iron deposition was done on the basis of scale from 0 to 4 in four different quadrants of the slide15.

RESULTS

Iron deposition in the epiphyseal cartilage inside the chondrocytes and in the matrix is established in the study. Perl’s staining demonstrated the iron as dark blue color deposits as amorphous deposits as well as in the clusters. Scoring of iron deposition in chondrocytes and matrix was analyzed (figure). There was no iron deposits in group A which was control group. Some iron deposition was seen in experimental group B which was scored as 1 and in group C it was scored as 2 (3 slides only). It was maximum in group C. The mean ± SD A group was 0.00 ± 0.00, in group B it was 0.20 ± 0.44 and in group C it was 1.30 ± 0.48 (table-I). Inter-group comparison (Post Hoc analysis) was considered statistically significant (p < 0.05) by Tukey test for comparison.
In the Perl’s stained slides iron deposition was obvious besides the other changes like change in the height of hypertrophy and proliferative zones and change in the diameter of chondrocytes. These changes were also more pronounced in the experimental group C.

**DISCUSSION**

It is proven in a previous study that the toxicity caused by the accumulation of iron in body tissues can result in cirrhosis, hepatic carcinoma, heart failure, diabetes mellitus, and osteoporosis.

Current study shows that surplus maternal iron can be accumulated in the growth cartilage after crossing placenta. It is in accord with earlier studies showing iron accumulation in bone and cartilage and synovium. The amount of iron in cartilage is related with deteriorating clinical and imaging status of joint, as seen in haemophilic arthropathy. Considerable iron deposition inside the cartilage is established by Perl’s staining, typically within and around chondrocytes, but also in lacunae without any evident active chondrocyte. Synovial iron accumulation is also seen in Hereditary hemochromatosis patients and in patients with Rheumatoid arthritis. It is assumed that the iron deposition seen in the knee synovial membrane would come from blood entering the joint. Besides iron, other mineral deposition in cartilage matrix always occurs before it occurs in bone because it provides the necessary, firm substrate for osteoblasts deposition.

Comparable results were seen in another study (E.T omaszewska et al, 2019) showing maternal nutritional supplementation and its accumulation in fetal bones and growth plate. Maternal intake of dried fermented rapeseed meal increased the bone volume comparatively and decreased trabecular number and trabecular space in femoral epiphysis as well as increased the thickness of hyaline cartilage. The maternal nutrition changed the structure of mineral content of bone, as X-ray diffraction analysis shown the decrease of the mean size of the hydroxyapatite Nano crystallites in boars maternally supplemented with dried fermented rapeseed meal. This could be the effect of ionic substitutions of Ca ions in the hydroxyapatite structure, as the content of macro and micro components in ashes of bone samples was different in treatment groups. Inclusion of DFRSM to the diet of pregnant sows re-
In the decrease of the concentration of Ca and P, and the increase of concentration of Cu in the blood plasma of their offspring.  

**CONCLUSION**

This study proves that injudicious iron supplementation through pregnancy results in deposition of iron in epiphyseal growth plate of the fetus and it can have damaging effects on bones of fetus.  

**CONFLICT OF INTEREST**

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

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