ORIGINAL ARTICLE

Modifying Role of Somatotrophin On Trabecular Width & Osteoblast Count of Irradiated Long Bones of Young Albino Rats

Abdul Latif Panwhar[*], *Furkhanda Jabeen Dahri*[**], *Razia Tariq Qureshi*[***], *Mevo Khan Zardari*[****]

ABSTRACT

Objective: To evaluate the modifying effect of Somatotrophin in preservating the harmful effect of α-radiation on trabecular width and osteoblast count in long bones of albino rats litters.

Study Design: Experimental study.

Place & Duration: Experimental work was completed in six month duration at Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC) Karachi.

Material & Methods: 30 newborn 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 inj. Somatotrophin (Growth Hormone). Each group was further divided into two subgroups according to their respective period of treatment i.e. 2 and 4 weeks respectively. At the end of their respective period of study the rats were anaesthetized by ether, dissection was done and their long bones humerus and femur taken out, transferred, fixed and processed. 5µ longitudinal sections were cut with rotatory microtome. The tissues stained with Goldner's trichrome for visualization of osteoblast cells and trabeculae.

Results: A highly significant decrease in osteoblast count and trabecular width was noted in irradiated subgroups compared with control. And highly significant increase in osteoblast count and trabecular width was noted in Somatotrophin treated subgroups as compared to irradiated subgroups.

Conclusion: Irradiation causes destruction of bone marrow, trabeculae and osteoblast cells while Somatotrophin reverses the damage.

Key words: Long bones, Trabeculae, Osteoblast cells, Somatotrophin.

INTRODUCTION:

Radiation therapy involves treating the cancer with ionizing radiation; for certain localized causes, it may be curative. Ionizing radiation can be delivered by radiation emitted from decay of radioactive isotopes or by high-energy radiation beams.[1] Ionizing radiation is either penetrating (x-rays,γ-rays), or non-penetrating.

[*] *Associate Professor Antomy Department PUMHSW Nawabshah-SBA.*

[**] *Associate Professor Deptt. of Surgery PUMHSW Nawabshah-SBA.*

[***] *Associate PRofessor Deptt. Gyne & Obst. PUMHSW Nawabshah-SBA*

[****] *Professor & Chairman Phsiology Deptt. & Dean of Basic Medical Sciences PUMHSW, Nawabshah-SBA*

Correspondence to:
Dr. Abdul Latif Panwhar
Associate Professor, Anatomy Department
PUMHSW Nawabshah. SBA

Penetrating radiation affects the skin and deeper tissues, while non-penetrating radiation affects the skin alone.[2] Damage to underlying bone can be a major complication of radiation therapy, whether alone or with chemotherapy. Radiation changes resulting from serious complications have been reported in several bones.[3] The cell nucleus is considered the primary target for the lethal effects of ionizing radiation.[4] Radiation damages DNA, rapidly dividing cells are more vulnerable to injury than the quiescent cells. Tissues with a high rate of cell division, such as gonads, bone marrow, lymphoid tissue and the mucosa of gastrointestinal tract, are extremely vulnerable to radiation, and the injury is manifested early after exposure.[5] Dose limiting complications of radiotherapy include skeletal abnormalities and disturbances in skeletal development

within the irradiated field.[6] Sensitivity of cells to radiation varies directly with their reproductive power.[7]

Growth hormone causes growth of almost all tissues of the body that are capable of growing. It promotes increased sizes of cells and increased mitosis, with development of greater number of cells and specific differentiation of certain types of cells such as bone growth cells and early muscle cells.[8] Growth hormone stimulates increased deposition of protein and increased growth in almost all tissues of body, its most obvious effect is to increase growth of skeletal frame. This results from multiple effects of Growth hormone on bone, including: increased deposition of protein by the chondrocytic and osteogenic cells that cause bone growth, Increased rate of reproduction of these cells, and a specific effect of converting chondrocyte into osteogenic cells, thus causing deposition of new bone.[8] Growth hormone reduces radiation associated mortality.[9]

MATERIAL & METHODS:

Thirty newborns of Albino rats were taken for this experimental work, they were weighed and marked on 1st post natal day and divided into 3 groups, i-e, A, B and C each comprising of 10 animals. Each group was further divided into two subgroups, i-e, A1and A2; B1 and B2; C1and C2 as their respective 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 given laboratory feed and water ad libitum. Rats 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) weighed, and treated according to their grouping. The rats were treated as under:

Group-A (A1 and A2), was observed as control. Group-B (B1 and B2), rats given 5 Gy irradiation 2.02 min. from 60-unit cobalt chamber,[10,11] at the Department of Radiotherapy JPMC Karachi, at the commencement of study. Group-C (C1 and C2), rats were given Radiation and injection Somatotrophin at the dose of 0.3µg/gm body wt. of animal for their respective period of study.[12] After treatment, all the animals watched daily for their health status. The animals were weighed weekly. After 2 and 4 weeks, the, animals were sacrificed under ether anaesthesia. They were fixed on dissecting board and dissected. Their long bones Humerus and Femur were taken out and transferred and fixed in 10% formalin fixative for 48 hours, then kept in 5% formic acid for 24 hours, then processed and embedded in paraffin. The bones were oriented horizontally and 5µ longitudinal sections were cut with rotatory microtome. Sections were floated on the surface of warm water at 42°C in water bath. Then cut sectioned were transferred on albuminized glass slides and were put on hot plate overnight at 37-40°C for fixation of tissues. The tissues were stained with: Goldner's trichrome stain, to visualize trabeculae, osteoblast and osteoclast cells. The width of trabeculae in section, apparently seen of marked thickness was measured out by ocular micrometer scale under 8X ocular and 40X objective. The osteoblast cells were observed just below the EGP, and counted with the help of ocular counting reticule in 10 different randomly selected areas of tissue section under 8X ocular and 40X objective of light microscope. The statistical significance of the difference of various quantitative changes between the experimental and control groups was evaluated by student t-test by utilizing computer software SPSS-20.

RESULTS:

Both gross and microscopic observations were made, in all groups. The microscopic changes were observed in 5µm thick, Goldners trichrome stained sections. The following observations and results were recorded for statis-tical analysis. Both bones in both subgroups showed numerous trabeculae, long and slender in shape, some interconnected, projecting from calcifying matrix to the metaphysis, and were lined by regular rows of osteoblast cells. The marrow was highly cellular and was filled with myeloid elements (figure-1). On observing Goldners

trichrome stained sections, in both humerus and femur of both subgroups B1 and B2, there was loss of trabeculae in the diaphysis, and the persisted trabeculae were short. The osteoblast cells were scanty lining the borders of trabeculae and osteocytes disappeared from lacunae. There was decrease in myeloid elements (Fig-2). The mean trabecular width of Humerus measured in subgroup B1 and B2 showed significant decrease in subgroup B1 and highly significant decrease in trabecular width of subgroup B2 as compared to control A1 and A2 respectively (Table-1). The mean trabecular width of femur measured in subgroup B1 and B2 showed highly significant decrease in subgroup B1,there was significant decrease in trabecular width of subgroup B2 as compared to control A1 and A2 respectively (Table-1). The mean osteoblast cell count in Humerus of subgroup B1 and B2 showed a highly significant decrease in cell count in both subgroups as compared with control subgroup A1 and A2 respectively (Table-2). The mean osteoblast cell count in femur of subgroup B1 and B2 showed a highly significant decrease in cell count in both subgroups as compared with control subgroup A1 and A2 respectively (Table-2). On Goldners trichrome stained sections, in both humerus and femur of both subgroups C1 and C2, the trabeculae were observed to be long and slender and thickened, with lacunae containing osteocytes, and the borders lined mainly with osteoblasts, inter trabecular spaces filled with marrow elements (Fig-3). The trabecular width measured in humerus of subgroup C1 and C2 showed moderately significant increase as

Fig.1 Goldners trichrome stain in 5µm thick section of femur of control group at 400X magnification

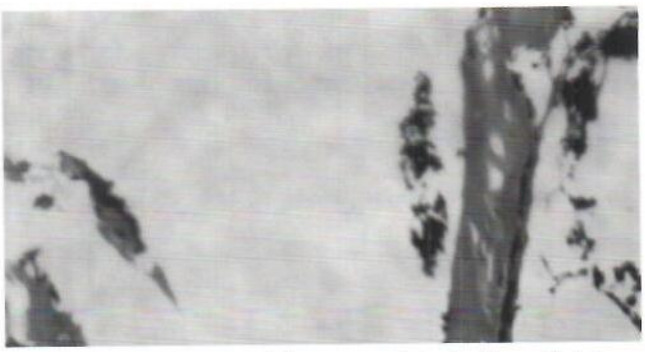

Fig.2 Goldners trichrome stain in 5µm thick section of femur of irradiated group B at 400X magnification

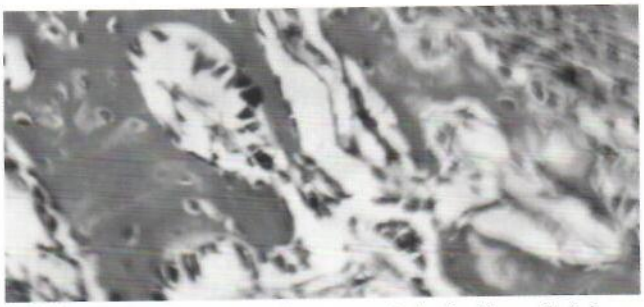

Fig.3 Goldners trichrome stain in 5µm thick section of femur of Radiation+ GH treated (C group) at 400X magnification

compared to irradiated subgroups B1 and B2 respectively. There was insignificant decrease of trabecular width in humerus of both subgroups C1 and C2, as compared to control subgroups A1 and A2 respectively. The mean trabecular width of femur showed moderately significant increase in subgroup C1, and highly significant increase in subgroup C2 as compared to irradiated subgroup B1 and B2 respectively. There was highly significant decrease of trabecular width in femur of subgroup C1 and significant increase in subgroup C2 as compared to control A1 and A2 respectively (Table-1). The mean osteoblast cell count in Humerus showed a highly significant increase in cell count in subgroup C1 and moderately significant increase in subgroup C2, as compared with irradiated subgroup B1 and B2 respectively. There was significant decrease in subgroup C1 and C2, when compared with control. The mean osteoblast cell count in femur of subgroup C1 and C2 which showed a highly significant increase in cell count in both subgroups C1 and C2, as compared with irradiated subgroup

B1 and B2 respectively. There was highly significant decrease of cell count in femur in subgroup C1 and moderately significant decrease in subgroup C2, when compared to control subgroups A1 and A2 (Table-2).

TABLE- 1

***Mean Trabecular width (µm) of Long Bones in different Groups of Albino Rats at variable time interval**

Groups	Subgroups	Treatment given	2 weeks		4 weeks	
			Humerus	Femur	Humerus	Femur
A (n=10)	A1 (n=5)	Control	78.5±2.69	84±1.69	–	–
	A2 (n=5)		–	–	68±2.0	71.5±1.69
B (n=10)	B1 (n=5)	Radiation	53±3.48	46.5±2.57	–	–
	B2 (n=5)		–	–	49±2.03	56.5±1.0
C (n=10)	C1 (n=5)	Radiation+Growth Hormone	74±1.87	65±1.11	–	–
	C2 (n=5)		–	–	66.51±2.04	77±2.15

Statistical analysis of differences in mean trabecular width in Humerus and Femur bones between different groups at variable period

Statistical comparison	Humerus P-value	Femur P-value	Statistical comparison	Humerus P-value	Femur P-value
B1vsA1	<0.01***	<0.001****	B2vsA2	<0.001****	<0.01***
C1vsB1	<0.01***	<0.01***	C2vsB2	<0.01***	<0.001****
C1vsA1	>0.05*	<0.001****	C2vsA2	>0.05*	<0.03**

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

TABLE- 2

*Mean Osteoblast number of Long Bones in different Groups of Albino Rats at variable time interval

Groups	Subgroups	Treatment given	2 weeks		4 weeks	
			Humerus	Femur	Humerus	Femur
A (n=10)	A1	Control	72±0.44	74.2±1.28	--	--
	A2		--	--	52.4±1.43	53.4±1.69
B (n=10)	B1	Radiation	22.4±1.36	17.8±1.49	--	--
	B2		--	--	27.4±1.12	23±1.18
C (n=10)	C1	Radiation+Growth Hormone	53.2±3.29	64.2±1.35	--	--
	C2		--	--	43.4±2.97	41.4±1.66

Statistical analysis of differences in mean osteoblast number in Humerus and Femur bones between different groups at variable period

Statistical comparison	Humerus P-value	Femur P-value	Statistical comparison	Humerus P-value	Femur P-value
B1vsA1	<0.001****	<0.001****	B2vsA2	<0.001****	<0.001****
C1vsB1	<0.001****	<0.001****	C2vsB2	<0.01***	<0.001****
C1vsA1	<0.01***	<0.001****	C2vsA2	<0.04**	<0.01***

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

B1 and B2 respectively. There was highly significant decrease of cell count in femur in subgroup C1 and moderately significant decrease

DISCUSSION:

Radiation consists of cosmic radiation and radiation emitted from radioactive substances present in the ground or commercial sources.[13] Radiations are used in medical treatment and diagnostic procedures. It can be used in combination with surgery and/or chemotherapy to provide permanent control or death of a tumor.[11] Somatotrophin can stimulate growth of different tissues, such as skeletal and soft tissues, by increasing number of cells.[9] Lloyd et al.,[14] used mice for radiation, and observed a profound and prolonged loss of trabeculae in long bones. Matsouka et al.,[12] reported that low dose irradiation on femur of wistar rats, is capable of inducing apoptosis in bone marrow cells and endonuclease activation manifested by subsequent fragmentation of DNA and RNA, and the growth hormone administration reverse this process. Wikipedia,[15] reported that growth hormone directly stimulate the division and multiplication of chondrocytes of cartilage. Parker and Berry,[16] reported that radiation induced suppression of bone marrow function. According to Guyton and Hall,[8] Somato-trophin strongly stimulates the osteoblasts, there-fore, the bones can continue to become thicker throughout life. This study was in agreement with Furstman[17] that in group B animals the structural changes were observed inhumerus and femur from 2 to 4 weeks, and the cartilage cells were disorganized, and trabeculae in metaphyseal and cortical bone were either absent or decreased and persisted trabeculae were reduced in thick-ness. Osteoblasts were less, and osteocytes were destructed or absent from lacunae, the bone marrow was deficient.

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 long bones of young albino rats. Gamma radiation produces destruction of trabeculae, osteoblasts, osteocytes and bone marrow elements in diaphysis of long bones of rats, which can be minimized by Growth hormone. So, this research suggest that adverse effects of irradiation need special cautions for human subjects and this study may act as base line for extension of project for humans.

REFERENCES:

1. Walker BR,Colledge NR, Ralston SH, Penman ID. Oncology in: Davidson's Principles and Practice of Medicine, 22nd edition, Churchil Livingstone, Elsevier, Edinburgh London. 2014;259-82.
2. Rosai J. Bone and Joints in: Rosai and Ackerman's Surgical Pathology, 10th edition, Mosby Elsevier, Edinburgh, London. 2011;2013-2104.
3. Kumar P and Clark M. Environmental Medicine in: Kumar & Clark, Clinical Medicine 8th ed. Edinburgh, Saunders Elsevier. 2012; 929-36.
4. Madrid O, Varea S, Peraz IS, Gomez-Garcia L, Miguel ED, Segura G and Perona R. Growth hormone protects against radiotherapy-induced cell death. Eur J Endocrinol. 2002;147:535-41.
5. Kumar V, Abbas A, Aster J. Environmental and Nutritional Diseases in: Robbins and Cotran Pathologic Basis of Disease. 9th edition, Volume I, Haryana India, Elsevier. 2014;403-50.
6. Pateder DB, Eliseev RA, O'Keefe RJ, Schwarz EM, Okunieff F, Constine LS, Puzas JE and Rosier RN. Role of autocrine growth factors in radiation damage to epiphyseal growth plate. Radiat Res. 2001;155(6):847-57.
7. Essman SC, Lattice J, Cook JL., Turnquist S and Kuroki K. Effect of 153Sm- Ethylene diaminetetramethylenephosphonate on physeal and articular cartilage in Juvenile Rabbits. J Nucl Med. 2003; 44(9): 1510-5.
8. Guyton AC and Hall JE. Endocrinology and Reproduction in: Text book of Medical

Physiology Philadelphia, Pennsylvania. 2011;881-1027.
9. Tekin SB, Ertekin MV, Erdogon F, Sezen O, Karslioglu I, Gepdiremen A, Serifoglu K and Atlas S. Is growth hormone a radioprotective agent? JEADV J Eur Acad Dermatol Venereol. 2006; 20(3): 293-8.
10. Koc M, Tayasi S, Buyukokuroglu ME, Bakan N. Melatonin protects rat liver against irradiation-induced oxidative injury. J Radiat Res. 2003; 44(3): 211-5.
11. Nash H. Radiation therapy in Dogs, Cats, and other small animals. Veterinary Services Department. 2008; 1-4.
12. Matsouka P, Mylonas P, Papandoniou E, Dimitropoulou I, Floratou K, Alexandridis T and Kardamakis D. Abdominal Radiation Initiates Apoptotic Mechanism in Rat Femur Bone Marrow Cells in vivo that is Reversed by IGF-1 Administration. J Radiat Res. 2008; 49(1)41-7.
13. Prasad KN, Cole WC, Hasse GM., Radiation Protection In Humans: Extending the concept of as low as achievable from dose to biological damage. Br J Radiol. 2004;77:97-9.
14. Lloyd SAJ, Bandstra ER, Travis ND, Nelson GA, Bourland J D, Peanut MJ, Gridley's, Willey JS and Bateman TA. Spaceflight-relevant types of ionizing radiation and cortical bone: Potential late effect? Adv Space Res. 2008; 42(12):1889-97.
15. URLhttp://en.Wikepedia.org/wiki/Growth_hormone, 2008 dated 6/2/2008.
16. Parker RG and Berry HC. Late effects of therapeutic irradiation on the skeleton and bone marrow. Cancer. 1976;37(2):1162-71.
17. Furstman LL. Effect of Radiation on Bone, J Dent Res. 1972;51(2)596-604.