ORIGINAL ARTICLE

Effect of Radiation on epiphyseal Growth Plate on Growing Long Bones, with Protective Effect of Somatotrophin (Growth Hormone) in Young Albino Rats

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ABSTRACT

Objective: To evaluate possible effects of Growth hormone in preventing the harmful effects of radiation on epiphyseal growth plate of growing long bones of young albino rats.

Study Design: Experimental study.

Place & Duration: Experimental work was completed in Six months duration in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC) Karachi.

Material & Methods: 30 litters of 10 days age of albino rats were taken for this study. They were divided into three groups: Group A (Control), Group B was given 5Gy gamma radiation and group C was given radiation and injection somatotrophin (Growth hormone). Each group was further subdivided into two subgroups according to their respective time period of treatment i-e., 2 and 4 weeks respectively. At the end of their respective period of study animals were anasthetised by ether. Dissection was done and their long bones i,e., humerus and femur were taken out and transferred, fixed, processed and embedded in paraffin. 5µm thick longitudinal sections were cut with rotatory microtome. The tissues were stained with Alcian blue- Haematoxylin and Eosin stain for measurement of epiphyseal growth plate.

Result: A highly significant decrease in mean epiphyseal growth plate was noted in irradiated subgroups as compared with control. And highly significant increase of thickness in epiphyseal growth plate was noted in growth hormone treated subgroups as compared to irradiated subgroups.

Conclusion: Irradiation causes destruction and reduction in epiphyseal growth plate. Growth hormone reverses the damage.

Key Words: Long bones, Epiphyseal growth plate, Irradiation, Growth hormone.

INTRODUCTION:

Since his appearance on earth, man has been exposed to ionizing radiation, emanating from natural radionucleotides in the environment, or as the cosmic rays from space[1]. Radiation therapy is a component of curative therapy for a number of malignant diseases. X-rays and gamma rays are the form of radiation most commonly used to treat cancer[2]. Rapidly dividing cells are more radiosensitive than the quiescent cells[3].Radiation therapy plays an important role as part of multimodality treatment for a number of childhood malignancies. Dose limiting complications of radiotherapy include skeletal abnormalities and disturbances in skeletal development within the irradiated field[4].In children treated for cancer, radiation has a direct effect on the epiphyses, which results in disruption of growth plate architecture and contributes to the impaired growth by a yet unknown mechanism[5]. Ionizing radiation affects all phases of physeal activity, but especially chondrocytes and small blood vessels. It is well known that growth plate is very sensitive to irradiation[6]. Irradiation of the epiphyseal plate of a growing long bone produces limb shortening and bowing[7].

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Normal tissue damage is the main dose limiting factor in clinical radiotherapy[8,9] Radiobiological studies have identified several radioprotective compounds some of which are non- toxic to humans[10]. Growth hormone is an anabolic hormone with effects on growth, differentiation and metabolism of cells. Treatment with growth hormone reduces radiation associated mortality[11].There is currently substantial interest in growth hormone as a protective agent against radiation related normal tissue injury[11-12]. The present study was designed to study the effects of Somatotrophin (growth hormone) on epiphyseal growth plate in irradiated long bones of young albino rats.

MATERIAL AND METHODS:

This experimental study was conducted at Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC) Karachi, from Jan to June 2009. 30 newborn litters of Albino rats were obtained from Animal house BMSI, JPMC Karachi. The animals (litters) were weighed and marked on 1st post natal day and divided into 3 groups, i-e, A, B, C each comprising of 10 animals. Each group was further divided into two subgroups, i-e, A1and A2; B1 and B2; and C1and C2 according to their respective time period of treatment, i-e, 2 and 4 weeks respectively. Each subgroup comprised of 5 animals, and was kept in separate cages along with mothers for milk feeding. The mothers were given laboratory feed and water ad libitum. Animals were kept in experimental room for 10 days prior to commencement of study, for acclimatization to the experimental conditions with 12 hours light and dark cycle. Animals were watched daily for their health status. On 10th post natal day animals (litters) were weighed, treated and allowed to survive for their respective period of study. In the present study animals were treated as under:

Group-A(A1 and A2), animals served as control.

Group- B (B1 and B2), animals received irradiation at the dose of 5 Gy for 2.02 min. from 60-unit cobalt chamber13,14, at the Department of Radiotherapy JPMC Karachi, at the commencement of study.

Group- C (C1 and C2), animals received Radiation and injection Somatotrophin with a dose of 0.3µg/gm body weight15 for their respective period of study.

After treatment, all the animals were watched daily for their health status on the basis of their activity and weight gain or loss. On completion of their respective period of treatment animals were sacrificed under ether anesthesia. Dissection was done and long bones Humerus and Femur were taken out, transferred and fixed in 10% formalin fixative for 48 hours, then were kept in 5% formic acid for 24 hours and then were processed and embedded in paraffin. The bones were oriented horizontally for longitudinal sections. 5µm thick longitudinal sections were cut with rotatory microtome. Sections were floated on the surface of warm water at 42°C in water bath. Then tissues were transferred on albuminized glass slides, the slides were put on hot plate overnight at 37-40°C for fixation of tissues.

The tissues were stained with, Alcian blue-Haematoxylin and Eosin to observe the regularity of cartilage columns at growth plates, trabecular network and blood vessels under 8X ocular and 10X objective of light microscope. The sections were observed for measuring the thickness of epiphyseal growth plate (EGP) with the help of ocular micrometer scale. 10 areas were randomly selected from each growth plate. The statistical significance of the difference of various quantitative changes between the experimental and control groups was evaluated by student "t" test. All the calculations were done by utilizing computer software SPSS (Special Package for Social Science) version 10, through Microsoft Excel in Window 2000xp.

RESULTS:

The microscopic changes were observed in 5µm thick Alcian blue-Haematoxylin and Eosin, stained sections. The following observations and results were recorded for statistical analysis. Alcian blue-H and E stained sections, the epiphyseal growth plate in humerus and femur of both subgroups A1 and A2 was regular in outline with chondrocytes scattered in the intercellular

matrix; the proliferating layer, made up of flattened cells arranged in parallel columns; hypertrophied cells also arranged in regular columns; and calcifying matrix adjacent to the diaphysis, regular in outline with a highly organized system of blood vessels was observed. Secondary ossification center was observed in both ends of bone, which was filled with trabecular network, and measured (Figure: 01 & 02).

Humerus and femur bones in both subgroups B1 and B2, the structural changes were observed in epiphyseal cartilage. Thickness of epiphyseal plate was reduced and distorted; there was considerable disorganization of cell columns of both the proliferating and hypertrophied layers and a relative increase in intercellular matrix appeared. Blood vessels just beneath the epiphyseal growth plate were disappeared. The mean epiphyseal growth plate thickness in the Humerus of subgroup B1 and B2 showed a highly significant ($P<0.001$) decrease in subgroup B1, and moderately significant ($P<0.002$) in thickness in subgroup B2 as compared to control A1 and A2 respectively (Table-1).The mean epiphyseal growth plate thickness in the femur of subgroup B1 and B2 showed a highly significant ($P<0.001$) decrease in thickness in this group as compared to control A1 and A2 respectively (Table-1).

The Humerus and Femur in both subgroups C1 and C2, there were restoration of structural changes in epiphyseal cartilage. There was considerable reorganization of cell columns of both the proliferating and hypertrophied layers. The calcifying matrix was narrow and regular in outline. The vascular invasion was appeared and myeloid elements filled the marrow spaces. The mean epiphyseal growth plate thickness in the Humerus and Femur of subgroup C1 and C2 showed a highly significant ($P<0.001$) increase in thickness in both subgroups as compared to irradiated subgroups B1 and B2 respectively (Table-1).There was highly significant ($P<0.001$) decrease in epiphyseal growth thickness in Humerus and Femur of both subgroups, when compared with control subgroups A1 and A2 respectively (Table-1).

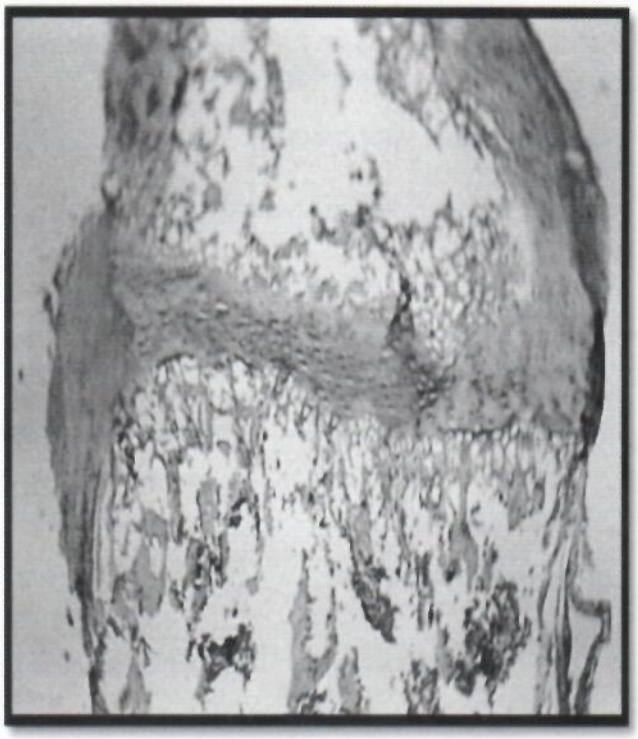

Figure No. 01: (Effect of Radiation on epiphyseal growth plate of humerus)

Figure No. 02: (Radio protective of growth hormone on epiphyseal growth plate of irradiated humerus)

Table 1: Mean Thickness of Epiphyseal Growth Plates (EGP in um) of Long bones in Different Groups of Albino rats at Variable Time Intervals

Groups	Subgroups	Treatment given	2 Weeks		4 Weeks	
			Humerus	Femur	Humerus	Femur
A	A1(n=5)	Control	555.2±0.87	545.2±1.54	----	---
	A2(n=5)		---	---	451.9±0.82	460.3±0.97
B	B1(n=5)	Radiation	165±1.32	172.6±1.58	---	---
	B2(n=5)		---	----	242.7±0.63	255.2±1.30
C	C1(n=5)	Radiation+	405.4±5.81	448.6±2.02	---	---
	C2(n=5)	Growth Hormone	----	---	426.1±1.55	429.1±1.41

***Mean±SEM±**

Statistical analysis of differences in mean thickness of EGP of long bones in different groups at variable time interval

Statistical comparison	Humerus P-value	Femur P-value	Statistical comparison	Humerus P-value	Femur P-value
B1vsA1	P<0.001****	P<0.001****	B2vsA2	P<0.01****	P<0.001****
C1vsB1	P<0.001****	P<0.001****	C2vsB2	P<0.001****	P<0.001****
C1vsA1	P<0.001****	P<0.001****	C2vsA2	P<0.001****	P<0.001****

Key:*non-significant, **significant, ***moderately significant, ****highly significant

DISCUSSION:

Radiations are used in medical treatment and diagnostic procedures. Radiation therapy can be used in combination with surgery and/or chemotherapy to provide permanent control or death of a tumor[14].In radiation therapy high energy rays are used. The gamma radiation produces anatomical and pathological alterations in bone growth. Several investigators have used experimental gamma radiations in animals. Nunia et al.[16], had used Swiss albino mice for whole body gamma irradiation. Lloyd et al.[17], used C57BL/6 mice and observed the effects of ionizing radiation on cortical bone. Growth hormone can stimulate growth of different tissues, such as skeletal and soft tissues, by increasing number of cells[12]. In the present study, the group C animals were protected by growth hormone. In group B animals the structural changes were observed in epiphyseal cartilage, thickness of growth plate was decreased in both humerus and femur from 2 to 4 weeks, and the cartilage cells were disorganized. This study is in agreement to Furstman[18], who used 6 Gy total body irradiation on Holtzman strain weanling rats, three days after exposure, the epiphyseal cartilage was narrower than the control epiphyseal plate, the number of cells decreased in both proliferating and hypertrophied layers. The cell columns were arranged irregularly with relative increase in intercellular matrix. This study is also in agreement with Argüelles et al[6], who used rabbits for irradiation and observed after one week, that there was marked alteration of the whole growth plate of the irradiated knee. There was marked disorganization of columnar arrangement with

isolated chondrocytes with signs of necrosis as well as degeneration of matrix. The degenerated zone showed an obvious reduction of thickness. It is also correlated with the findings of Eifel et al[9], who observed that the effect of radiation on epiphyseal bone growth is one of the most important dose-limiting factors in radiotherapeutic management of children with malignant neoplasm

Matsouka et al[16], reported that low dose irradiation on femur of wistar rats, is capable of inducing apoptosis in bone marrow cells , the growth hormone administration reverse this process. As described by Standring et al[19], Growth hormone is required for normal interstitial proliferation in growth cartilage, and hence increases in stature. Reduction of growth hormone production in the young leads to quiescence and thinning of growth plates. In the present study in group C, the structural changes in epiphyseal cartilage were restored; blood vessels and myeloid elements were normally present. As suggested by Ganong[20], in young animals in which the epiphysis have not yet fused to the long bones, growth is stimulated by growth hormone. Chondrogenesis is accelerated, and the cartilaginous growth plates widen, they lay down more bone matrix at the ends of long bones.

CONCLUSION:

In the light of above considerations the net result suggest that injurious effect of radiation occur more frequently at a dose of 5 Gy in growing bones of young albino rats. Irradiation can cause cellular damage, but the Growth hormone, restores the growth. The present study suggest that adverse effects of irradiation need special cautions for human subjects and the study may act as a base line for the extension of project for humans.

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