ORIGINAL ARTICLE

Effects of Iron Supplementation on the Height of the Zones of Growth Cartilage of Rat

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ABSTRACT

Objective: To study the effects of iron supplementation in pregnancy on the height of zones of epiphyseal growth plates of off springs.

Study Design: Laboratory based randomized control trial.

Place and Duration of Study: This study was conducted at Department of Anatomy, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad from March 2016 to November 2016.

Materials and Methods: Five lactating rats with ten pups were selected for control group A1and experimental groups B1 and B2 each. Experimental groups were given iron supplementation daily throughout the pregnancy. Mothers of group B2 were given oral iron during pregnancy as well as during lactation. Control group was on normal diet throughout the pregnancy.

The infant rats were allowed to reach seven weeks of age till dissection. They were weighed before euthanasia. Right femur of each rat was removed for the epiphyseal growth plate analysis. Femurs were processed, embedded and stained with Hematoxylin & Eosin, Perl's stain, and toluidine stain for histological study. The heights of hypertrophy and proliferative zones were analyzed histologically and statistically.

Results: The height of hypertrophy zone and proliferative zone of epiphyseal plates of long bones were measured. Mean values of the heights of hypertrophy in group A1 276.19 ±43.61, B1 136.73±3.58and B2 205.00±12.76. Mean values of the heights of proliferative zones in group A1 377.29±50.73, B1 202.89±11.56 and B2 281.07±11.96 were taken. The heights in hypertrophy and proliferative zones were statistically decreased in group B1 and B2 as compared to group A1.

Conclusion: Indiscriminate iron supplementation during pregnancy and lactation can decrease the height of hypertrophy zone and proliferative zone of epiphyseal growth plates of long bones of the off spring.

Key Words: *Growth Plate, Hypertrophy Zone, Iron Supplementation, Proliferative Zone.*

Introduction

Iron is an essential nutrient required by each cell of the body. The deficiency as well as excess of iron in the body has clinically significant effects.[1] Iron deficiency can be due to either increased requirements of body as in pregnancy and period of rapid growth or inadequate iron supply. Iron deficiency is the most common cause of anemia.[1] Availability of iron in the diet, with the food fortification and the use of iron supplementation are methods which can correct iron deficiency anemia. Iron excess in body may be associated with excess dietary intake. Iron overload is associated with problems like ineffective erythropoiesis. Clinically, systemic iron overload can present as liver disease, diabetes mellitus, gonadal insufficiency and other endocrine disorders, cardiac dysfunction, arthropathy, and increased skin pigmentation.[2] There is a negative effect of excess iron on bone structure, which exposes patients to fractures.[3] There is a decrease in osteoblast activity due to iron . Iron supplementation is given during pregnancy as well as during lactation routinely even in nonanemics.[4] Excess iron intake during pregnancy is associated with reduced fetal growth. Fetuses of pregnant women with iron intake in the third trimester have a significantly lower biparietal diameter, abdominal circumference and femur length than the fetuses of mothers taking iron in the second trimester.[5] Excessive iron can lead to oxidative damage and decrease in the absorption of

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copper and zinc, which are the important micronutrients for fetal growth.[5] There is an association between elevated maternal hemoglobin and adverse birth outcome, including low birth weight, preterm birth, and small for gestational age at birth in addition to anemia.[6] Effects of iron supplementation on the growth plate need to be evaluated further . Growth plate is responsible for the elongation of bones.[7,8]

As iron supplementation is given as a routine during pregnancy and lactation so its effects on the height of hypertrophy and proliferative zones of epiphyseal growth plate of femur of new born will be determined with the help of present study.

Materials and Methods

This laboratory based randomized controlled trial was approved by Ethical Committee, of the Army Medical College Rawalpindi. It was conducted at the anatomy department of AMC Rawalpindi from March 2016 to September 2016 in collaboration with the National institute of health (NIH) Islamabad. Twenty adult Sprauge Dawley rats (sixteen female and four male) with average weight of 250gms and average age of seven weeks were selected for the study. The animals were kept at standard temperature21±2 ċ in a room maintained on 12 hour light/dark cycle.[9] A battery powered fan was used to maintain the temperature. They were fed on standard lab diet and water ad libitum.

They were kept on breeding.[10] Presence of vaginal plug was checked in the dames daily.[11] Its presence confirms mating and considering it the first day the pregnant rats of both experimental groups B1 and B2 were started with oral iron supplementation daily in water. Sytron syrup was given in a dose of 0.5ml once daily for each pregnant rat throughout pregnancy till the day rat delivers through spontaneous delivery. The pups of group B2 were separated with the mothers on iron supplementation throughout the lactation as well.[12] Ten pups were separated as control group which was group A1, ten pups in experimental group B1and ten pups in experimental group B2. Control group was kept on normal laboratory diet.

On completion of 7 weeks of age the rats of all the three groups were weighed and killed by euthanasia by ether inhalation. Right femurs were removed. Bones were fixed in 10% formalin decalcified in 2% EDTA solution. Lower ends of femurs were separated for evaluation of growth plate. Processed into 5-micron thick sections using rotary microtome. Staining with Hematoxylin & Eosin, Perl's stain[13] (for detection of iron deposition), and toluidine stain was done.[14] Slides were observed under the light microscope for histological analysis of the height of hypertrophy and proliferative zones and measured with image J.software.[15] The height of the hypertrophy and proliferative zones was measured at three different areas of epiphyseal plate (in the Centre and two extreme zones). Average of these three readings was recorded as final height. Firstly the boundary between reserve and proliferative zone and proliferative hypertrophic zone had to be identified first. Between the proliferative and hypertrophy zone it was identified by the first considerably enlarged chondrocytes proportional to the proliferative cells. The limit between the reserve and the proliferative zones was also recognized by the upper border of the proliferative columnar organization, till the beginning of hypertrophy zone.[16] Data was analyzed using SPSS version 21. Parameter was expressed as mean and standard deviation. The means were compared for significance among groups using one way analysis of variance (ANOVA) followed by post hoc Tukey test for comparison. P value less than 0.05 was considered significant.

Results

The mean ±SD of height of hypertrophy zone in groupA2 (figure-1) was 276.19 ±43.61, Mean± SD of group B1 (figure-3) was 136.76±3.58um (table-1). The mean ±SD of group B2 was 205.00±12.76um (table-I).

The p-value for the height of hypertrophy zone was < 0.005 (table-II) which is statistically significant. In proliferative zone Mean ±SD of group A2 was 377.29±50.73um (table-1), Mean± SD of group B1 (figure-2)

was 202.89± 11.56um and Mean ±SD of groupB2 (figure-2) was 281.07 ±11.96 (table-1), The p-value for height of proliferative zone was < 0.005(table-1) which is statistically significant.

Discussion

Supplementation of mothers with iron during pregnancy and lactation without checking the serum iron level can have damaging effect on the

Table I: Showing Comparison of Mean Value of Height of Hypertrophy and Proliferative Zones

	Group A1 Mean ±SD (n=10)	Group B 1 Mean ± SD (n = 10)	Group B 2 Mean ± SD (n = 10)	Group A1 vs. B1 p-value	Group A1 vs. B2 p-value	Group B1 vs. B2 p-value
Height of hypertrophy zone	276.19±43.61	136.73±3.58	205.00±12.76	< .001	< .001	< .001
Height of proliferative zone	377.29±50.73	202.89±11.56	281.07±11.96	< .001	< .001	< .001

P value <0.05 is statistically significant

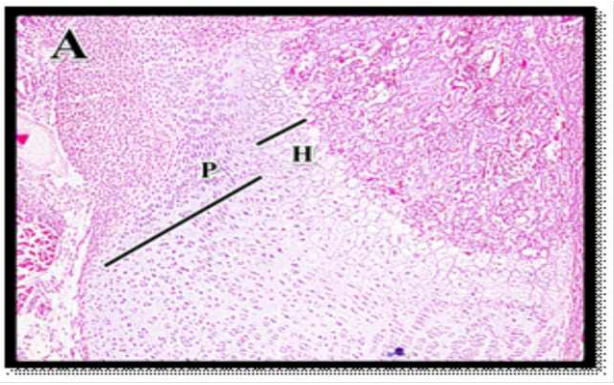

Fig 1: H&E Stain at 10X. Photomicrograph of Histological Section of Growth Plate of Control Group A1 Showing Height of Hypertrophy and Proliferative Zones

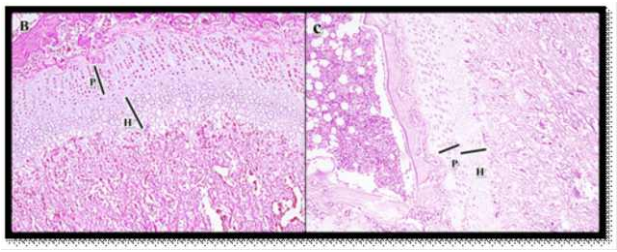

Fig 2: H & E at 10x. Photomicrograph of Histological Section of Growth Plate of Experimental Groups B1 (B) and Group B2(C) Showing Hypertrophy Zone(H) and Proliferative Zone (P)

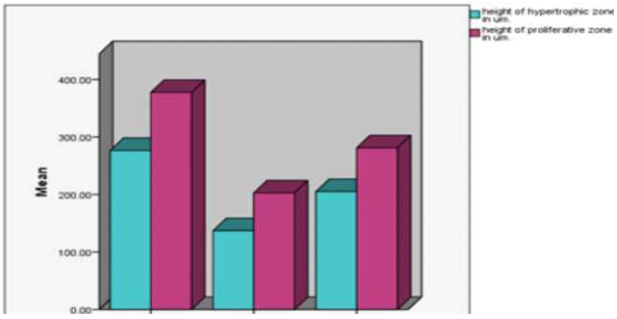

Fig 3: Cluster Bar Chart Showing Comparison of Mean Values of Height of Hypertrophy and Proliferative Zones in Micrometers (along Y-axis) among the Groups (along X-axis), Control Group A1 and Experimental Groups B1 and B2

longitudinal growth of the long bones of the offspring via its effects on epiphyseal growth plate. In the current study iron administration during pregnancy and lactation resulted in the reduction of height of the hypertrophy zone and proliferative zone of growth plate of long bones of the off spring as compared to the control group of rats. Effects on the height of hypertrophy and proliferative zones of growth plates of experimental groups B1 and B2 are significant statistically. The body has limited capacity to excrete iron, and iron in excess can lead to long-term damage to tissues.[17] Iron supplementation in non-anaemic pregnant women may not be beneficial. This may permit the consideration of a personalized approach for antenatal iron supplementation, especially in non-anaemic women.[18] Current study shows that injudicious iron supplementation of mothers during pregnancy and lactation significantly reduces. the height of hypertrophy and proliferative zones of epiphyseal growth cartilages of long bones of the off springs. It is in accordance with the previous researches showing the orally supplemented iron can easily cross the placental barrier and accumulates in the fetal tissues. A study shows that multiple doses of positively-charged nanoparticles given over several days resulted in significantly increased fetal deaths and accumulation of iron in the fetal liver and placenta.[19]

It is also in accordance with the previous studies showing the height of the hypertrophy zone is the main contributor of the growth plate height.[20] The growing cartilages are affected more as compare to the adult cartilage. Iron can be detected in isolated chondrocytes after a short culture period in the presence of either iron or haemoglobin, which negatively influences DNA content and proteoglycan synthesis rate. This inhibiting effect is reversible in the absence of a pro-inflammatory signal.[21] The decrease in the height of hypertrophy zone and the height of proliferative zone in the iron supplementation groups are comparable with the earlier study which shows the effects of iron on the bone.[22]

Conclusion

This study recommends that Indiscriminate iron supplementation of rats throughout pregnancy and lactation without checking serum iron levels can

affect the height of the hypertrophy and proliferative zones of epiphyseal growth plate of long bones of the off springs.

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