Nandrolone Decanoate and Tamoxifen Related Histomorphometry: A study in line of Osteoporosis

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Abstract

Objective: To compare the histomorphometric data (in line with osteoporosis) between Tamoxifen treated and Nandrolone decanoate treated female Albino rats through an innovatively modified parameter using SEM (scanning electronic microscopy).

Methods: An animal experimental research study was done in which the subjects were ageing six female Albino rats, 8-12 months of age. The animal study was carried out in strict accordance with the guidelines of the Institutional Animals Ethics Committee (IAEC) and all standard dietary protocols were observed. The Albino rats were divided into two main groups comprising of three rats in each group. Group "A" was Tamoxifen treated, 5mg/kg body weight s/c daily and Group "B" Nandrolone decanoate treated 3 mg/kg body weight, I/M per week. Both groups were treated for six weeks. On completion of treatment the animals were sacrificed, dissected and bones were removed. Quantitative and qualitative data was developed through SEM.

Results: The histomorphometric data in this study was Specific Bone Surface (SBS) Specific Eroded Surface (SES) and Total Bone Surface (TBS)/Total Eroded Surface (TES). Comparison of these parameters was done by the SEM for the two groups of Albino rats i.e. group A; Nandrolone decanoate treated and group B; Tamoxifen treated. The data obtained was evaluated statistically and no significant variation was found between the two groups.

Conclusion: The histomorphometric data shows that the effects of Nandrolone decanoate and Tamoxifen in female Albino rats are similar in terms of structural measurement such as SBS, SES and TBS/TES.

Keywords: Nandrolone decanoate, Tamoxifen, electron microscope, histomorphometry, bone mineral density (AASH & KMDC 18(1):1;2013)

Introduction

Osteoporosis is one of the commonest, multifactorial disorder of bones, heterogeneous in nature, characterized by a reduction in bone mass and volume along with certain abnormalities in architecture[1]. These changes may be due to inadequate synthesis of bone matrix composed of organic as well as inorganic fraction, or excessive loss of these components.

The Involutional/postmenopausal osteoporosis is a major health problem for the ageing female population worldwide[2]. These bony changes are a part of ageing process usually in the post menopausal (post-reproductive) age group. Hormonal deficiency is considered to be one of the main factors leading to this problem. It has been estimated that 75 million females all over the world may fall in this category[3]. The problem is illustrated by the fact that approximately 15% of them are expected to have a vertebral fracture and 20% will suffer from hip joint fracture and in some places like Scandinavia this risk may exceed to 25%[4], probably due to combination of factors, including low ultraviolet (UV) exposure. Postmenopausal osteoporosis is also termed as "Type-1 Osteoporosis"[5], a process occurring in a subset of post menopausal women who typically range in age from 51 to 65 years that leads to marked morbidity and mortality mainly due to decline in circulating estrogen. However, estrogen replacement therapy has numerous undesirable side effects including increased risk of breast and uterine cancer.

Tamoxifen, triphenylethylene derivative (TPE), is a synthetic non-steroidal estrogen analogue, first reported to be a potent estrogen in rats[6]. A successful modification of its stilbene nucleus resulted in a compound with anti-estrogenic properties[7].

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Tamoxifen thus has both estrogenic as well as anti-estrogenic properties.

Nandrolone decanoate an Anabolic-Androgenic steroid (AAS),is a synthetic compound which is based on the structure of Testosterone, and has been used to treat various conditions such as reproductive dysfunction, breast cancer and anaemia[8].Three basic modifications are made to the structure of Testosterone[9] in order to enhance its delivery, potency and slow down its rate of degradation. Similarly, sex steroids play an important role in maintaining skeletal mass in humans and rats[10]. The role of anabolic steroid the Nandrolone decanoate in relation to positive protein turn over and calcium metabolism has also been documented[11].

Experiments with current therapy for osteoporosis indicate that to avoid fractures it may be necessary to look beyond bone mineral density, to factors such as bone structure, remodeling, strength and repair. Many experts now agree that bone measures need also to be explored in the treatment and therapy evaluation.

The present study was basically designed to develop an in depth high profiled index of the bony structural changes, mapped out through innovatively modified parameter based on SEM (scanning electronic microscopy), developed in terms of relation to resorptive changes as well as the unaffected trabecular surface. Hence, the objective of this animal experimental study was to compare the histomorphometric data (in line with osteoporosis) between Tamoxifen treated and Nandrolone decanoate treated female Albino rats by SEM.

Material and Methods

This Animal Experimental Study was conducted at Basic Medical Sciences Institute (BMSI), Jinnah post graduate Medical Centre (JPMC), HEJ, Centralized Science Laboratory Karachi University and facilitated by Agha Khan Medical University which provided the chemicals. Six female Albino rats of post reproductive age were used. They were divided in two groups A and B; sample size was six (6)/group i.e. right and left side femur in each group i.e 12 femurs of these six rats, were included in group A and B respectively. Experimental Tamoxifen treated was designated as group "A" and Nandrolone decanoate treated was designated group"B"

The guidelines of the Institutional Animals Ethics Committee (IAEC) were strictly followed for the rats involved in this study. Ageing female Albino rats 8-12 month old, weighing 200-250 grams, were provided by the HEJ Research Institute of Chemistry, University of Karachi and housed in Perspex cages. Animals were placed in an environmentally controlled room at temp (25 ± 2°C).The standard laboratory diet containing wheat, floor, milk powder supplement of minerals, vitamins etc, was given to the rats as per protocol of the Institutional Animal Ethics Committee.

Animals were divided into two main groups comprising of three animals each. Group "A" Tamoxifen treated, was given 5mg/kg body weight/ animal s/c daily[12,13], and Group "B" Nandrolone decanoate treated, was given 3 mg/kg body wt. I/M per week for six weeks [14]. On completion of treatment the animals were sacrificed, dissection was done and bones were taken out. Following which, quantitative and qualitative data was developed through SEM.

Twelve specimens of femoral head were used for qualitative and quantitative assessment. Sample was coated on Auto-coater from Jeol Japan model number JFC-1500, with gold up to 300A. The images were taken on SEM from Jeol Japan model JSM 6380 A. A virtual grid with 494 squares, each square of 0.25 mm sq. area size, was superimposed over the generated SEM image, this allowed determination of the ratio of squares containing Eroded surface "ES" assessed in the form of Inter-trabecular gaps to squares with "U" unaffected trabecular surface marked as bone surface "BS"(BS/ES) [15].

Results

In this study bone micro-architecture "Trabeculae" found in femoral head was analyzed and mapped out in terms of "BS". Similarly the "inter-trabecular gaps" referenced for resorptive changes were viewed and measured (Fig 1, 2 & 3). In addition the pattern of orientation of plates and rods (trabeculae) was also determined, thus qualitative as well as quantitative data were explored concretely. The captured data, evaluated is shown in Table1 and Fig1, 2 and 3.

The Specific Bone Surface (SBS) was determined as follows: Mean trabecular length in terms of "Bone Surface (BS)" was mapped out along the inter- trabecular gap. Resorptive (R) or Eroded surface (ES) by longitudinal axis measured at six different well marked Specific loci per exposure of SEM of varied sizes 120-300 micrometer with a magnification which was generated at 10 kv .

Similarly, mean trabecular width in terms of "Bone Surface (BS)" vertically across two correspondingly placed trabecular gap=Resorptive-Eroded Surface (ES) by transverse axis measured at six different well marked Specific loci /places per exposure of SEM, size 120-300 mic.m .Thus (BS) mean area was calculated statistically 6.7333 with STD error of mean 0.4277 in group "A" and 6.7833 with ATD error of mean 0.6195 in group"B". Thus there was no significant difference ($p>0.05$) between the two groups.

Specific Eroded Surface (SES): Mean width and length of Inter-trabecular gap (ES OR RS) itself was also mapped out. This was derived from the trabecular gap in a similar fashion and the respective area thus commuted was 5.9500, STD error of mean 0.7582 in "A" and 5.9600, STD error of mean 0.4014 in "B", ($p>0.05$).

Calculation of the Total Bone Surface (TBS)/Total Eroded Surface(TES) was done by the total Area in relation to BS as well as ES per SEM exposure determined and deducted mathematically. The total area in relation to BS was 206.6500, SEM 1.825 in "A" and 210.833 mm, SEM 1.480 in "B". The ES was 36.0333 mm, STD error of mean1.133 in "A" and 36.0333,STD error of mean 1.452 in group "B", again $p>0.05$ b/w two groups.

The collected data were analyzed, and statistical comparisons were performed by SPSS (version 16.0).Comparative analysis of the values was done by using independent t-test (Table-1). After the administration of Tamoxifen 5mg/kg birth weight and Nandrolone decanoate 3mg/kg birth weight in six female, age and weight matched rats with sample size twelve (12) femurs in toto, the values when compared statistically between the two group were found to be non-significant ($p>0.05$).

Table 1. Comparision of histomorphometric data (millimeter) between Tamoxifen treated (group A) and Nandrolone Decanoate treated (group B).

Group of Subjects	N	Mean ± STD	STD. Error Mean	p-value	95% Confidence interval of the difference	
					Lower	Upper
Bone Surface Group A	6	6.7833 ± 1.0477	0.42772	0.948	-1.62736	1.72736
Group B	6	6.7333 ± 1.51745	0.6195			
Eroded Surface Group A	6	5.95 ± 0.75829	0.30957	1	-1.12956	1.12956
Group B	6	5.95 ± 0.98336	0.40146			
Ratio BS/ES Group A	6	1.145 ± 0.19957	0.08148	0.837	-0.2709	0.32757
Group B	6	1.1167 ± 0.26151	0.10676			
Total ES Counted Group A	6	39.6 ± 2.77561	1.13314	0.082	-0.53806	7.67139
Group B	6	36.0333 ± 3.5579	1.45251			
Total BS Counted Group A	6	206.65 ± 4.47202	1.8257	0.105	-9.42093	1.05427
Group B	6	210.833 ± 3.62694	1.48069			
Ratio TBS / TES Group A	6	5.2383 ± 0.47055	0.1921	0.119	-1.45371	0.19371
Group B	6	5.8683 ± 0.77368	0.31585			

The Histomorphometric study on six variables was compared between the two experimental arms. No significant difference found regarding bone surface (BS) and Eroded surface (ES) ($p>0.05$).

Discussion

The present study is basically designed to develop an in depth high profiled nano scale concrete index of the bony structural changes, mapped out through innovatively modified parameter based on SEM, developed in terms of relation to resorptive changes as well as unaffected trabecular surface.

In this experimental study design, trabeculae appeared to be found relatively slightly reduced in various point loci of ageing female albino breed (Tamoxifen treated group as compared to Nandrolone treated group). However, the average (mean) size of inter-trabecular gap=resorptive or eroded surface (ES) was found to be non-significant in both groups. The measurements were quantified through the "SEM" image, virtual grid of "594"squares, each of 0.25 mm developed on superimposed image.

Gentzschi G et al[15] used resorption lacunae for quantification instead of inter-trabecular gaps that have been the main focus of attention in our study, thus clearly a differential criteria has been worked out for quantitative evaluation (Fig 3). In order to testify its applicability this study has been executed with the trabecular bone dissected from femoral head region of rat bone.

Experiments with current therapies for osteoporosis indicate that to avoid fractures it may be necessary to look beyond BMD (Bone mineral density) to factors such as bone structure, remodeling, strength and repair. Many experts now agree that "BMD" should be used as one of the sole markers for treatment efficacy, but other bone measures need also to be included in the treatment therapy evaluation. In women, osteoporosis associated with the dissipation of estrogen at menopause is viewed as osteoclast mediated. The end result is perforation of trabecular plates, uncoupling of bone formation bone resorption, and loss of bone mass[16].

The agents currently approved by the Food and Drug Administration (FDA) for the prevention and/or treatment of osteoporosis are calcium, hormone replacement therapy (HRT), the selective estrogen receptor modulator (SERM1) raloxifene, the bisphosphonates alendronate and risedronate, and calcitonin. The comparative efficacy and safety of these agents, all of which block the activity of bone resorbing osteoclasts, have been well documented[16,17,18].

Although HRT has been the main line of defense against osteoporosis associated with menopause, concerns have recently arisen regarding the long-term risk of breast cancer, coronary disease, and pulmonary embolism despite the antifracture efficacy of HRT[19]. A promising new addition to agents used for the treatment of osteoporosis is recombinant human parathyroid hormone 1-34 (rhPTH(1-34)[1]. This anabolic agent, which stimulates bone turnover with a substantial net increase in bone formation, is currently being reviewed by the FDA for marketing approval[20, 21] .

Resorption Lacunae (RL) are stressors that can increase the risk of mechanical failure in a trabecular network. Quantification of "RL"has previously been described through the parameter ER(Eroded Surface)/BS(Bone Surface) as established by light microscopy but results have been inconsistent and contradictory.

In this study "Trabeculae" appeared to be of similar size and thickness on large scale i.e. in terms of TBS and TES in both groups (Table 1). In comparison to Tamoxifen treated group, the systemic administration of Nandrolone decanoate, electron microscopy sections revealed relatively increased thickness with reduced inter-trabecular gaps and well organized anastomosing pattern in the treated group in few specific loci. Our studies are well in match with the results of Hamdy et al.[22] who took [21] patients with idiopathic osteoporosis as determined by radiology and dual energy X-ray absorptiometry, and were given 50 mg Nandrolone decanoate IM weekly, then bone densitrometry was done on lumber spines and left femur.

The identification of estrogen and androgen receptors in osteoblasts was established, by a