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Original Article

Changes in Serum Estradiol Levels and Histomorphological-Evaluation of Cortical Thickness in Long Bones of Mice Induced by Anastrazole and Protective Effect of Olive Oil

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ABSTRACT

Background: Breast cancer is the second major cause of death among women worldwide. Drugs like anastrazole are effective standardized therapy for postmenopausal breast cancer patients. Anastrazole causes estrogen deficiency by inhibiting enzyme Aromatase. Estrogen has an important dynamic role in growth as well as development of bone, both in men and women. Olive oil can be used as non-hormonal therapy to improve skeletal health by maintaining bone mineral density in patients taking anastrazole.

Objective: The aim of this study was to find out protective effect of olive oil in bone loss induced by the use of anastrazole.

Materials and Methods: Sixty female albino mice, 6-8 weeks of age were used in this experimental study. Aromatase inhibitor drug anastrazole was given alone and in combination with olive oil once daily for 30 consecutive days. Mice were divided into three groups.Group 1(Control group) which were given normal diet only, Group 2 (Drugged mice group) and Group 3 (Drugged + Olive oil group). Blood samples were taken by cardiac puncture and ECLIAmethod was used for serum estradiol estimation. Femur bone specimens were obtained and examined for histomorphological evaluation of cortical thickness.Study duration was from March – December 2019**.**

Results: Different parameters of all the groups were measured before and after experiment. After experiment, group 1mice showed increased levels of serum estradiol (18.25± 5.01pg/ml), as compared to group 2 (9.21± 2.12pg/ml) and group 3mice (14.79± 2.53pg/ml). Group 1 mice had femur weight of 0.08 ± 0.01 grams as compared to group 2 (0.05± 0.006 grams)and group 3mice (0.07± 0.008 grams). Mean cortical thickness of group 1 mice was (273.00 ± 15.67µm), that of group 2 was (138.00± 19.89µm), and that of group 3 mice was (189.50±14.31µm).

Conclusion: The results showed beneficial and protective effects of olive oil in anastrazole induced bone loss in female albino mice.

Key Words: breast cancer, estrogens, aromatase inhibitor, osteoporosis, olive oil.

Introduction

Cancer is one of main causes of mortality worldwide. As population grows the overall number of cases of cancer and mortality increases[1]. Regardless of the improvements in cancer treatments, it is still the prominent and the most important cause of death in the world[2,3]. It is considered 2[nd]commonly detected cause of female mortality [4,5,6].

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In 2012, an approximately, 1700,000 (1.7 million) cases and over half a million (521,900) mortalities occurred worldwide frombreast cancer[7-8].Higher incidence is predicted inhigher income groups[9,10,11].

Throughout Pakistan,the breast cancer is one of the most dominant and commonly diagnosed cause of malignancy in females. In 2012, estimated 5-year incidence of the disease in Pakistan was 119,710 cases including 34,038 new identified cases and 16,232 deaths [12,13].In the United States, in 2012, above 200,000 women were detected having carcinoma breast. In the United States, therefore, it is crucial to monitor the long term consequences especially bone health of successfully treated breast cancer patients[14].

Breast cancer treatment and prognosis options are usually based on staging, vascular and lymphatic spread, histological grade, presence or absence of hormone receptors, ERBB2 over expression and concomitant diseases. Patient's age and status of menopause are also important factors regarding treatment.

Nearly 70% of breast tumours have hormonereceptors (oestrogen or progesterone), therefore in order to reduce the risk of relapse, anti-hormonal treatments are frequently used. Anti-hormonal and chemotherapeutic agents can induce osteoporotic changes and thus have a destructive effect on bone health. Hence, in such patients, the bone health management is a key factor for care and survival [15].

Osteoporosis is a "silent "disorder as there is usually no distinct threatening or alarming signs, till a fracture occurs. The overall yearly incidence of osteoporotic fractures is higher compared to carcinoma breast, cardiac arrest and stroke combined.

Aromatase inhibitors are endocrine drugs used in breast cancer and they exert their action by blocking the production of oestrogen [15].Anastrozole is an effective and potent non-steroidal aromatase inhibitor.Aromatase inhibitors block the enzyme Aromatase which catalyzes a key aromatization step in the synthesis of estrogen. They are largely used in post-menopausal patients having hormone receptor positive (estrogen receptor) cancer [16,17,18].They are associated with severe loss of bone and fractures [19].

Olive oil and its phenolic compounds, bothin vivo and in vitro have antioxidant properties [20,21]. The consumption of phenols may prevent bone cells damage caused by oxidation and has positive impact on bone mineral density.

Objectives

The study aimed to observe the protective effect of olive oil in bone loss secondary to decreased estradiol levels after anastrazole administration.

Materials and Methods

The study was approved by the ASRB (Advanced Study and Research Board, DIR/ KMU AS&RB/EM/000892) and the Khyber Girls Medical College Ethical Board, after approval by the Graduate Study Committee (GSC) of Khyber Girls Medical College.

It was an analytical experimental study conducted from March 2019 to December 2019. The study was carried out in animal houseof "Pakistan Council for Scientific and Industrial Research (PCSIR), and Pathology department of KGMC, Peshawar.A total of 60 female albino mice were included in this study.Only non-pregnant mice, 6-8 weeks of age were included, while male albino mice were excluded from the study. Non probability consecutive sampling technique was used. Sixty mice were segregated into 3 groups. In group 1(control group) there were total of 10 mice. They were on routine standard diet only.In group 2 (drugged group) there were 25 mice and were on drug anastrazole, to whom dose of 0.1mg/kg bodyweight was given once daily. In group 3 (drugged + Olive group) there were 25 mice and they were given 1ml olive oil daily along with 0.1mg/kg/per day of anastrazole.

Blood was collectedby cardiac puncture forestrogen estimation.Oestrogen estimation was done by Enhanced "Chemiluminiscence" Immunoassay (ECLIA). ECLIA is an immunoassay which uses electrochemical compounds to generate. The test was performed on Cobaselecsys e411 analyzer by ROCHE diagnostics. Femur bone was collected for histomorphologicalevaluation of cortical thickness.The standard occulargraticule and stage micrometer were used to measure the cortical thickness of the femur bones, of all experimental albino mice.

Ocular or eye-piece graticule/reticule is a glass disc, placed in the eye-piece of the microscope. It is 10mm long with regular 100 sub- divisions, called ocular units (o.u). The engraved side of the ocular graticule faces downward. Eye-piece graticule divisions are arbitrary and need to be calibrated for each power, because it shows different values for same object depending on magnification used. For this purpose, stage micrometer is used with calibrated known measurement. In this project, calibration was done for low power i.e. 10X. Stage micrometer is (1 x 3 cm) glass slide or glass ruler placed on the stage of the microscope. The length of the stage micrometer is 10mm with 100 divisions. Therefore,

100 divisions = 10mm or

1 stage units (s.u) = 1/10 = 0.1mm

1mm = 1000 µm and

0.1mm = 100 µm therefore, 1 stage unit = 100 µm

At 10X, standard measurement of ocular graticule = 100 divisions

10 o.u = 1 stage unit = 100µm

Therefore 1 ocular unit = 100/10 = 10µm

Slide microscopy was done for measurement of cortical thickness.

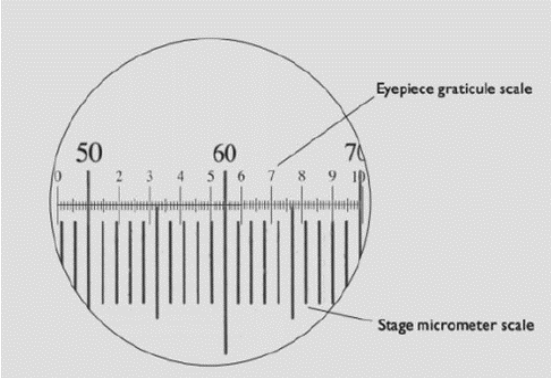

Figure: 1: Calibration of ocular graticule and stage micrometer at 10x magnification

Data was analysed using SPSS version 23. Means and standard deviationswere calculated for continuous data like weight, length of femur bone, estradiol levels and cortical thickness of femur bone. The means of different variables like estradiol levels, femur length, femur weight and mean cortical thickness of all three groups were compared by applying one way anova. A level of ≤ .05 at 95% confidence interval was taken as significant.

Results

The study results showed that anastrazole given alone caused significant reduction in serum estradiol levels and considerable loss of cortical thickness of femur bone (osteoporotic changes). The extent of damage was observed by gross parameters like weight of mice, length, weight, color and texture of femur bone. The results of group 2 and 3 were compared with group 1 i.e. control group given normal, standard diet and tap water. Comparison of group 2 and group 3 was also done. Group 3 treated with olive oil revealed less adverse effects in bones as matched with anastrazole treated group 2 having worst adverse impact on bones.

Table 1: Mean serum estradiol levels (pg/ml) of all mice groups.

Groups	Mean	Std. Deviation	Sig
Control (10) and Drugged mice (20)	18.25	5.01	<.001#
	9.21	2.12	
Control (10) and Drug and Olive oil(20)	18.25	5.01	<.014#
	14.79	2.53	
Drugged mice (20)and Drug and Olive oil (20)	9.21	2.12	<.001#
	14.79	2.53	

#: One way anova, Sig: Significance level

There was a highly significant difference of serum estradiol levels among all the groups. On comparison, there was significant difference (<.001) between the group 1 (control group) and group 2 (drugged group) and same difference between group 2 (drugged group) and 3 (drugged + olive oil group). While the significance level between group 1 (control group) and 3 (drugged + olive oil group) was <.014.

Table 2: Mean femur bone lengthof all mice groups.

Groups	Mean	SD	Sig
Control (10) and Drugged mice (20)	1.57	0.050	0.04#
	1.52	0.040	
Control (10) and Drug and Olive oil(20)	1.57	0.050	0.005#
	1.50	0.062	
Drugged mice (20)and Drug and Olive oil (20)	1.52	0.040	0.62#
	1.50	0.062	

 # One way anova, SD: Standard deviation, Sig: Significance level

There was significant difference of 0.04 between the mean femur lengths of group 1 and group 2 and a highly significant difference of 0.005 between group 1 and 3 but there was no significant difference (0.62) between group 2 and 3.

Table 3: Mean cortical thickness and weight of femur of all experimental groups

	Groups	Mean	SD	Sig		Mean	SD	Sig
Femur cortical thickness (μm)	Control (10) and Drugged mice (20)	273.00	15.67	<0.001#	Mean femur bone weight (Grams)	0.08	0.01	<0.001#
		138.00	19.89			0.05	0.006	
	Control (10) and Drug and olive oil (20)	273.00	15.67	<0.001#		0.08	0.01	0.001#
		189.50	14.31			0.07	0.007	
	Drugged mice (20)and Drug and Olive oil (20)	138.00	19.89	<0.001#		0.05	0.006	<0.001#
		189.50	14.31			0.07	0.007	

#: One way anova, N: Number, SD: Standard deviation, Sig: Significance level

Mean cortical thickness of femur was greatest in group 1 (control group) while in group 2 (drugged group) it was least of all. On the contrary in group 3 (drugged + olive oil group) it was greater than group 2, though lesser than control group.The mean femur weight of group 1 was greatest of all. Among treated groups the mean weight of group 3 was more than that of group 2.

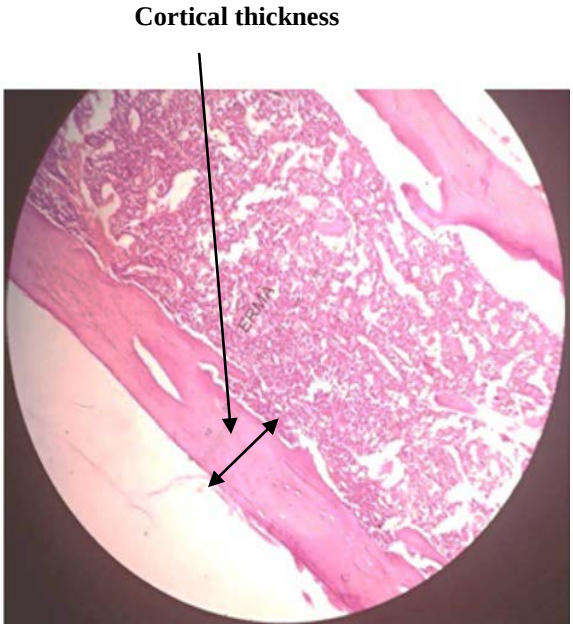

Figure 2A: Cortical thickness of control group

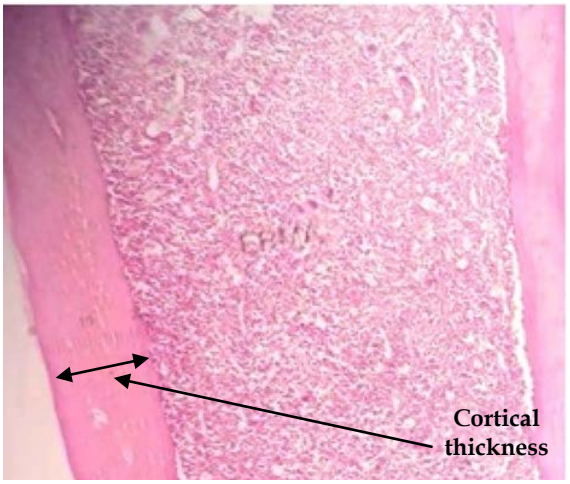

Figure 2B: Cortical thickness of group 2

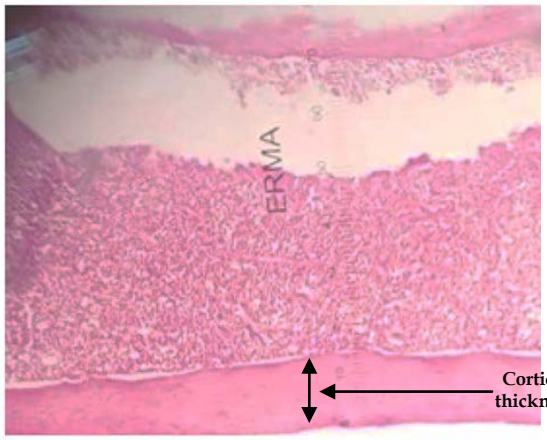

Figure 2C: Cortical thickness of group 3

Discussion

This study was conducted to evaluate the impact of theanticancer drug anastrazole on bones, commonly prescribed in postmenopausal, hormone sensitive (HR+) breast cancer patients. Moreover, study aimed at evaluation of efficacy and effectiveness of olive oil on bone mineral density (BMD) in those mice which received anastrazole.

The study results were supportive of protective role of olive oil against bone damages caused by osteoporosis in general. This research showed considerable loss of weight of mice and femur bone in group 2 which was treated with anastrazole. This finding was consistent with the study conducted by Gaillard et.al [22], which showed loss of weight, bone pains, osteoporosis and increased risk of fracture. Another study, ATAC trial (median follow-up, 68 months) conducted by Richard Eastell et al, showed increase bone loss associated with 5 years therapy with anastrazol[23]. In ATAC trialstudy, fracture rates were higher in groups treated with anastrazole (2.93%) as compared to tamoxifen (1.9%), with a highly statistically significant difference of <0.0001.The same fact was observed in another study conducted by Amr a Rezq et al. in 2010, showing that, the bone mineral contents were improved by dietary intake of olive oil[24]. Olive oil increases the calcium and phosphorus in bone and decreases the urinary calcium excretion in mice.

Our study showed marked reduction in cortical thickness of the femur bone of mice treated with Anastrazole (group 2) as compared to control group (group 1) and olive oil treated group (group 3). The same findings were also found in a study conducted by FlMarcu et al [25], in which, 47 bone tissue samples were collected. Twenty seven bone samples were collected from femoral head and 20 were collected

from the vertebral body of 60-70 years old dead patients. The results showed marked thinning in trabecular bone. On the other hand, mice treated with olive oil along with anastrazole (group 3) showed marked improvement in cortical thickness, proving efficacy and effectiveness of olive oil regarding bone health management in breast cancer patients receiving aromatase inhibitors like anastrazole. This effect of olive oil was also documented by another trial, conducted to estimate the protective role of olive oil against osteoporosis in ovariectomized rats [26]. Our experimental trial showed marked reduction in serum estradiol level in anastrazole treated group. Same results were also reflected in Folkerdet al and Jamal Zidan et al studies [27, 28]. Group 3 mice treated with olive oil showed improved serum estradiol levels as compared to group 2, showing that olive oil has beneficial role regarding bone density by improving estrogen level.Studies support that dietary intake of olive oil plays an important role in improving bone health due to its anti-oxidative and anti-inflammatory properties [29].

Conclusion

Our experimental trial concluded that, aromatase inhibitor drug anastrazolecaused marked reduction of serum estrogen levels in the female albino mice which led to decreasedlength, weight and cortical thickness of femur bones. The study also showed protective effects of olive oil on bone health by increasing estrogen levels and thereby improving different bone parameters including cortical thickness and weight. This protective effect of olive oil is helpful in reducing the risk of future fractures.

Recommendations

It is recommended that olive oil should be made a part of diet in general and especially in patients treated with aromatase inhibitors.

Conflict of Interest

Authors bear no conflict of interest.

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A. Conception/Study designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion

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