IRON SUPPLEMENTATION DURING PREGNANCY AND ITS EFFECTS ON EPIPHYSEAL GROWTH PLATE OF NEWBORN RAT: A HISTOLOGICAL STUDY

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ABSTRACT

Objective: To study the effect of iron supplementation during pregnancy on epiphyseal growth plate of Sprague dawley rat pups.

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 Mar 2016 to Nov 2016.

Material and Methods: Eight female and two male Sprague Dawley rats, 3-4 months old were selected and divided into two groups and kept for breeding. Pregnant rats were divided into two groups. Four pregnant rats in each group. Group A1was control group and group B1 was experimental group. Iron supplementation was given once daily throughout pregnancy till the time of delivery. Iron was given to the experimental group in syrup form (Sytron syrup containing iron as sodium feredetate). Each 5ml of sytron syrup contains 27.5mg of elemental iron content[1]. The dose was mixed in water given to the animal. Maternal body weight (wt.) was recorded at the start and the end of experiment.

As the rat pups were born, they were weighed and euthanized. Right femur of each rat pup was removed for the epiphyseal plate analysis. It was processed, embedded and stained with Hematoxylin & Eosin, Perl's stain for histological study. Hypertrophy and proliferative zone length were histologically and statistically analyzed.

Results: Height of hypertrophy and proliferative zone was measured. Mean values of the heights of two zones were taken. Heights of hypertrophy and proliferative zones were considerably decreased in group B1 as compared to groups A1.

Conclusion: Indiscriminate iron supplementation to the rats throughout pregnancy without checking serum iron levels can disturb the longitudinal growth of epiphyseal plate of femur. The height of the hypertrophy zone and proliferative zone was significantly reduced in iron supplementation group as compared to the control group.

Keywords: Height of hypertrophy zone and proliferative zone, Iron supplementation.

INTRODUCTION

Iron is a mineral that is naturally present in many foods, added artificially to food and available as a dietary supplement. Iron supplementation is given during pregnancy to fulfill the extra iron needs of the developing fetus. Recommended dose of iron in pregnancy is 60mg to 120mg daily[2]. Iron supplementation during pregnancy[3] with normal iron status can lead to iron toxicity[4] to the mother and growing fetus[5]. Iron administration as a routine during pregnancy and childhood can be hazardous to the growth of long bones[6]. Possible effects on mother[7] include gestational diabetes, hypertension and metabolic syndromes[7]. The effect of iron overdose on hematopoietic stem cells involved cellular apoptosis[5]. There is a decrease in osteoblast activity due to iron overdose[6]. Excess iron intake during pregnancy is associated with reduced fetal growth[8,9]. There is an association between elevated maternal hemoglobin and adverse birth outcome, including low birth weight, preterm birth, and small for gestational age birth[10].

The harmful effects of iron overdose need to be assessed further especially on the growth plate of long bones. The fully matured growth plate is organized into five horizontal zones. Growth

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Received: 27 Oct 2016; revised received: 19 Dec 2016; accepted: 27 Dec 2016

plate is situated between the epiphysis and diaphysis at both ends of long bones. It is cartilaginous structure which is responsible for the elongation of bones[11].

The rationale of this study is to analyze effects of iron supplementation during pregnancy on the height of hypertrophy and proliferative zones of epiphyseal growth plate of femur of new born.

MATERIAL AND METHODS

This study was approved by Ethical Committee, of the Army Medical College Rawalpindi. This laboratory based randomized and controlled trial study 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 were selected by non-probability convenience sampling technique. Ten adult Sprauge Dawley rats (eight female and two male) were divided into two groups by random no table. Average weight was 250 to 300 grams and average age was six to eight weeks. The animals were kept at standard temperature 21 ± 2 ċ in a room maintained on 12 hour light/dark cycle[12]. 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 as instructed by NIH. Presence of vaginal plug was checked in the dames daily[13]. Its presence confirms mating and considering it the first day the pregnant rats of iron supplemental group 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[3]. Ten pups were separated as control group which is A1 and ten pups in experimental group B1.Control group was kept on normal laboratory diet. On the day one, the newborn pups 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[14] for detection of iron deposition, and toluidine stain was done[15]. Toludine blue stain was done to observe the amount of matrix. Slides were observed under the light microscope for evaluation of the hypertrophy and proliferative zones heights and

Table: Showing comparison of mean value of height of hypertrophy and proliferative zones among groups A1 and B1.

	Group A1 Mean ± SD (n = 10)	Group B1 Mean ± SD (n = 10)	Group A1 vs. B1 *p*-value
Height of hypertrophy zone	310.8 ± 65.16um	233.83 ± 24.81um	0.003
Height of proliferative zone	453.08 ± 108.82um	357.67 ± 23.35um	0.014

p-value <0.05 is statistically significant.

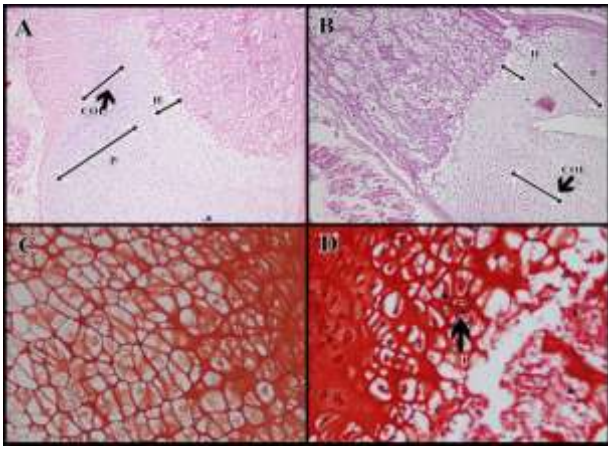

Figure-1: Photomicrograph of histological section of growth plate (10X) of control group A1 (A) and experimental group B1 (B) showing hypertrophy zone (H) and proliferative zone (P),column of chondrocytes (COL). while control group A1 (40X) on Perl's staining with no iron deposition (C). Iron deposition (I) is seen in group B1 (40X) in (D).

measured with image J.software[16]. Heights were measured at three different areas of epiphyseal plate right, left and central area and average of these three readings was recorded. Data was analyzed using SPSS version 21. Parameter was expressed as mean and standard deviation. Significant difference was determined using independent sample t-test. A *p*-vlaue<0.05 was considered significant.

RESULTS

In the study two groups each comprising ten subjects were used. Height of hypertrophy zone was measured in 10X objective magnification. The height was measured at three different zones of growth plate (right, left and center) and the boundary between proliferative and hypertrophic zones was identified by the first chondrocytes that had a significantly increased size relative to the proliferative cells[17]. The border between the reserve and the proliferative zones was identified by the upper margins of the proliferative columnar arrangement, till the beginning of hypertrophy zone[17]. In hypertrophy zone, Mean ± SD of group A1 is 310.8 ± 65.16um (fig-I).

Mean ± SD of group B1 is 233.83 ± 24.81um (table). The *p*-value for the height of hypertrophy zone is ≤0.005 (table) which is statistically significant In proliferative zone. Mean ± SD of group A1 is 453.08 ± 108.82um (table), Mean ± SD of group B1 is 357.67 ± 23.35um (table), The *p*-value for height of proliferative zone is ≤0.005 (table) which is statistically significant (fig-II).

DISCUSSION

Excess iron can lead to oxidative stress which can damage various tissues. In the current study iron administration during pregnancy resulted in the reduction of length of hypertrophy and proliferative zone as compared to the control group of rats. Previously the effects of iron on bone metabolism and osteocyte function have been observed[18]. In the current study, effects of iron supplementation on rats during pregnancy were observed especially on the longitudinal growth of epiphyseal plate of femur. Role of iron in osteoblast impairment has been noticed in iron-related osteoporosis[4].

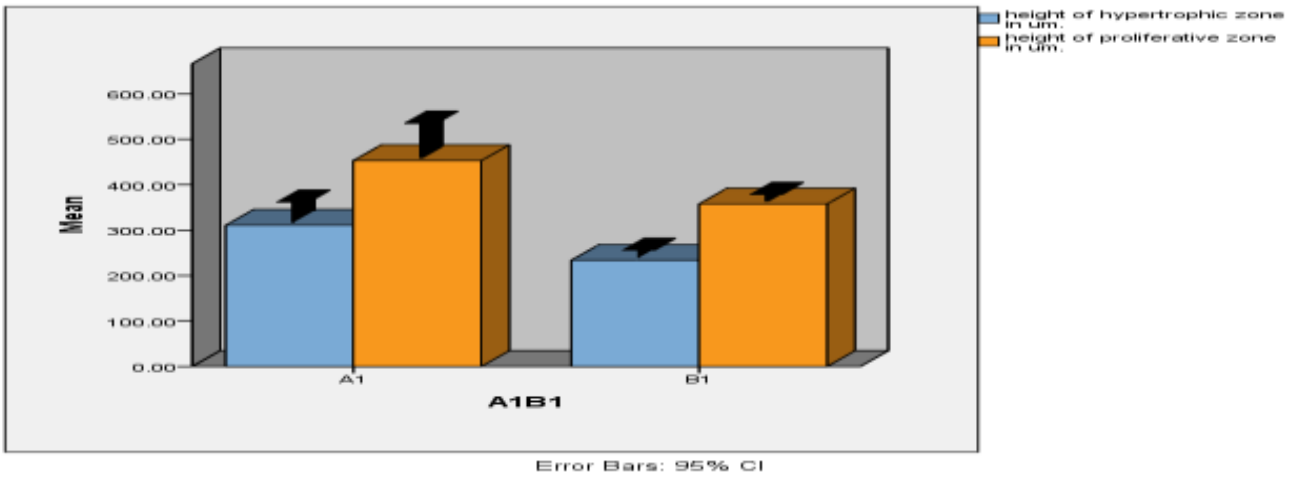

Figure-2: Simple bar chart showing comparison of mean values of heights in micrometers (along Y-axis) of hypertrophy and proliferative zones among the groups (along X-axis), control group A1 and experimental group B1.

In this study effects of iron supplementation during pregnancy on the height of hypertrophy and proliferative zone of epiphyseal growth plates of rat pups are compared with that of control group. There was statistically significant reduction in the heights of the zones in iron supplemental group. It is also in accordance with the previous studies on rats which showed that the iron excess affects the height of growth plate mainly by affecting the size of chondrocytes and

amount of matrix. Height of the hypertrophy zone is the main contributor of the growth plate height[19]. Chondrocyte proliferation has been typically considered as the major contributor of longitudinal growth. A positive correlation between the size of chondrocytes in the terminal hypertrophic zone and longitudinal growth rate has been demonstrated in both physiologic and pathologic conditions[20]. Height is determined by the hydrated chondrocyte size as well as the amount of matrix. Iron causes statistically significant reduction in height of the zone[21]. Probable reason could be the deposition of iron in chondrocytes and matrix. Clustering of chondrocytes, accompanied by a decrease in proteoglycan content[6]. This leads to decreased matrix deposition by the damaged chondrocytes. It is comparable to a study conducted on mice in which osteoarthritis was induced in ten weeks old mice and also seen in hemochromatosis. Iron excess leads to increased oxidative stress. Oxidative stress then induces inflammatory changes, in a dose-dependent fashion, which then mediate bone loss through changes in bone remodeling[22].

CONCLUSION

This study recommends that indiscriminate iron supplementation of rats throughout pregnancy without checking serum iron levels can disturb the longitudinal growth of fetus. The height of the hypertrophy zone and proliferative zone were significantly reduced in iron supplementation group as compare to the control group.

ACKNOWLEGMENT

Hafsa Abid a remarkable young budding researcher who remained intimately involved in approval phase of research proposal and subsequent data collection cum analysis.

CONFLICT OF INTEREST

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

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