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HISTOMORPHOMETRIC COMPACT-CANCELLOUS ANALYSIS IN RELATION TO AGING FEMALE ALBINO RATS

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ABSTRACT

Osteoporosis is a world wide well known metabolic bone disease featured as vulnerable fracture fragility associated by dropped bone mass.. Ageing female Albino rats were used in this experiment in order to detect changes and effects of the applied medicines; An image analytical method was designed for the metamorphic measurements regarding cancellous and cortical compact bony fraction. The transitional evaluation was studied from cancellous to compact through light microscopy,the effects of treatment of tamoxifen and nandrolonedecanoate were also analysed. The distance was taken from area of transition to peripheral rim/periosteum for compact bone and similarly trabeculi were also mapped out, thus their respective width/thickness was calculated statistically,several points in terms of area of interest (multiple discrete zones) explored through an individual bone cross section and comparative matching was done between the groups and found to have significant difference of cortical thickness in case of combination therapy treated group versus the other groups.

Key words: Osteoporosis,heterogeneity,FDA,TGF,ovariectomized rats.

INTRODUCTION

Osteoporosis is a widely ageing bone disease that has affected more than 75million people worldwide; characteristically featured decreased level of strength, low architectural mass with relative increased sensitivity to fracture and micro-damages (Cummings and Melton, 2002). There is an impact of internal bone mass upon the overall internal milieu mechanics of bone, capacity toward fracture healing and the tissue biochemical strength (Judex*et al.*, 2003). The quantitative data explored in terms of small sized bony fractures, micro-damages strongly co-relate with compositional as well as micro-architectural changes. A great many recent mode of researches have principally been focusing nanoscale topography also in terms of mineralized crystalline ground substance i.e. collgens and calcium crystalloids, their geometrical pattern of orientation, numerical concentration etc, which in turn tend to develop substantial relation as to map out and determine qualitative aspects.

Animal models like overiectomized rats have been used exceedingly to study various aspects related to bone research (Kalu*et al.*,1984; Newman *et al.*,1995). Estrogen-decline leads to cause negative effects like thinning of trabeculi and distortion of normal connectivity pattern (Rodgers *et al*., 1993), ageing(Weiss *et al*., 1991; Grynpas*et al*., 1993), delayed fracture healing (Ferguson*et al.*, 1987), and affected calcium homeostasis(Riond*et al.*, 1995), untoward effects of drug treatment (Aerssans*et al.*, 1994) etc. . The macaques, rodents other non human primate models have undergone an exhaustive mode of experimental trial in order to attain a semi-ideal search of animal from a certain specific set of research related to human skeletal biology.They have been sorted out quite an effective and meaningful way for the evaluation of hormonal as well as non-hormonal therapeutics as to prevent/slow down or treat the process of aging osteoporosis, yielding promising results that were found to be profoundly consistent in order to up-grade clinical approach and usefulness. Great insights into cortical and cancellous architectural changes with respect to treatment regimens have been developed,improved and modified as part of the due course of research paradigm.

FDA (1994) calls for detailed analysis and assessment of noval compounds and combinations (drugs/chemicals or medicines etc.) probe out their usefulness by recommending large and small (rodents) animal models with evolving larger scale comparison data, it is however a difficult task and the selection of an appropriate animal model for these studies is quite challenging. The idea of an ideal model encompassing broader aspects of bone research, could meet the major objectives of research is still a wild goose chase.Nevertheless, long term as well as short term efforts in terms of experimental trial and error have been under way, yet kept going on , our current experiment is also one of the example of this objective goal.

Harper was firstwho introduced Tamoxifen,triphenylethylene derivative(TPE), a synthetic non-steroidal estrogen analogue, characteristically a potent estrogen in rat (Harper and Walpole,1966). It was made to undergo

successful modification of its stilbene nucleus resulted in a compound with anti-estrogenic properties as well (Ugwumadu and Nauren, 1998), hence estrogen and anti-estrogen both causing dual effects.

A series of Androgenic steroids (AAS) with anabolic role are synthetic compounds which have got core structure containing testosterone, are applied in the treatment of many conditions such as anaemic disorders,reproductive dysfunction, carcinoma of breast etc. In order to enhance the anabolic potency with slow rate of degradation useful modifications are made to its core structure of Testosterone. The role of sex steroids is quite significant in the strength and maintenance of skeleton both in human and rodents (Turner *et al.*, 1994). Anabolic steroid produce positive protein turnover and regulate calcium metabolism. In this study the transitional evaluation was mapped out in cancellous as well as compact bone areas through light microscopy ,the effects of treatment of tamoxifen and nandrolonedecanoate were also analysed in the given context.Purpose of study is to assess and compare the effects of tamoxifen and nandrolonedecanoate in combination on compact cancellous morphology of bones in case of ageing female Albino rats.

MATERIALS AND METHODS

In this study female Albino rats of post-reproductive/post-menopausal(Farris *et al.*,1962) age 8 to 10 month old were selected.All animals were maintained on standard laboratory diet containing wheat,flour,milk-powder,supplement of minerals,vitaminsetc,allowed free access to waterand kept under controlled lighting schedule. The experiment was carried out under approved animal care protocol.

Animals were divided into four groups by making punch holes in their ears. Each group comprised three rats of similar age,sex and weight.

Group A: control kept on corn oil vehicle given sub cutaneously daily for four week.

Group B: Tamxifentreated, giventamoxifen 5mg/kg per animal sub cutaneously daily for four week.

Group C:Nandrolonedecanoatetreated,given 3 mg/kg body wt.(Gerez*et al* ., 2005) intra- muscularly for four week.

Group D: combined tamoxifen and nandrolonedecanoate treated(4 week).

On completion of four week treatment animals were sacrificed under chloroform anesthesia; dissection was done , bones excised,sections of upper end,lower end(epiphyses) ,diaphysis (shaft) were taken then fixed in 10% formalin .Later on bones were processed as under. Decacification,dehydration,clearing,infiltration,emedding,sectioning,staining.Routinelyused haemotoxylin and eosin stains have been used.Twelve bones i.e. right and left side femur and humerusfrom each group were used.

An image analytical method was designed in this experiment for the metamorphic measurements regarding cancellous and cortical compact bony fraction(Christopher *et al.*,1997).The method of light microscopy was used to examine the effects of treatment of tamoxifen and nandrolonedecanoate and the transitional evaluation from cancellous to compact.In this context the distance was taken from area of transition to peripheral rim/periosteum for compact bone and similarly trabeculi were mapped out, thus their respective width was calculated;several points in terms of area of interest (multiple discrete zones)exposed through the individual bone cross section were selected and comparative matched data was measured on statistical basis among the groups.

RESULTS

Control/sham group

The mean trabecular width of humerus,and femur in control/sham group measured 7.60 and11.80 µm, respectively(Table 3,4). The mean cortical width in femur and humerus measured was 29.50 and 25.00µm(Table1 and 2; Fig.1).

Tamoxifen treated group

The mean trabecular width of humerus ,and femur in this group measured was 9.62and 11.20µm,respectively. The mean cortical width in femur and humerus measured was 32.00 and 29.00µm respectively(Table1,2; Fig.2).

Nandrolonedecanoate treated group

The mean trabecular width of humerus,and femur in this group measured was 10.90 and 14.85µm respectively(Table3,4) The mean cortical width in femur and humerus measured was36.00 and 28.00µm,respectively(Table 1,2; Fig.3).

Combination treatment(nandrolonedecanoate and tamoxifen treated) group

The mean trabecular width of humerus,and femur in this group measured was 12.10 and15.15µm(Table 3,4) respectively. The mean cortical(compact) width in femur and humerus measured was46.00 and 31.00µm, respectively(Table 1,2; Fig.4).

The cortex of femur was found to have significant difference(cortical width/thickness),greater thickness compared to rest of the other groups.Relatively more well organized anastomoting pattern of trabeculi were observed in femur,the difference may be based on heterogeneity as well as receptor modulation(Brennan *et al.,*2011).

DISCUSSION

Great many advancements and useful proceedings in line of bone biology under went quite successfully with the advent of new technical procedures, so the process of exploration has become simple and more corroborative e.g. shift of in vivo complex and more difficult study to vitro (ex-vivo) has eased the mode of exploration ; as for example design of culture media in relation to bone cells . Animal models have already been and shall play a central role investigating patho-physio dynamics of bone , similarly the bone metabolic state , fracture and micro-damages will be quite usefully explored in the light of application of different drugs or chemicals. Moreover, before establishing efficacy of any drug for clinical trials in terms of osteoporosis, the Food and Drug Administration(FDA, 1994)now requires marked acceptable utility of the applied regimen with regard to number of advantageous effects by testifying small(rodent) animal and larger animal strata under set criteria of intra-cortical bone remodeling (Thompson*et al.,* 1995).The rat by far has been one of the commonest used animal because of number of positive facts, easy availability, cost effectiveness (cheaper in price),conveniently cared and nurtured , having short life span, its genetic, metabolic and mechanical loading profile help bone research and aging changes in the light of human biological domain;results found to be of great help and assistance in the benefit and comparative wellness against homosapiens(Moskilde, 1995).

Different models have been used e.g., the aged rats(Vanderschueren*et al.,* 1993),the growing rats(Aerssens*et al.,*1994), and the mature young ovariectomized rat (Wronski*et al.,* 1985), and the aged ovariectomisedrats(Ibbotson *et al.,* 1992). Certain other species can be replaced against rats like mouse, guinea pigs etc (Aerssens*etal.,*1994; Weiss *et al.,* 1991).The choice of an appropriate animal model is an up hill challenge , the suitability of model substantially depend upon the objective tasks of study though there is no concept of ideal model might give considerably excellent coverage to research protocol. Such standard norm could encompassing multidimensional aspects of research related to bone disorders such is yet to be discovered; allanimal models have their own plus and minuses. In this study bone microarchitecture i.e. trabecular and compact area found in humerus and femur was evaluated, the qualitative and quantitative data/ changes were mapped out in concrete manner. The trabeculi in humerus of control group found to be relatively thinner, less well oriented and reduced in thicknesss in various loci as compared to that of femur of control; during oestrogen deficiency, bone mass and trabecular micro-framework get degraded and distorted by way of trabecular thinning, micro-damages and disturbed trabecular micro-architectural connectivity (Parfitt*et al.,* 1983). The aging estrogen deficit state lead to bring about extensive marked micro-damages within bone tissue (Dai *et al.,* 2004). The resorptive gaps were also larger as compared to the femur of control.Similarly tamoxifen treated and nandrolone given groups reveal better and improved changes, however no marked difference were developed and results remained non-significant. However when these drugs were given in combination form, the femur showed significant variation as a result the size of the trabeculi found increased as compared to other groups, similarly their pattern of arrangement was found well connected anastomosing with reduced resorptive gaps or lacunae .our studies are well matched with Hamdy*etal* who worked on 21 patients suffering from osteoporosis of idiopathic origin having used radiology and DEXA,given 50 mg nandrolonedecanoate intra-muscular per week on left femur and lumber vertebra.A nuclear binding assay determined the presence of estrogen and androgens receptors in osteoblasts of bones (Bonnelye and Aubin, 2002; Kousteni*et al.,* 2001) nandrolone might operate through “estren mechanism” exploit these receptors as 19NT (Nor-Testosterone) also taken as nandrolone (Centrella*et al*., 2004). the biological activity of estren on the skeleton of in sex organ ablated mice ,elderly male and females humans and in castrated primates,ratsetc has also been documented.(Centrella*et al.,* 2004; Kousteni*et al.,* 2002).

Tamoxifen works through the activation of gene coding TGF-b3 (transforming growth factor) a protective protein maintain bony texture in animals (Butta*et al.,* 1992). The changes differ in some respects with the study of Ke*et al.* (1995).This study provides direct evidence that combination treatment affects cortex of the femur markedly and a significant difference is observed on statistical ground,estrogen deficiency in aging state induced noticeable alterations in the mineralized as well as organic fractionated environment of bone, the trabeculi across certain

specific anatomical sites/loci.Healthy bones reveal heterogenic distribution of mineral component and this heterogeneity might be one of the key modulator operate through receptors in osteocytes, the current study reveals that this heterogeneity might be one of the factor yielding difference in the cortical region of femur. In addition, direct modulation of osteocyte and process of secondary mineralisation might be maintained by oestrogen receptors (Batra*et al.*, 2003). When levels of circulating oestrogen get declined in post menopausal period. It is known that osteocyte apoptosisis is upregulated during oestrogen decline phase (Kousteni*et al.*, 2001) and which in turn lead to infilling of the remaining cavities, known as micro-petrosis (Boyde*et al.*, 1998). Further studies are required however to authenticate if these mechanisms contribute to the differences observed.The localized increase in mineral heterogeneity may occur as a result of site specific bone remodeling activity or mechanical loading pattern. While back ground studies on the sheep cohort have reported region l(site specific), variability in trabecular micro-architecture having observed various anatomical regions of the vertebrae of healthy sheep in comparison(Kennedy *et al.*, 2009). Increase in heterogeneity within trabeculae were site specific,the decreased estrogenic level makes shift in the spatial configuration of calcium,which is linked up to the intensity of the degree of surface remodeling i.e. high turn-over rate ,as characteristically observed in osteoporosis ; such increased heterogeneity may be a transient shift and the normal heterogeneity would be restored by almost complete normalized mineralization under the effects of these drugs over a certain period of time.

Table 1. Femur compact bone
(mean cortical width in µm)..

	Mean cortical width	St.D
A	29.50	1.16
B	32.00	6.30
C	36.00	8.43
D	46.00	1.83

Table 2. Humerus compact bone
(mean cortical width in µm).

	Mean cortical width .	St..D
A	25.00	5.14
B	29.00	2.56
C	28.00	9.35
D	31.00	1.91

Table 3.HumerusTrabeculi
(mean cortical width in µm).

	Mean cortical width	St.D
A	7.60	0.03
B	9.62	0.05
C	10.90	0.09
D	12.10	0.08

Table 4. Femur Trabeculi
(mean cortical width in µm).

	Mean cortical width	St.D
A	11.80	3.96
B	11.20	1.01
C	14.85	2.95
D	15.15	1.05

One other study reported that oestrogen deficiency in post meno-pausal phase cause amplification in the mineralized fraction in jaw ,so number of controversies that are yet to be probed into , similarly certain confounders like mechanical loading profile of an animal , bio-cellular activity are believed to impact heterogeneity (Batra*et al.*, 2003). All these kind of findings light upon the need of getting tissues from different loci/sites/regions exploring many more bones in order to achieve concrete results and could reach at positive end point.

Conclusion

The present study suggests that group A and B treated group trabeculae was found to have maintained similar level of thickness as that of control, while combination treatment in group D (combination therapy **(**tamoxifen and nandrolone) was found to have significant increase in cortical thickness compared to rest of the othergroups, this may be a matter of site specific distribution and modulation of receptors or mechanical loading factor .However heterogeneity may also matters which is site specific. Further studies are required in this context in order to substantiate the use of combined therapy as treatment regimen of better choice in osteoporosis.

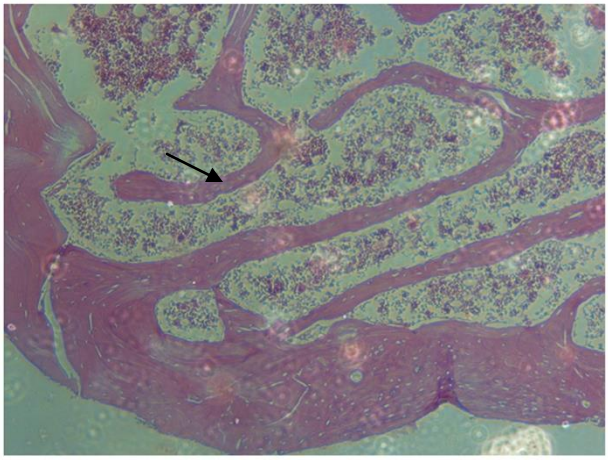

Fig.1. Photomicrograph H and E stained section from Group A arrowshowing trabeculae.

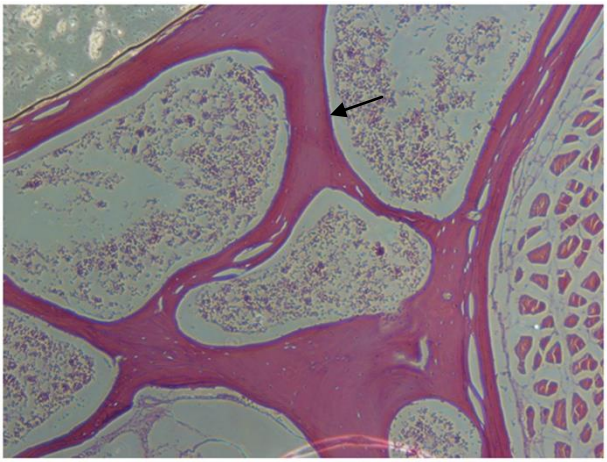

Fig.2. Photomicrograph H and E stained section from Group B arrowshowing trabeculae.

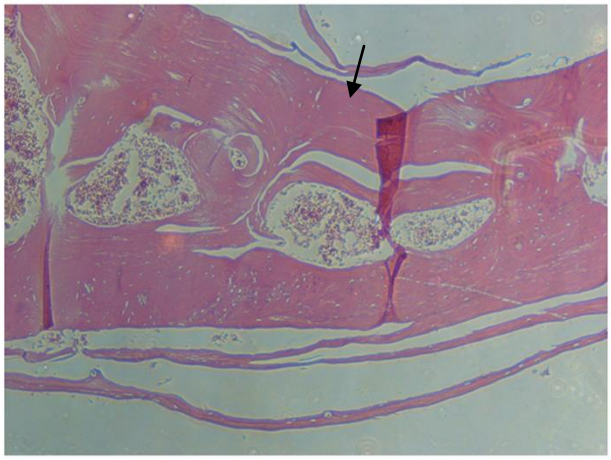

Fig.3. Photomicrograph H and E stained section from Group C arrowshowing cortical bone.

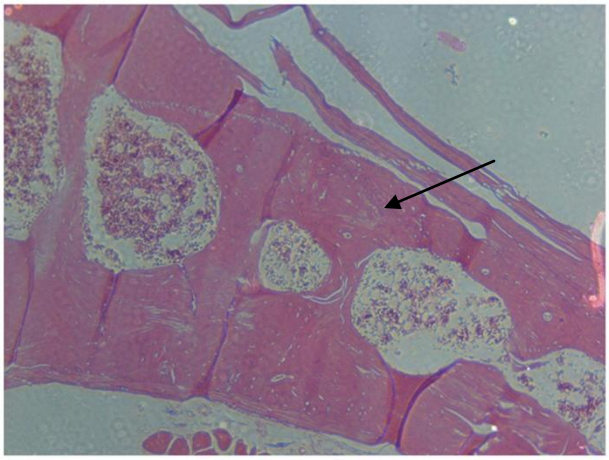

Fig.4. Photomicrograph H and E stained section from Group D arrowshowing cortical bone.

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