Iraqi National Journal of Nursing Specialties, Vol. 29 (1),

Effect of Atorvastatin on Bone biochemical markers in dyslipidemic patients, Basra, Iraq

تأثير عقارالا تورفاستاتين على المؤشرات الحيوية للعظام في المرضى الذين يعانون من داء الشحام،في البصرة-العراق

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المستخلص:

الهدف : وكان الهدف من الدراسة لتقييم عمل الأتورفاستاتين (20 ملغ / يوم) على المؤشرات الحيوية لعظام الرجال المصابين بداء الشحام.

المنهجية: وقد أجريت هذه الدراسة بين ايار 2015 وتشرين الاول من 2015 في مستشفى البصرة العام في البصرة، العراق، لتقييم الدور الهام للاتورفاستاتين 20ملغم من شركة فايزر الدوائية(لبتور,المانيا) على المؤشرات الحيوية للعظام. وقد شارك بالدراسة ثلاثين رجل من الذين ادخلوا للمستشفى نتيجة المشاكل الطبية المتنوعه. وقد تم تشخيص جميع المرضى المصابين بالشحام مسبقا من قبل الطبيب المختص في الطب الباطني. وجميع المرضى تقل أعمارهم عن 55 سنة من العمر. وكانت معايير الشمولية للمجموعة التجريبية للمرضى الذين استخدموا الأتورفاستاتين لمدة ثلاثة أشهر على الأقل. ثم قارن فريق الدراسة إلى تطابق سن الرجال الأصحاء . تمت مقارنة بين المؤشرات الحيوية بين المجاميع. تم التعبير احصائيا باستخدام المعدل ± الانحراف المعياري تم استخدام اختبار t للمقارنة بين المتغيرات.

النتائج: وكان متوسط العمر لمجموعة الستاتين هو 44.5 ± 5.9 ومجموعة الاصحاء او السيطرة هو 45.7 ± 4.4 بعد ثلاثة اشهر من علاج الأتورفاستاتين، كما في الجدول (1) والاشكال من (1-8) تبين بصورة مفاجئة ان مستوى مجموع الكولسترول في الدم أعلى بكثير من مستوى مجموعة الاصحاء او السيطرة. كما تبين ان مستوى الدهون قليلة الكثافة انخفض انخفاضا كبيرا باستخدام العلاج ولكن لا يزال أعلى بكثير من قيم مجموعة الاصحاء. كما ان قيمة الدهون عالية الكثافه أعلى بكثير من القيم الأولية. ولكن مستوى هذه الدهون ، وبعد العلاج؛ كان لا تختلف كثيرا عن مجموعة الاصحاء الطبيعية. اما الدهون الثلاثية. لم تتغير بشكل كبير عن طريق العلاج وقيمته لا يزال أعلى من مجموعة الاصحاء. لم يتغير مستوى فيتامين دي بشكل كبير عن طريق العلاج وكان قيمتها قلت بشكل ملحوظ من مجموعة الاصحاء. بينما ارتفع مستوى الاوستيوكالسين بشكل ملحوظ عن طريق العلاج. وكان هذا الارتفاع بشكل ملحوظ بالمقارنة مع القيمة الأولية ومع أن مجموعة الاصحاء الطبيعية. مستوى التايلوبيبتيدات ذات الامينات الطرفية لم تتغير بشكل كبير عن طريق العلاج وقيمتها لا تزال اعلى من مجموعة الاصحاء والقيم الاولية (قبل بدء العلاج).

التوصيات: اوصت هذه الدراسة بوجود الآثار الإيجابية للأتورفاستاتين (20 ملغ / يوم) على المؤشرات الحيوية للعظام على الاقل بعد ثلاثة أشهر من العلاج.وكذلك اوصت باستخدام عقار الاتورفستاتين لتقليل خطر الكسور بالنسبة لمرضى داء الشحام.

Abstract

Objective: The aim of the study was to estimate the action atorvastatin(20mg/day) on bone biochemical markers dyslipidemic men.

Methodology: This study was conducted between May 2015 and November 2015 in Al-Basrah General hospital in Basra, Iraq, to evaluate important role of atorvastatin (20mg/day)(Lipitor® Pfizer Pharma GmbH.Germany) on bone biochemical markers. Thirty men patients who had been admitted for a variety of medical problems included in the study. All the patients had previously been diagnosed with Dyslipidemia by specialist physician in internal medicine and all patients age below 55 years . the inclusion criteria for the experimental group was use of atorvastatin for at least three months; the study group was then compared to age-matched healthy men .bone biochemical markers were compared between groups. Results are expressed mean ± SD; statistically Student's *t* test was used to compare variables.

Results: The mean age of the statin group was 44.5 ± 5.9 and control group was 45.7 ± 4.4.After 3 months treatment with atorvastatin, as in table (1) and figures (1-8) **S**urprisely level of serum total cholesterol remain significantly higher than value of normal healthy group. LDL value significantly reduced by treatment but still significantly higher than values of normal group. HDL value were significantly higher than that of its initial values, but level of HDL ;after treatment; was not significantly different from that of normal group. Triglycerides was not significantly changed by treatment and its value still significantly higher than normal group. Vit D level was not significantly change by treatment and its value was significantly lower than normal group. Osteocalcin was elevated significantly by treatment; and this elevation was significantly as compared with its initial value and with that of normal group. Amino Terminal telopeptide procollagen type I (NTX-I) was not significantly change by treatment and its value still significantly higher than normal group and before treatment values. Also body mass index value was not significantly changed by treatment.

Recommendations: Further studies are recommended to confirm the positive effects of atorvastatin and other statins on bone biochemical markers with larger number of patients and longer period of follow up. Also other bone formation and resorption markers are recommended to determine the expected mechanisms of action of statins on bone remodeling processes.

Key Words: dyslipidemia, osteoporosis, atorvastatin, NTX-I, Osteocalcin

Introduction

Osteoporosis is a disease affecting health characterized by reduction in bone mass density mainly affecting women after menopausal period[1] . Decrease bone mass density make patients in the end suspected for fractures as a result of imbalance between the bone-forming action of osteoblasts and the bone restorative action of osteoclasts [2]. In addition it characterized by a decline in the quality and quantity of both the cortical and cancellous bone, this is called a whist disease because it usually does not cause any pain or symptoms until a bone actually breaks[3,4].The tissues of bone has a unparalleled potential for recovery to its original states completely after damage[5].However, large flaw caused by "trauma, cysts, neoplasms, infections or congenital defect" may not renewed spontaneously and the use of surgical or pharmacological therapy is required for complete renewed[6]. The osteoporosis as adisease is mor common in women than men[5]. There are number of drugs that used to prevent or treat osteoporosis including "bisphosphonates (oral, injection and intravenous drip(;SERMs (selective oestrogen receptor modulators), strontium ranelate and calcitonin" However, some of them have appropriate effects, and some even lead to further complications [3]. Therefore the need for a new effective and more safe compound for osteoporosis is essential. Statins, which are usually indicate to treat and prevent CVD (cardiovascular disease) may be used for treatment of osteoporosis[3].There are different new markers of bone turnover can be useful to control the efficacy of therapy and to predict risk of fracture like serum osteocalcin (OC),and the procollagen type I N-terminal propeptide (PINP). The most of these markers of bone resorption are disintegrat products of type I collagen and include the pyridinolines ;carboxy-terminal cross-linked telopeptides of type I collagen (CTX-I and ICTP), and amino-terminal crosslinked telopeptide of type I collagen (NTX-I)[7]. Different diseases may lead to development of osteoporosis like type I diabetes, hypertension, metabolic syndrome, ischemic heart disease, dyslipidemia and vitamin D deficiency [8]. Dyslipidemia is the most crucial risk factors for cardiovascular diseases which are the most common cause of death[9]. osteoporosis and dyslipidemia are two important medical issues with high spread in modern population. At this time, cholesterol is most important cause for atherosclerosis which affect a large number of victims around the world. The connection between dyslipidemia and bone metabolism can be explained by different mechanisms, two links are highlighted :dyslipidemia is the basis for vascular calcifications as a result of advanced atherosclerosis of the blood vessels; oxidized low-density lipoproteins (LDL) inhibit differentiation of pre-osteoblasts into osteoblasts[10,11]. Statins are antilipidemic drugs with inhibit3-hydroxy methyl glutaryl coenzyme A (HMGCoA) reductase, the rate limiting enzyme in cholesterol synthesis. Statins is drug of choice in the treatment of hypercholesterolemia as well as for primary and secondary coronary artery disease prevention. Besides reduced cholesterol synthesis and reduced mevalonate production, they have pleotropic effects apart from their hypolipemic effects. These effects are anti-inflammatory, vasodilatation, antithrombotic antioxidative and antiproliferative effects .The effects of statins on bone mineral metabolism are of highly occur with post-menopausal women that have risk for coronary artery disease development[12].

The mechanisms behind effect of statins on the bone involve : reduce the signaling proteins that responsible for osteoclastic activity and increased expression of morphogenetic bone protein 2 gene . These mechanisms in addition to reduction of cholesterol could be the main reason for statins therapy in osteoporotic women. Furthermore, Statins may inhibit osteoclast proliferation in vitro, but this effect considered minor as compared with the effects on osteoblasts[13]. It has been shown that atorvastatin effect on the expression of the osteoblastspecific genes COL1A1, osteocalcin, and BMP2 in cell cultures. also statin treatment cause cell proliferation arrest which is another important new finding were statins promote osteoblast differentiation, the inhibit of osteoblast apoptosis, and the blocking of osteoclastogenesis[14].

Methodology:

This study was carried out at Al- Basra General Hospital from May 2015 to November 2015. thirty patients with dyslpidemia were participated in this study (male)The mean age of the statin group was (44.5 ± 5.9). Apparently healthy subjects were selected to participate as

a normal group for comparison (control) with same age group and same sex (male) the mean age of these subjects was (45.7 ± 4.4). Diagnosis was made by a specialized physician in internal medicine deepened on history. Disposable syringes and needles were used for blood collection[13].After 12 hours fasting , venous blood samples, about 10 ml were collected from patients before test and from healthy volunteers in plain tubes. After allowing the blood to clot at room
temperature for 15 min, blood samples were centrifuged at 3000 rpm for 15 min. Fresh serum was used for the assessing the plasma levels of osteocalcin (ng/ml) by kit provide by (abcam company;USA),the plasma levels of N terminal Telopeptides of type I Collagen(NTX1ng/ml) was assessed by kit provide by company (mybiosource,US) and the plasma levels of25(OH)Vit.D (ng/ml)was assessed by kit provide by company (alpco,US). ELISA was performed according to manufacturer's protocol company all of them depend on principle Enzyme linked immunosorbent assay(ELISA),meanwhile the serum levels of total cholesterol(mg/dl) ;high density lipoprotein HDL-cholesterol(mg/dl);low density lipoprotein (LDL-cholesterol) and triglyceride (TG) (mg/dl) were measured by using kits of GIESSE (Italy) and identified by spectrophotometric methods. Method for statistics was used to compare the results. Body mass index was measured by divided weight in Kilogram over height in square meters (Kg/m^2). Results are expressed mean ± SD; statistically Student's *t* test was used to compare variables.

Results:

The data in table (1)and figures(1-8)of study group before treatment showed a significant higher serum level of totalcholesterol; LDL-cholesterol; triglycerides and NTX-I than that normal healthy groups .meanwhile serum levels of HDL-cholesterol;25(OH)vit D and osteocalcin were significantly lower than value of normal healthy groups. After 3 months treatment with atorvastatin, as showed in table (1) and figures (1-8). After three months treatment with atorvastatin 20mg daily, in the study group, **S**urprisely level of serum total cholesterol remain significantly higher than value of normal healthy group. LDL value significantly reduced by treatment but still significantly higher than values of normal group.
HDL value where significantly higher than that of its initial values . but level of HDL ;after treatment; was not significantly different from that of normal group. triglycerides; was not significantly change by treatment and its value still significantly higher than normal group. Vit. D level was not significantly change by treatment and its value was significantly lower than normal group. Osteocalcin was elevated significantly by treatment; and this elevation was significantly as compared with its initial value and with that of normal group. NTX-I was not significantly change by treatment and its value still significantly higher than normal group and before treatment values. BMI value was not significantly change before and after treatment.

Table 1 : Effect of treatment with Atorvastatin 20mg/day, on Lipid profiles(mg/dl) ;25(OH) Vit.D(ng/ml) ;Osteocalcin(ng/ml);BMI and NTX-I(ng/ml) in patients with Dyslipidemia, untreated group of .patients (control), initial values and after 3 months of treatment

After 3months		Initial values		Variables
Study gp	Control gp	Study gp	Control gp	
222.5 ± 64 a	171.3 ± 27.2	224.2 ± 38.6 **a**	167 ±23.1	**Total serum (Cholesterol (mg/dl**
*a 38.4 ± 166.2	135.5 ± 12.8	182.8 ± 21.5 **a**	8.7 ± 131.5	**LDL Cholesterol ((mg/dl**
*19.9 ± 39.6	45.6 ± 8.5	29 ± 12.8 **a**	8.4 ± 49.3	**HDL Cholesterol ((mg/dl**
279.8 ± 82.7 a	150.6 ± 24.5	250 ± 40.8 **a**	146.4 ± 18.7	**Triglycerides (mg/dl)**

8.8 ± 4.4 a	12.7 ± 8.9	7.3 ± 2.9	10 ± 6.7	**25 (OH) Vit D**
35.2 ± 13.9 a*	26.9 ± 9.7	18.3 ± 14.2	23.7 ± 8.5	**(Osteocalcin (ng/ml**
38.8 ± 23.3 a	18.2 ± 13.8	31.6 ± 18.7 **a**	19.7 ± 12	**(NTX-I (ng/ml**
N/A	N/A	37.1 ± 6.6	36.4 ± 4.8	**BMI (kg/m^2)**

* Significant (p value<0.05) as compared with its initial values
a significabt at P<0.05 as compared with Control values
N/A not measured or available
LDL:Low Density Lipoprotein,HDL:High Density Lipoprotein
NTX-I:N-Terminal tylopeptide procollagen type I
BMI:Body Mass index

Figure (1): Total cholesterol (mg /dl)

Effect of atorvastatin20mg/day on total cholesterol **(mg/dl)** in patients with, dyslipidemia untreated group of patients and after 3 months of treatment as compared with control group.

Figure (2): Low density lipoprotien- cholesterol (mg/ dl)

Effect of atorvastatin on low density lipoprotein (LDL-Cholesterol) **(mg/dl)** in patients with, dyslipidemia untreated group of patients and after 3 months of treatment as compared with control group. **LDL: Low Density Lipoprotein**

Figure (3): Triglycerides (mg / dl)

Effect of atorvastatin on triglyceride **(mg/dl)** in patients with, dyslipidemia untreated group of patients and after 3 months of treatment as compared with control group.

Figure (4) : High Density Lipoprotein (mg / dl)

Effect of atorvastatin on high density lipoprotein(HDL-Cholesterol) **(mg/dl)** in patients with, dyslipidemia untreated group of patients and after 3 months of treatment as compared with control group.

Figure (5): 25(OH) VitD(ng /ml)

Effect of atorvastatin on **25 (OH)Vit D(ng/dl)** in patients with, dyslipidemia untreated group of patients and after 3 months of treatment as compared with control group.

Figure (6): Osteocalcin (ng / ml)

Effect of atorvastatin on Osteocalcin **(ng/dl)** in patients with, dyslipidemia untreated group of patients and after 3 months of treatment as compared with control group.

Figure (7): N-Terminal tylopeptide procollagen type I

Effect of atorvastatin on **Aminoterminal telopeptid(NTX-I)(ng/dl)** in patients with, dyslipidemia untreated group of patients and after 3 months of treatment as compared with control group.

Figure (8) : Body Mass Index

Body mass index for control and study group

Discussion:

In this study, lipid profile and body mass index wasn't significantly changed as compared with initial values(before treatment) and control groups except with LDL-cholesterol which significantly lowering as compared with initial values but level still significantly higher than those values observed in normal control group. An explanation for such finding that statins lower serum LDL cholesterol by inhibiting 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase in the liver and thus indirectly cause an increase in the number of LDL receptors in the liver cells, which leads to an increased removal of LDL cholesterol from the blood[15].On the other hand, HDL value where significantly increased, the interpretation for these disturbances in lipid profile attributed to major inherent reasons lipid test results vary, even in the very short-term. The first is biological and the second is analytical. Large changes in lipid values may also be due to secondary causes of dyslipidemia. Many studies have also documented a seasonal variation[16]. Other biological factors that contribute to a patient's cholesterol level include: Within day variation, Age and gender, several factors that occur before or during blood collection, Venous occlusion and so on.[17-19].

Regarding Vit D level was not significantly change by treatment with atorvostatin and its value was significantly lower than normal group, this came in tune with results of different studies that have not shown any effect of statins on vitamin D concentration [20-22].In contrast to our study, other studies indicate a specific relationship between atorvastatin and serum level of vitamin D with clear improvement in 25-hydroxy-vitamin D 25(OH)D levels after the use of statins [23-26]. Measurement of osteocalcin levels may gave more clear idea about bone formation and it is a positive predictor of osteoblasts activity [27]. In this study osteocalsin level was elevated significantly by atorvostatins. The beneficial effect of atorvastatin on bone formation can be explained by different mechanisms. The inhibition of mevalonate synthesis prevents the synthesis of isoprenoids, which are used by osteoclasts to modify and activate intracellular proteins, the inhibition of prenylation alters osteoclast activity [3,14]. Also statins increase gene expression of bone morphogenetic protein-2, a protein capable of increasing osteoblast maturation and bone formation[28]. In addition it has been shown that statins stimulate bone formation in several *In vitro* studies were statins increase the number of osteoblasts and the amount of new bone formation in mouse skull bones [29]. Our results regarding osteocalcin came in tune of many studies. Chan et al 2001, found a significant increase in serum osteocalcin concentration after 4 weeks of therapy with simvastatin[30]. These results were consistent with another study that analyzed the age-dependent effects of atorvastatin on biochemical markers of bone turnover in a randomized controlled trial in postmenopausal women and suggested beneficial effects of statins on bone

turnover exclusively in older individuals[31]. Furthermore, numerous recent studies have demonstrated statins' beneficial effects on bone structure. In a retrospective case-control study by Wang et al., statin was associated with a50% reduction in hip fracture[32]. On the other hand, Sirola et al. examined the potential of statin therapy to prevent early postmenopausal bone and found no significant effect[33]. However longer duration of therapy was required and this is the major limitation of the study.

It has been shown that statins have pleiotropic effects other than lowering cholesterol, such as "stimulation of nitric oxide (NO) production, anti-apoptosis, anti-proliferation and immunomodulation" .Several retrospective and longitudinal analysis show that statin use associated with a significant reduction of fracture risk[34,35]. Recent studies have shown that these drugs are also act on bone formation by increasing expression of BMP-2, and it is also statins might be useful for fractures and osteoporosis treatment [3], depend on its effect on HMG-CoA reductase enzyme inhibition may increase bone density and decrease risk of fractures. In contrast to this study, Observational studies, which found no evidence between statin use and reduction of bone fracture risk[36]. One large systematic review, including 3220 postmenopausal women, found no correlation of statins with increased BMD, or reduced fracture risk[37].However, another study conducted by Chissas et al. demonstrate that low-dose simvastatin showed a negative effect in bone healing in the bone fracture model of male New Zealand rabbit[38]. In general oral treatment with therapeutic doses of atorvastatin showed positive effects on treatment of osteopenia and mild dyslipidemia[39].

The correlation of effects of statin on bone with their lipid-lowering effects is still conflicting. Some studies reported that the lipid-lowering effects of statins were related to their effects on bone[40], while other studies revealed no causal relationship[41].

Recommendations

Further studies are recommended to confirm the positive effects of atorvastatin and other statins on bone biochemical markers with larger number of patients and longer period of follow up. Also other bone formation and resorption markers are recommended to determine the expected mechanisms of action of statins on bone remodeling processes.

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