

Analysis and detection of microporosities in aging bones in context to pharmaceutical treatment: A study in line of osteoporosis

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Abstract: The objective of the current study was to evaluate the effects of nandrolone decanoate in terms of morphological changes in the diaphyseal region of appendicular skeleton long bone (femur) of aging female Albino rats with the help of innovatively designed histomorphometric tool of study being introduced first time mapped out microporosities (lacunae) which tend to intensify as part of advanced aging denoting deterioration in osseous tissue. In fact our research trial has been in the line of involuntional osteoporosis especially common in post-menopausal aged women, as to find out appropriate drug usefulness in the care and cure of this diseased state as per recommendation of FDA. An animal experimental research trial was done in strict accordance with the guidelines of the Institutional Animals Ethics Committee (IAEC) and all standard dietary protocols were observed. Rats were separated in two groups comprising of five rats in each. Group A was control and group B was nandrolone decanoate treated 3mg/kg body weight I/M on daily basis for four week duration. On completion of treatment animals were sacrificed, dissection was done and bones were excised. Qualitative histological assessment was worked through SEM, whereas the Quantitative data was constructed through a special company fitted software in electron microscope, model "jeol JSM-6380", the procured data was evaluated statistically and high degree significant variation was detected b/w control and experimental groups. The histomorphometry validated relatively reduced index of BLD in experimental/treated case signifying less resorptive activity in aging bones with the use of nandrolone decanoate.

Keywords: SEM, BLD, nandrolone decanoate, histomorphometry, lacunae.

INTRODUCTION

Bone debilitation, deterioration and damage is a hallmark of aging especially in females being emerged globally as a magnified public health concern. Aging women are prone, tend to undergo involuntional/post menopausal osteoporosis, a leading well recognized poorly controlled and treated diseased state prevailing world wide. In this condition architectural frame gets vulnerable to fracture because of insufficient synthesis or excessive disintegration of matrix, bone volume also tends to diminish along with drop in mass (Nevitt, 1994). Higher prevalence of risk factors for the disease have escalated the burden. An estimated 200 million women worldwide have osteoporosis. Across the globe, an osteoporotic fracture is estimated to occur every 3 seconds, while a vertebral fracture happens every 22 seconds (bone, 2012). Comparing people suffering from osteoporosis, Pakistan stands at 5th position across the world (Osteoporosis, 2014). It's a key clinical challenge as to find out effective prophylaxis and treatment against the osteoporotic diseased state progression.

In advanced age microporosities in the form of excavations/lacunae do increase in bones both in size and numbers found to signify gender independent resorptive manifestations (Carsten *et al.*, 2005). The mechanical

properties, fluid shear power do get modified with the change in geometrical pattern of the lacunae (Fritton and Weinbaum, 2009). Inherently any change to the internal micro-morphology of the matrix has the potential to modulate bone's mechanical properties, similarly the intracortical matrix pore size does modulate strength. The smaller pores may be better able to absorb shear forces on the other way larger pores are weaker usually result in microcracks (Cheng *et al.*, 2009; Herman *et al.*, 2010).

In the context as per FDA recommendation toward animal trial studies aging female rats tend to have preferential matchable morphology, physiology of reproduction, skeletal biology, pattern of haversian remodeling in bones as that of human (Zoetis *et al.*, 2003; Burr, 1992). Aging Female rats, laboratory rodents are found to have midlife cessation of ovulatory cycles (Wise, 1983; Gosden *et al.*, 1983). The period of persistent vaginal cornification (PVC), which is characterized by low to moderate level of estradiol, progesterone, luteinizing hormone, absence of corpora lutea in ovaries (Everett, 1939; Lu *et al.*, 1979; Nelson *et al.*, 1981). The loss of follicles results in drop of estradiol level similar to those seen in ovariectomized rats (Vom Saal *et al.*, 1994).

In the prescribed direction of research and experimentation varied pharmaceutical interventions approved by FDA like Alendronate (bisphosphonates), anabolics like parathormone injectables, triphenyl

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ethylene derivatives raloxifene, droloxifene, tamoxifen etc have been tried out in the ovariectomized rat model (Body, 2002; Hotchkiss *et al.*, 2001) their effects are found to be in considerably good match with the agents used in the clinical trials on women with post-menopausal osteoporosis. Nandrolone decanoate is synthetically prepared core anabolic steroid bearing androgenic properties resemble with testosterone in bit of its modified forms (Hall, 2005) have been proved to work well in treating various reproductive dysfunctions, anaemic states, mammary gland carcinoma (Thiblin and Paterson, 2005), the nandrolone does generate positive protein turn over and useful enhancement in calcium metabolism (Johansen *et al.*, 1989).

Our principal focus of interest was to observe and analyze the effects (morphological changes) in aging female rat bones in the context of treatment with nandrolone decanoate. As it is derived from estren which is found to be unique regarding mode of action, one way it works through sex-nonspecific non-genotropic mode; as a pro-hormone steroid it regulates both estrogenic as well as androgenic receptor by transcriptional signaling (estren) mechanism (Kousteni *et al.*, 2001; Michael *et al.*, 2004).

METHODS AND MATERIAL

The holistic research logistics and procedure facilitation involved Department of Zoology, HEJ, Centralized Science Laboratory, University of Karachi.

Animals

The relatively old middle aged eight to twelve month female albino rats, weight 250 to 300gm wer picked out on random basis, animals were separated into age matched control and treated groups (5/group). group A (control) was put on corn oil vehicle as placebo 0.02 cc s/c, group B (experimental) received injectable nandrolone decanoate, the dose applied in this study was the therapeutic equivalent derived from other trial studies in rats, 3mg/kg body wt. (Gerez *et al.*, 2005) via intramuscular route. We injected it on daily basis for four week period. Animals were maintained under environmentally controlled room temp, fed on standard purified laboratory dietary regimen comprising 0.8% Calcium, 0.7% Phosphorous, floor, wheat, and fortified by vitamins, kept in standard cages. All rats were allowed access to a water *ad libitum*, subjected to a 12-h light: dark cycle as per protocol and guidelines of the Institutional Animals Ethics Committee (IAEC).

On completion of four week treatment animals were sacrificed under chloroform anesthesia; dissection was done, the bones (femur)bi-sidedly placed in hind limbs extracted both from control and experimental group rats, tiny pieces (n=50/group) of bone extirpated from diaphyses were utilized for analysis and mapping through Scanning Electron Microscope (SEM).

BONE lacunar dimensions (BLD)

The BLD a newly introduced histomorphometric parameter designed and developed to facilitate further future researches, depicting and mapping out dimensions of lacunae (excavations)/ inter-trabecular spaces. Infact we have developed a noval index to quantify these lacunae/micro porosities through electron microscopic image, an Inbuilt soft ware inside SEM model “Jeol JSM-6380” was utilized to generate values of measurement in micro-meter automatically with arrows showing axial dimensions of the lacunar spaces both vertically and transversely across (fig. 1). Seven different sites/ observational fields were viewed per specimen. One exceptional feature of this soft ware is that it maintains and does not change values of measurement irrespective to the change in the level magnification, any change in magnifying power does not alter the measured values i.e measurements remain constant throughout thus lacuna dimensions were mapped out and matched quite concretely.

RESULTS

The collected data statistics was performed on SPSS version 20, independent t-test was applied. Values were represented as mean \pm SD (n=50/group).

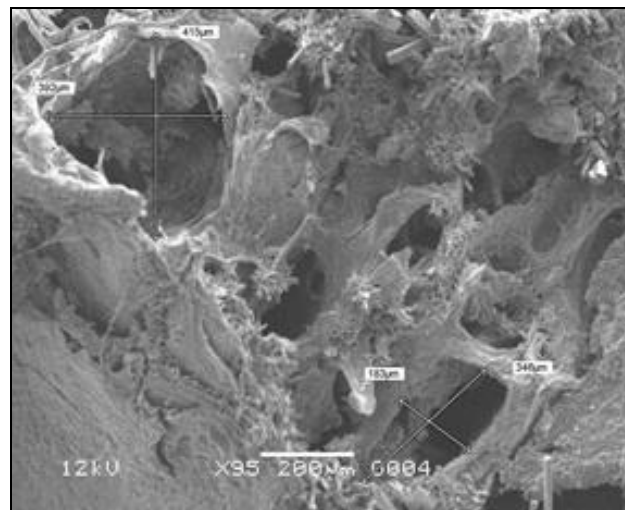


Fig. 1: SEM image of 10KV (femur-diaphyses) group A, Showing lacunae, Vertical and transverse arrows denoting histomorphometric mapping.X95.

The respective lacunae dimensions measured in osseous specimen diaphyseal sites of control group “A” recorded as mean BLD 187.20 ± 88.70 SD in μm , where as in case of treated group “B” the mean values were relatively on lower side $100.21 \pm 43.65 \mu\text{m}$, the outcome was in the form of highly significant ($p < 0.001$) decrease in the dimensions of experimental group bones.

Micro porosities (lacunae) appeared to be of regular symmetry and shape in nandrolone treated specimen (fig.

2), as compared with control which have revealed enhanced size of the lacuna (fig. 1).

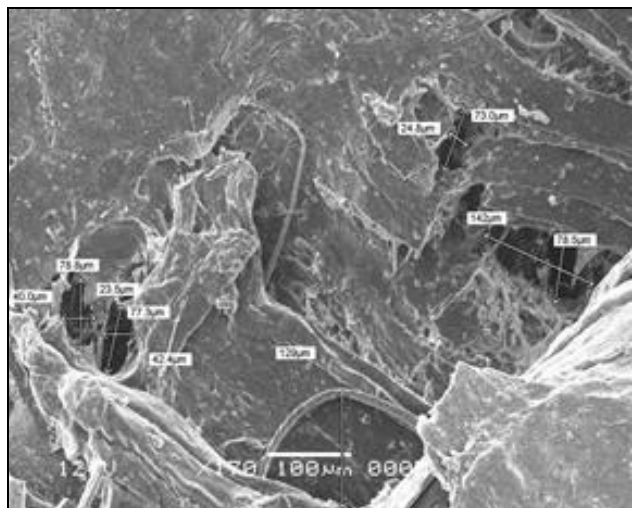


Fig. 2: SEM image of 10KV (femur-diaphyses) group B, Showing lacunae, Vertical and transverse arrows denoting histomorphometric mapping.X170.

DISCUSSION

our research trial results imply matched uniformity with other experiments performed by (De vries *et al.*, 1994) on young juvenile rats who underwent surgically induced menopause like state, the tibilon another anabolic steroid was administered into this breed found to suppress resorptive changes along with preservation of appendicular and axial trabecular skeletal micro architecture. tibilon works upon androgenic as well as estrogen receptors in bones (Ederveen and Kloosterboer, 1999) thus bringing about incremental rise in the organic and to some extent the inorganic fraction of bone matrix.

Several long duration animal lab. Studies conducted on female monkeys in relation to the use of selective estrogen receptor modulators failed to maintain endocortical matrix density and the process of cancellous bone formation was also impeded (Brommage *et al.*, 2001; Lees *et al.*, 2002) thus differ with our study results.

In our experiment nandrolone decanoate treated (group B) bones revealed considerable better organized geometry and configuration (fig.2), large lacunae were detected to be in low numerical proportion. The lacunae (microporosities) mapped out BLD signature low resorptive state as compared against its control counterpart (fig.1). Larger pores are weaker usually result in microcracks. In fact matrix pore size does modulate strength. The smaller the lacuna or microporosity the better would be its capability to withstand mechanical forces (Cheng *et al.*, 2009; Herman *et al.*, 2010).

In case of control (group A) the overall microarchitecture looked disturbed and distorted, relatively larger sized excavations are marked with vague appearance (fig. 1). During estrogen deficient state in aged animals phenomenon of receptor modulation occurs leads to cause alteration in trabecular core mineralized milieu, configurational changes and process of secondary mineralization also gets precipitated (Batra *et al.*, 2003; Caverzasio *et al.*, 2008). Whereas group B manifest better morphology. Our study trial case has had profound consistent pattern as that of (Jerome *et al.*, 1997), in that experiment female cynomolgus monkeys were given anabolic steroid nandrolone decanoate, histomorphometry denoted halting of osteopenia progression in axial skeletal bones. In a similar context clodronate (bisphosphonates) was introduced in aging cynomolgus macaques with estrogen deficient state, appendicular bone tibia was assessed found to have specific cortical enhancement (Itoh *et al.*, 2000) thus concrete conformity shown with our diaphyseal site results (fig. 2). Number of new mechanisms are attributed to the functional output of nandrolone. The Nor-Testosterone (19 NT) also known as nandrolone a potent androgen derived from estren helps maintaining bone mass with in skeletal micro-environment in sex organ ablated mice (Kousteni *et al.*, 2002). The bone cells osteoblasts contain both androgenic as well as estrogenic repertoire of gene expression, an interesting new observation that testosterone prevent bone disintegration in ovariectomized female rats through the process of aromatization as well by acting through these receptors in osteoblasts (Ebeling, 1998; Ederveen and Kloosterboer, 1999; Ederveen *et al.*, 2001).

CONCLUSION

The aging dynamics impact the pattern of bone remodeling kinetics, high turnover resorptive activity galvanize changes. The nandrolone decanoate might help reverse and normalizing these kinetics, In this relation advance studies are recommended to endorse up to what extent nandrolone have their impetus in terms of differences observed in our current set of study. Our study may serve towards indexing approximate level of bone quality and the relationship over other various aspects of post-menopausal osteoporosis.

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