Published by :
http://www.ijert.org

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 8 Issue 08, August-2019

The Effect of Selective β-Blocker Bisoprolol on Osteocalcin (OC) Hormone Level in Human

[1]Abeer Mansour Abdel Raoul , [2]Dr. Sedra Ahmed Othman , [3]P H. Ahmad Shoab Karmoosh
[1,2,3]Ministry of Health and Environment,
Mosul, Iraq

***Abstract*— Beta blockers have a well-recognized antihypertensive action that is mediated through a reduction in cardiac output and in the release of rennin from the kidneys and inhibition of the action of endogenous catecholamine on beta-adrenergic receptors Bisoprolol fumarate (BF) is a synthetic β1-selective (cardio selective) adrenoreceptor-blocking agent. Human study has included the use of beta–blocker (β-blocker) Biscor® (BF) on osteocalcin (OC) hormone level. Osteocalcin (OC) is a large peptide that is manufactured by osteoblasts, odontoblasts and some chondrocytes. It connects to hydroxyapatite and much of it is deposited in the bone matrix. Aim of study Assess the effect of BF tablet at different doses and durations on serum OC hormone level in treated patients .**

Subjects and methods. The study was performed on total number of (67) female subjects; their ages were selected between (30-60) years old. Those subjects were divided into three groups and utilized the same drug Biscor ® BF tablet from (United Company / Jordan) at different doses and durations

Result: no significant results of BF tablets on OC was shown at the three treated groups

Conclusion- lowered level of OC level were influenced by age, dose of BF tablet and duration of drug.

Keywords:- Osteocalcin, Bisoprolol, bone healing, osteoporosis

INTRODUCTION

The β-adrenergic receptor blockers are an important and versatile class of drugs widely used in cardiovascular therapeutics. The beta- blockers (β- blockers) are also used to treat numerous cardiovascular disease states[1]. B-blockers have a well-recognized antihypertensive action that is mediated through a reduction in cardiac output and in the release of rennin from the kidneys and inhibition of the action of endogenous catecholamine on beta-adrenergic receptors [2] .Bisoprolol fumarate (BF) is a synthetic β1-selective (cardio selective) adrenoreceptor-blocking agent. Chemically, it is (±) -1-[4-[[2-(1-methylethoxy) ethoxy] methyl] phenoxyl-3-[(1-methyl ethyl) amino] -2-propanol.Serum Osteocalcin (OC): a large peptide that is manufactured by osteoblasts, odontoblasts and some chondrocytes. It connects to hydroxyapatite and a large amount of it is deposited in the bone matrix. For the reason that OC fragments are released from the bone matrix at the duration of resorption, assays for circulating OC and its fragments mirror both bone formation and resorption. The precise function of OC in bone is still indistinguishable, but recent studies elevate the surprising possibility that it is a hormone that influences energy metabolism by modulating the production and action of insulin [3]-.The aim of this study was to assess the effect of Bisoprolol tablet at different doses and durations on osteocalcin level in human being.

SUBJECTS AND METHODS

The study was conducted at Ibn Sina Teaching Hospitals, Cardiovascular Department in at Mosul City. The ethics committee a Health of Nineveh Heath Office approved the study data collection enclosed a period of (4) months from August to February 2013.

Study design and patients enrollment: the study performed on total number of (67) female subjects, their ages were selected between (30-60) years old. Those subjects were separated to three groups and utilized the same drug Biscor ® BF tablet from (United Company / Jordan) but different doses and durations[4].

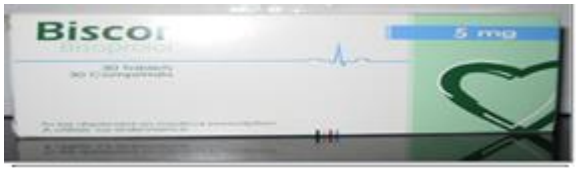

Figure (1) Biscor tablet 5 mg

• Chronic Group: this group integrated post-menopausal women that previously on same types of drugs, but at different doses and different durations of treatment more than (3) months. Chronic Group included hypertension, arrhythmia and anxiety disease.
• Follow-up Group: These group-integrated females diagnosed as hypertension or arrhythmia and anxiety and follow up for three month.
• Control group: This group included healthy females. The criterion of selection the controls were based on non existence additional diseases consequence on the results like diabetes mellitus, hyper and hypothyroidism and not used corticosteroid drug.

SUBJECTS GROUP'S EVALUATIONS:

Estimation of the study included the following information:

- **The questionnaire:** feature information about each patient was employed (case sheet in human). It consisted of three parts: demographic data, medical history and anthropometric measurements:
- **Demographic Data:** questions of universal information about the age ; age of menarche, number of pregnancies, activity level, housing, smoking, exercising menarche, menopause, last menstrual cycle, family history of osteoporosis, smoking habit[5] .
- **Medical History:** questions about tendency for falling, surgical history and the use of hormonal therapy and other drugs.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 8 Issue 08, August-2019

- **Anthropometric indicators:** three changeable were preferred for anthropometric measurements of the subject matters: height, weight and body mass index (BMI).

❖ **Height-** patient height was in use and then duplicated[6]

❖ **Weight –** was taken without shoes and wearing negligible clothing by a digital balance scale (China) (Alissal *et al.*, 2014).

❖ **Body mass Index (BMI):** is a constructive way of classifying obesity. Body mass index was calculated by relinquished equation (dividing the weight in kilogram (Kg) by Height square in meters)(Luis *et al.*,2014):

$$\mathbf{BMI} = \frac{\text{Weight (Kg)}}{\text{Height (m)}^2}$$

BIOCHEMICAL ASSESSMENT:

Determination of serum hormone was measured with a commercial Enzyme-linked immune sorbent assay (ELISA) (My Bio Source, USA).

Figure (2): Kit of Osteocalcin Eliza

❖ Blood Collections: From all contributors could not eat, drink, or smoke before collection of blood two samples of blood were drawn. One sample was taken from control and chronic group at the base line visit. From the follow up group, two blood sample were collected, first sample at a first visit (before treatment with BF tablet with different doses) and the second sample was collected three months after the treatment(follow up group).A sample of 5 ml of blood was drawn and collected into gel tubes, reserved at room temp for 30min. and centrifuged for15min. (Remimoter, china) at (1000) rpm, then serum samples were eliminated and transmitted to fresh two eppendroff tubes to be stored at (-20°C) to avoid loss of bioactivity [7]

REAGENTS PREPARATION:

1. Bring all kit components and samples to room temperature (20-25 °C) before use.
2. Samples- predict the concentration before assaying. If concentrations are unknown or not within the detection range, a preliminary experiment is recommended to establish the optimal dilution. PBS (pH7.0-7.2) or 0.9%physiological saline can be employed as dilution buffer.
3. Wash solution-Dilute10mlof wash solution concentration 100×with990mlof deionized or D.W to prepare1000mlof wash solution1×. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.The1×wash solution is stable for two weeks at (2-8°C).
4. Do not dilute the other components which are ready to use.

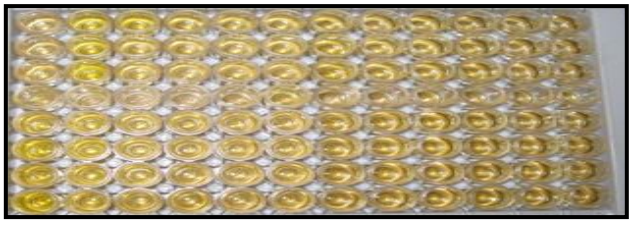

Figure (3) Result Of OC Kit

CALCULATION OF RESULTS:

1. The standard curve is used to determine the amount of samples.

2. First, average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.

3. Construct a standard curve by plotting the average O.D. for each standard on the horizontal (X) axis against the concentration on the vertical (Y) axis and draw the best fit curve using graph paper or statistical software to generate a linear regression, four parameter logistic or curvilinear regression of second degree. An X-axis for the OD and a Y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.

4. Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.

5. Standard curve for demonstration only. As figure below.

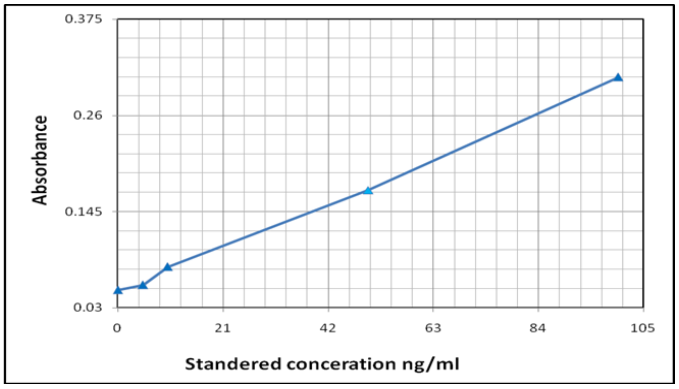

Figure (4) Standard curve of OC hormones

STATISTICAL ANALYSIS

The statistical analyses were achieved using statistical package for social sciences(SPSS)version 17.1.Standard statistical methods were used to determine the mean and standard error unpaired t-test was used one way. Analysis of variance (ANOVA) and (Duncan) test were used to identify statistical difference through comparison within the same and among groups P-value less than 0.05 was considered to be statistically significant

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 8 Issue 08, August-2019

RESULTS OF DEMOGRAPHIC CHARACTERISTICS OF THE STUDIED GROUPS:

A total of (67) female were included in the study .The individuals were distributed in 3 groups as shown in table (1): **Control group. Follow-up group. Chronic group.** In the control group, 14 females were accidentally preferred for the relationship with the other diseased groups and not complained from fracture and were not on long corticosteroid therapy comparison with the disease group Follow up group 23 female that were used BF tablet for a first visit and were followed up for 3 months, and the chronic group 30 female that were using BF tablet for more than 3 months[7]

Table 1: The Demographic Distribution of The Studied Groups:

Parameters	Control	Follow up	Chronic	P-value
No.	14	23	30	
Age (year)	43.21 ± 2.805 a	51.90 ±1.340 b	47.30±2.130 Ab	0.013
Age of menarche(year)	12.71± 0.286 a	13.73± 0.197 b	13.39± 0.224 B	0.018
Postmenopausal (year)	2.47 ± 0.95 a	4.43 ± 0.770 a	4.6 ± 0.95 A	0.340
Height- (cm)	164.2±1.638 a	162.13± 1.218 a	161.96 ±1.876 A	0.629
Weight(Kg)	68.79±2.448 a	77.53±2.458 b	79.78± 3.193 B	0.052
BMI (kg/cm2)	25.567± 0.983 a	29.4987±0.913 b	30.6209±1.340 B	0.021

*Data represented as mean ± SE.

*Different letters (a, b, ab) horizontally mean out put of Duncan′s test significant differences at p<0.05 within group

Table 2: The Demographic Distribution of Past Medical History of The Studied Groups

Parameters	Control	Follow up	Chronic	P-value
No.	14	23	30	
Fracture				
No Fracture	(14)100%	(23)76.7%	(30)82.6%	0.103
Yes Fracture	(14) 0%	(23)23.3%	(30)17.4%	
Family osteoporosis				
No osteoporosis	(14)85.7 %	(23)63.3%	(30)60.9%	0.114
yes osteoporosis	(14)14.3%	(23)36.7%	(30)39.1%	
Smoking				
non Smoker	(14)85.7 %	(23)100 %	(30)100 %	0.066
Smoker	(14)14.3%	(23)0 %	(30)0 %	
Corticosteroid use				
User	(14)7.1%	(23)10.0%	(30)30.4%	0.100
Non User	(14)92.9%	(23)90.0%	(30)69.6%	
Rheumatoid arthritis				
No Rheumatoid arthritis	(14)100%	(23)46.7%	(30)56.5%	0.004
Yes Rheumatoid arthritis	(14)0%	(23)53.3%	(30)43.5%	
Secondary osteoporosis				
No osteoporosis	(14)100%	(23)96.7%	(30)82.6%	0.103
Yes osteoporosis	(14)0%	(23)3.3%	(30)0.103	

The Demographic Characteristics of the studied groups demonstrated that age of control group was significantly different from chronic and treated groups. The control females were significantly younger because they were chosen to be healthy, with no any past medical history and the weight and BMI of the control group female were significantly higher than the follow up and chronic group in the table (2) illustrated that control group has no history of fracture, rheumatoid arthritis or secondary osteoporosis.

CONSEQUENCE OF BF ON STUDIED GROUPS:

The mean serum OC concentration in control group was not significant than that of the follow-up group (before) treatment by biscor® BF before treatment

Table 3:Comparison of Serum OC Concentration Level Between the Control and Follow-up Group Before Treatment.

Parameters	Control	Follow up(before)	P-value
No	14	23	
Oc conc. (ng/ml)	15.62857 ± 5.537816	16.900000 ± 5.3442296	0.355

* Data represented as means ± SE

- In relationship of serum OC concentration level between the control and follow up(after) group. the mean serum OC concentration in control group was higher than mean serum concentration in Follow up(after)group, but significant statistical difference was found between the two groups(p=0.687) as shown in table 4.

Table 4: Comparison of Serum OC Concentration Level Between the Control and Follow-up Group after treatment

Parameters	Control	Follow up (after)	P-value
No	14	23	
Oc conc. (ng/ml)	15.628571 ± 5.537816	12.0826087 ± 0.003	.687

*Data represented as means ± SE.

In assessment of serum OC Concentration Level between the control and Chronic group. the mean serum OC concentration in control group was higher than mean serum OC concentration in chronic group, statistical significant difference was found between the two groups(p=0.000) as shown in table 5

Table 5:Comparison of Serum OC concentration Level between the Control and Chronic group

Parameters	Control	Chronic	P-value
No	14	30	
OC conc. (ng/ml)	15.628571 ± 5.537816	17.596000 ± 5.1712812	0.000

* Data represented as means ± SE.

Table illustrates the mean of follow up group(before) was higher than mean serum OC concentration in chronic group, no statistical significant variance was[found between the two groups (p= 0.127) as be evidence for table 6

Table 6: Comparison of Serum OC concentration Level between the Follow up and Chronic Group

Parameters	Follow up (before)	Chronic	P-value
No	23	30	
OC conc. (ng/ml)	0.135 ± 0.0321	0.091 ±0.004	0.127

*Data represented as means ± SE.

Comparison of serum OC concentration level between these two group. The mean serum OC concentration in

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 8 Issue 08, August-2019

follow up(after) group was lower than mean serum OC concentration in chronic group; no statistical significant difference was found between the two groups (p=0. 540)as shown in table 7.

Table 7: Comparison of serum OC concentration Level between the Follow up and Chronic group

Parameters	Follow up (after)	Chronic	P-value
No	23	30	
oc conc. (ng/ml)	0.087 ± 0.003	0.091 ±0.004	0. 540

Data represented as means ± SE.

The result of BF treatment on serum OC concentration level. The mean OC concentration in follow-up(before and after) group was diminished after treatment for three months with Biscor ® BF for 3 months, but the diminish was not significant (p=0.143) as shown in table 8.

Table 8: Comparison of Serum OC Concentration Level Before and After Treatment with BF Tablet.

Parameters	Follow up (before)	Follow up (after)	P-value
No	23	23	
oc conc.(ng/ml)	0.135 ± 0.032	0.087 ±0.003	0.143

Data represented as means ± SE.

DISCUSSION

According to the effect of *β*-blockers on bone, data of humans are limited and conflicting[8]. This study is so tried to detect probable role of *β*-blockers in the prevention of osteoporotic fractures on patients with cardiovascular diseaseas[9]. Many animal and human studies have been conducted to estimate the risk of fractures using *β*-blockers and different results have been obtained. Serum OC was measured as a specific product of the osteoblast [10] .Takeda (2002) reported that the *β*-blocker propranolol increased bone formation in oophorectomized female rats[11]. In a great population based case-control analysis, there was additional evidence that the current use of *β*-blockers is associated with a statistically and significantly decreased risk of fractures on both men and women taken alone as well as in combination with thiazide diuretics [12]. However, prospective data from the Danish Osteoporosis prevention study illustrated (20%) lower serum OC levels in women treated with *β*-blockers compared to untreated women and recommended that *β*-blocker use is linked to an increased risk of fracture and no change in bone density[13]. A study on bone turnover in normal postmenopausal women using *β*-blocker showed that bone densities in the lumbar spine and total proximal femur did not change significantly on either group[14]. In animal models, there was considerable evidence of sympathetic nerve fibers in bone tissue and functional adrenergic receptors in osteoblasts and osteoclasts which has achieve d on osteoblast proliferation, osteoclast development, and osteoblast maturation [15]. In contrast, sympathetic system inactivation in rats consequences in a significant diminish in osteoclast number and osteoclast activity. In a recent study on human osteoblasts, fenoterol, a beta-2 agonist, nearly doubled RANKL mRNA and this raise was inhibited by propranolol indicating that in human bone cells, bone turnover might be modulated by the sympathetic nervous system[16].For the numerous special pathways and signaling systems that are influenced by different medications utilized to treat hypertension this may have a positive effect on bone health. It is assumed, for scientists, that a great deal necessary and translation effort requires to be done to better understand the linkages between cardiovascular disease (in particular hypertension) and osteoporosis, clinicians, when their patients need hypertension treatment and their patient is also at high risk of fracture; there is some assistance about which agents if possible (loop diuretics, thiazides, cardio selective *β*-blockers, ACE inhibitors) to patient[17.]

Osteocalcin concentration in blood sera can not only advance the diagnostic potential of osteoporosis, but also be useful in its dissimilarity from osteopenia, OC concentrations are influenced by gender, age and diurnal variation OC exhibits a diurnal variation with a nocturnal peak, plummeting by as much as (50%) to a morning nadir. Males have higher OC concentrations; OC concentrations are superior in children. With the highest concentrations observed during periods of rapid growth[18]. OC levels are generally increased during menopause. Increased levels of OC have been reported on patients with high bone turnover osteoporosis and fractures [19]. OC concentrations have been reported to increase, decrease or remain unchanged with advancing age; feasible effect of the heterogeneity of circulating, studies demonstrated elevated levels of serum OC may be associated with increased activity of osteoblast[20]. De Verit *et al*., (2006) found that serum OC levels on postmenopausal osteoporotic women were significantly higher than on premenopausal non-osteoporotic women. Deficiency of calcium may lead to lowering of formation of hydroxyapatite crystals in osteoporotic women, consequently, decreased rate of bone mineralization, free OC may be existing for circulation in the blood, and this may clarify the increased concentration of OC in the serum of osteoporotic postmenopausal women [21.] Found that OC is a promising indicator of bone turnover useful in the diagnosis and follow-up of high turnover osteoporosis. Similar observations were reported by a number of other studies [(22, 23)] .Estrogens are fundamental for bone maturation and mineralization on both men and women. Many causes in females can cause estrogen deficiency, congenital estrogen deficiency, estrogen resistance. Direct action of estradiol on osteoclasts diminishes the development and the action of osteoclasts and enhances the activity of osteoblasts. Estrogen deficiency stimulates increased production and activity of osteoclasts, which perforate bone trabeculae, decrease their strength and increase fracture risk. The natural life of functional osteoclasts and thus the amount of bone that osteoclasts resorb may also be improved following estrogen deficiency. This recommends that estrogen may avoid extreme bone loss by restraining the life span of osteoclasts and encourages apoptosis of osteoclasts [20]. OC synthesis is known to be modulated by vitamin D that means OC synthesis depended on Vitamin D. Vitamin D deficiency stays unrecognized over a long period of time, it may be appropriate to monitor both Vitamin D and OC levels on patients at risk of developing osteoporosis.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 8 Issue 08, August-2019

Roodman,(1992) mentioned at a nearly step of the study and durations of uses of drugs and doses of drugs, bioavailability of *β*-blocker used clinically is in agreement with the doses previously tested in animal models[24] [25] .A number of *β*- blockers used by the patients. The *β*-blocker consumers have been aged and body weight-matched, consequence the *β*-blocker treatment on bone via bone formation or bone resorption. The studies on animal models are much better fixed to such an objective and recommend both a stimulation of bone formation and a reduction of bone desorption. In human, this variation may reproduce changeable bone effects of the sympathetic nervous system depending on local factors such as involuntary loading, muscle mass, hormonal effects and response of marrow cells to adiposeness [24].It was discovered that the BMD values on the jaw region with among patients receiving *β*- blocker treatment for more than 5years give better results compared with patients treated with calcium channel blocker. Whereas there may be differences in types of *β*-blocker used, dose, or duration [14].The effects of *β*-blockers on BMD have been previously investigated in several studies. The data from human studies about the effects of *β*- blockers on osteoporosis is approximately the similar. Although in some studies can expansions on BMD with *β*- blockers treatment have been proved [26]. In additional studies no special effects of these drugs on bone metabolism have been reported [27] [28] .However, clinical studies and investigations are needed to be established clinical data represents that the effects of *β*-blockers on bone in humans stay an open question. In human, the no significant result of BF tablet on OC could be differed on human field because of many different factors mentioned above.

REFERENCES:

[1] Piascik, M.T., Peter, W., Yagiel,J .A. , Dowd, F.J. ,Johnson, B.S., Mariotti,A.J., Neidle, E.A.(2011). *Adrenergic Antagonists*. In: pharmacology and therapeutics for dentistry. 6th Ed**, Mosby Elsevier USA** Pp: 106-116.

[2] Graham, S., Hammond, J.D, Gamie, Z., Polyzois,I., Tsiridis, E., (2008). *The effect of beta-blockers on bone metabolism as potential drugs under investigation for osteoporosis and fracture healing*. **Expert Opin Investig DrugsJ**.; 17(9):1281-99**.**

[3] Coates, P. (2013).*Bone turn over markers.* **Australian family physician J**.; 42(5):285

[4] Luis,B. L.,Silvia, M.L., Daniel, L., H.(2014).*The Effect on Bone Mineral Density in Patients with Osteoporosis and Obesity of Once-weekly Treatment with Risedronate/Vitamin D3 Combined in a Single Pill for 12 Months: A Post-marketing Study*.**AustinJ.of Endocrinology and Diabetes;** 1(4):2**.**

[5] Reid, I.R., Lucas, J, Wattie, D., Horne, A., Bolland, M., Gregory, D. James, S. D, Andrew, B.G. (2005). *Effects of A β-Blocker on Bone Turnover in NormalPostmenopausal Women: A Randomized Controlled Trial*. **Clinical Endocrinology & Metabolism J.;**90(9):5213

[6] Alissal,E.M. Alnahdi, W.A, Alama,N., Ferns,G.A. (2014). *Relationship between the Components of the Metabolic Syndrome and Measures of Bone Mineral Density in Post-Menopausal Women*.**Journal of Diabetes Mellitus**; 4(1):165

[7] Al-Nuiemi, Z.W (2012).*The Effect of Bisphosphonate Drugs on Ghrelin Hormone in Osteoporosis*, M.SC Thesis. **Mosul University, Dentistry College**. Mosul, Iraq.

[8] Tucker., A., Gulcan, E., Serdar, T., Enver, E., Aksakalli, E. (2009). *Nebivolol might be Beneficial in Osteoporosis Treatment: A Hypothesis*. **Trop J Pharm Res;** 8(2):181.

[9] Perez Castrillon, J.L., DeLuis, D.A., DuenasLaita, A. (2009). *Are beta-blockers useful in the prevention of osteoporotic fractures?*. **Eur Rev Med Pharmacology Sci. J**. 13(3):157.

[10] Zhang,W., Kanehara, M., Zhang, Y., Wang, X., Ishida, T. (2007).*β-Blocker and Other Analogous Treatments that Affect Bone Mass and Sympathetic Nerve Activity in Ovariectomized Rats*. **The American Journal of Chinese Medicin***;* 35(1):96.

[11] Takeda, S., Elefteriou, F., Levasseur, R. Liu, X., Zhao, L., Parker, K., Armstrong, D. (2002). *Leptin Regulates Bone Formation via the Sympathetic Nervous System*.**J. Epidemiology.**111 (3):305-317.

[12] Schlienger, R.G., Kraenzlin, M.E., Jick, S.S., Meier, C.R.(2004).*Use of beta-blockers and risk of fractures*.**JAMA J**.; 292(11):1326.

[13] Rejnmark, L.,Vestergaard, P., Mosekilde, L. (2006). *Treatment with beta-blockers, ACE inhibitors, and calcium channel blockers is associated with a reducedfracture risk: a nation wide case-control study*. **J Hyper;** 24(3): 581-589.

[14] Reid, I.R., Lucas, J, Wattie, D., Horne, A., Bolland, M., Gregory, D. James, S. D, Andrew, B.G. (2005). *Effects of A β-Blocker on Bone Turnover in NormalPostmenopausal Women: A Randomized Controlled Trial*. **Clinical Endocrinology & Metabolism J.;**90(9):5213

[15] Aitken, S.J, Landao-B.S., Ralston, A.I. (2009). *Beta2adrenoreceptor ligands regulate osteoclast differentiation in vitro by direct and indirect mechanisms*. **Arch.Biochem. Biophys**; 482(2):96-103

[16] Sliwinski, L., Folwarczna, J., Pytlik,M. ,Cegiela, U., Nowinska, B., Trzeciak, H., Trecciak, H. I. (2013). *Effects of propranolol on the skeletal system depend on the estrogen status*. **Pharmacological report J.**; 65(5):1345-1356.

[17] Ghosh. M., Majumdar.S. (2014). *Antihypertensive medications, bone mineral density, and fractures: a review of old cardiac drugs that provides new insights into osteoporosis*.**Endocrine spring J;** 46(3):397-405**.**

[18] Sacide, A., Abdullah E., Huseyin, K., Can, B., and Nurettin, A. (2012). *Osteocalcin for osteoporosis*. **Ann Lab Med J**.; 32(1):23-30

[19] Deftos, L., Wolfert, R., Hill, C., Burton, D. (1992). *Two-site assays of bone Gla protein (osteocalcin) demonstrate immunochemical heterogeneity of the intact molecule.***Clin ChemJ.;** 38(11): 2318-2321.

[20] De Venit,F.,Yazggan,p.,Geyikli,C.,Zer,Y.,Celik,A. (2006). Diagnostic value of TRAP5b activity in postmenopausal osteoporosis. Turkish-German Gynecol AssocJ; 7(2):120-124.

[21] Pino, J.D., Gomez, E.M., Rodriguez, M.M., Sosa, C.L., Cordero, M., Lanchares, J.L., Talavera, J.R. (2005). *Influence of sex, age, and menopause in serum osteocalcin (BGP) levels*. **J. of Molecular Medicine;** 69(24):1135-1138.

[22] Rosenquist,C.,Qvist,P.,Bjarnason,N.,Christiansen,C.,(1995). *Measurement of a more stable region of osteocalcin in serum by ELISA with two monoclonal antibodies*. **Clin ChemJ**; 41(10): 1439-1445.

[23] Ones, K., Schacht, E., Dukas, L., Cagler, N., (2007).*Effects of combined treatment with alendronate and alfacalcidol on bone mineral density and bone turnover in postmenopausal osteoporosis: A two years, randomized, multiarm, controlled trial*. **The Inter J. of Epidemiology**; 4(4):1-9.

[24] Roodman, G., (1992). *Perspectives. Interleukin-6: an osteotropic factor?***J Bone Miner Res;**7(5):475-478.

[25] Franck,O.,Grzegorz,S.,Olga.S.,Mathieu.F,Haixin,C.,Charles,E.Louis, H.,Susan,S.,Bryan, L.(2011). *Endocrine Regulation of Male Fertility by the Skeleton*.**Cell J**.; 144(5):796-809.

[26] Ilic,K. Obradovic, N. Vujasinovic,N. (2013).*The relationship among hypertension, antihypertensive medications, and osteoporosis: a narrative review*. **Calcif Tissue Int**.; 92(3):217–227.

[27] Yang, S., Nguyen, N.D., Center, J.R., Eisman, J.A, Nguyen, T.V. (2014). *Association between hypertension and fragility fracture: a longitudinal study*. **Osteoporos Int**.; 25(1):97–103.

[28] Cherruau, M., Facchinetti, P., Baroukh, B., Saffar, J.L. (1999) *Chemical sympathectomy impairs bone resorption in rats: a role for the sympathetic system on bone metabolism.***Bone.J**; 25(5):545–551.