Am J Transl Res 2021;13(6):6175-6181
www.ajtr.org /ISSN:1943-8141/AJTR0107244

Original Article

Efficacy of autologous bone marrow derived Mesenchymal stem cells (MSCs), osteoblasts and osteoblasts derived exosome in the reversal of ovariectomy (OVX) induced osteoporosis in rabbit model

Mir Sadat-Ali[1,3], Dakheel A Al-Dakheel[1,3], Haifa A Al-Turki[1,4], Sadananda Acharya[2]

[1]College of Medicine, Imam AbdulRahman Bin Faisal University, Dammam, Saudi Arabia; [2]College of Public Health, Departments of [3]Orthopaedic Surgery, [4]Obstetrics and Gynecology, Imam AbdulRahman Bin Faisal University, Dammam, Saudi Arabia

Received January 1, 2020; Accepted January 27, 2021; Epub June 15, 2021; Published June 30, 2021

Abstract: *Background*: A recent study showed that OVX-induced osteoporosis was reversed after injection of osteoblasts cultured from the bone marrow in rats. The present study evaluated the effect of injecting MSCs, osteoblasts, and exosomes isolated from osteoblasts for the treatment of osteoporosis in the rabbit model. *Methods:* Osteoporosis was created in 40 rabbits by performing ovareictomy at 6 months of age, and 1 mg/kg body weight of methyl prednisolone sodium succinate was injected daily for 8 weeks. Animals were fed twice daily and were given water ad libitum. MSCs and osteoblasts were grown from the bone marrow as per the methodology described earlier. From osteoblasts, exosomes were extracted. After the 15th day, MSCs (Group 2), osteoblasts (Group 3), and exosomes (Group 4) were injected into 5 animals each, and 0.5 ml of normal saline were injected into the control group (Group 1). After 12 weeks (11 months of age), all the animals were euthanized. The whole femur and the lumbar vertebrae 3-5 were dissected out and were subjected to radiological assessment using high-resolution peripheral quantitative computerized tomography (HRpQCT). All parameters of the bone volume, trabecular number, thickness, and spacing were assessed using SPSS (Statistical Package for the Social Sciences), version 21.0, Chicago, Illinois. A p value of <0.05 was considered Statistically significant with a confidence interval (CI) of 95%. *Results:* Structural indices of the osteoblasts-injected animals were significantly better than the control group for the distal femur. The most significant improvement was seen in the osteoblasts, MSCs, and exosomes group in that order. The p value of all parameters was <0.0001 in the osteoblasts group, whereas the total and bone volume had a lower p value in the MSCs group. In the osteoblasts group, the positive changes were similar in the distal femur and lumbar vertebrae, but with MSCs and exosomes, the changes were more pronounced in the vertebral spine than the distal femur. Conclusions: This study shows that autologous bone marrow-derived osteoblasts have the robust influence of reversing OVX-induced osteoporosis in rabbits.

Keywords: Osteoporosis, osteoblasts, mesenchymal stem cells (MSCs), exosomes

Introduction

Secondary to the loss of estrogen, osteoporosis occurs in the postmenopausal age group, in which bone loss exceeds bone formation [1-4]. Osteoporosis is a serious metabolic bone disease in the world that causes enormous morbidity and mortality due to fragility fractures that are secondary to osteoporosis [5]. In the US, the financial cost due to fragility fractures was $22 billion in 2008, and this might have increased much more since then [6]. The osteoporotic-related fractures in Saudi Arabia are the least-studied subject; hence, the real prevalence is still unknown. It was assessed that, by 2050, the lifetime cost of managing the fragility fractures of femurs in Saudi Arabia may be US$9.34 billion annually [7] due to the increasing prevalence of osteoporosis in the population [8-12].

The world's population is living longer compared to previous decades, and it has been reported that this may increase in the near future [13,

Table 1. The comparison between the control and the MSCs group

Parameter	Control Group	MSCs Group	*P* Value
Total Volume (TV mm^3) (VOX)	324.761±2.547	359.349±24.485	0.007
Bone Volume (TV mm^3) (VOX)	14.308±2.224	16.8±5.08	0.07
BV/TV (Vox) Relative Bone Volume (%)	0.044±0.007	0.47±0.01	0.001
Trabecular Number [1/mm] (VOX)	0.303±0.032	0.498±0.066	0.01
Trabecular Thickness [1/mm] (VOX)	0.171±0.008	0.175±0.003	0.001
Connectivity density, normed by TV [1/mm^3] (VOX)	0.848±0.137	4.643±0.654	0.001
Trabecular separation = marrow Thickness [mm] (VOX)	3.087±0.154	1.364±0.453	0.001
Total Volume (Tmm^3) (TRI)	322.547±4.696	357.146±24.219	0.001
Bone Volume (TV mm^3) (TRI)	14.260±1.540	16.729±5.158	0.001
TRI-BS	107.696±27.559	120.488±30.732	0.001
TRI-BS/BV	14.226±0.886	16.885±1.030	0.002
Trabecular number [1/mm] (TRI)	0.315±0.039	0.453±0.125	0.001
Trabecular Thickness [1/mm] (TRI)	0.062±0.002	0.135±0.009	0.001
Trabecular Spacing	3.060±0.215	0.590±0.119	0.001

VOX = Based on counting voxels; TRI = based on Triangularization of surface.

Table 2. Comparison between control and osteoblast for structural indices of distal femur

Parameter	Control Group	Osteoblast Group	*P* Value
Total Volume (TV mm^3) (VOX)	324.761±2.547	89.673±15.504	0.001
Bone Volume (TV mm^3) (VOX)	14.308±2.224	29.667±1.141	0.001
BV/TV (Vox) Relative Bone Volume (%)	0.044±0.007	0.691±0.071	0.001
Trabecular Number [1/mm] (VOX)	0.303±0.032	0.511±0.29	0.001
Trabecular Thickness [1/mm] (VOX)	0.171±0.008	2.631±0.461	0.001
Connectivity density, normed by TV [1/mm^3] (VOX)	0.848±0.137	5.30±0.369	0.001
Trabecular separation = marrow thickness [mm] (VOX)	3.087±0.154	1.124±0.186	0.001
Total Volume (Tmm^3) (TRI)	322.547±4.696	456.241±15.50	0.001
Bone Volume (TV mm^3) (TRI)	14.260±1.540	19.224±7.27	0.001
TRI-BS	107.696±27.559	176.055±12.024	0.001
TRI-BS/BV	14.226±0.886	15.619±1.074	0.001
Trabecular number [1/mm] (TRI)	0.315±0.039	0.510±0.24	0.001
Trabecular Thickness [1/mm] (TRI)	0.062±0.002	0.148±0.009	0.001
Trabecular Spacing	3.060±0.215	0.427±0.127	0.001

VOX = Based on counting voxels; TRI = based on Triangularization of surface.

14]. The increased longevity of the Saudi Arabian population is no different from the rest of the world. The increased care for osteoporosis patients in the coming years will add to the economic burden. The management of osteoporosis and fragility fractures prevention has remained the same since the last decade, and we still rely on old medications with complications [15-20]. The new approach to treating osteoporosis with customized treatment by stem cell therapy was achievable in animal studies and showed promising results in experimental animals [21-24].

The aim of this study is to assess the effect of MSCs, osteoblasts, and exosomes derived from osteoblasts in the rabbit model.

Methods

Institutional Review board of the Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia gave the ethical approval and the deanship of the scientific research funded this study. Osteoporosis was induced in 40 rabbits by performing ovareictomy at 6-month age and 1 mg/kg body weight of methyl prednisolone

Table 3. Comparison between control and exosome for structural indices of distal femur

Parameter	Control Group	Exosome Group	*P* Value
Total Volume (TV mm^3) (VOX)	324.761±2.547	287.513±2.909	0.1
Bone Volume (TV mm^3) (VOX)	14.308±2.224	14.214±1.630	0.1
BV/TV (Vox) Relative Bone Volume (%)	0.044±0.007	0.28±0.005	0.01
Trabecular Number [1/mm] (VOX)	0.303±0.032	0.369±0.05	0.01
Trabecular Thickness [1/mm] (VOX)	0.171±0.008	0.100±0.008	0.1
Connectivity density, normed by TV [1/mm^3] (VOX)	0.848±0.137	0.885±0.109	0.1
Trabecular separation = marrow thickness [mm] (VOX)	3.087±0.154	0.933±0.137	0.001
Total Volume (Tmm^3) (TRI)	322.547±4.696	181.508±2.878	0.1
Bone Volume (TV mm^3) (TRI)	14.260±1.540	14.202±6.128	0.1
TRI-BS	107.696±27.559	118.503±19.062	0.1
TRI-BS/BV	14.226±0.886	12.750±1.522	0.1
Trabecular number [1/mm] (TRI)	0.315±0.039	0.310±0.030	0.1
Trabecular Thickness [1/mm] (TRI)	0.062±0.002	0.101±0.003	0.01
Trabecular Spacing	3.060±0.215	0.966±0.233	0.1

VOX = Based on counting voxels; TRI = based on Triangularization of surface.

sodium succinate was injected daily for 8 weeks. Animals were fed twice daily as well as water ad libitum. Autologous bone marrow was used to isolate MSCs, and osteoblasts were separated as described [25]. Osteoblasts exosomes were extracted as described earlier [1]. On 15th day, 10^6 cells in 0.5 mL normal saline of MSCs (Group 2) and osteoblasts (Group 3) were respectively injected, in the exosomes (Group 4) 100 µg protein and in the control 0.5 ml of normal saline was injected in the saphenous veins.

At 12 weeks (11 months of age), all the animals were euthanized. The whole femur, Lumbar 3-5th vertebrae were dissected out and were subjected to radiological assessment using High Resolution peripheral quantitative computerized tomography (HRpQCT). All the parameters of the bone volume, trabecular number, thickness and spacing were assessed using the VOX = Based on counting voxels; TRI = based on Triangularization of surface. Statistical analysis was performed using the Statistical Package for Social Sciences software, version 21.0 (SPSS Inc, Chicago, IL, USA). Data was presented as a mean standard deviation (±SD). HRpQCT analysis was taken into consideration for each sample and was reproducible; a coefficient of variation (CV) was calculated as the standard deviation of the three repeated measurements divided by the subject mean. Moreover the precision error was calculated as root-mean-square (RMS) averages for each of samples and each parameter assessed. A p value of <0.05 was considered statistical significant with Confidence Interval (CI) of 95%. As a standard policy of the Imam AbdulRahman Bin Faisal University all the studies are monitored by The Monitoring Office for Research and Research Ethics (MORRE) which is instituted by the Ministry of Higher Education, Kingdom of Saudi Arabia.

Results

Osteoblast-treated animals had significant positive differences in most of the parameters compared to the control group. **Table 1** gives the comparison of the control to the MSCs group. Most the parameters were significant but the osteoblasts group had a highly significant p value between <0.001 to <0.0001. (**Table 2**). The exosomes group was only significant in trabecular number and trabecular separation (**Table 3**).

MSCs group was weakly significant in half of the parameters compared (**Table 4**).

While osteoblast group was again highly significant in all parameters between p<0.01 to <0.0004 (**Table 5**). For the exosome group (**Table 6**), most of the parameters were significant except the total volume and relative bone volume and bone surface assessed.

Figure 1 shows the HRpQCT for 4 groups of the distal femur. In the control group, the num-

Table 4. Comparison between Control and MSCs for structural indices of lumbar spine

Parameter	Control Group	MSCs Group	*P* value
Total Volume (TV mm^3) (VOX)	21.251±5.980	28.352±1.450	0.001
Bone Volume (TV mm^3) (VOX)	2.446±0.783	2.776±0.424	0.001
BV/TV (Vox) Relative Bone Volume (%)	0.086±0.028	0.098±0.015	0.01
Trabecular Number [1/mm] (VOX)	0.996±0.002	1.339±0.387	0.01
Trabecular Thickness [1/mm] (VOX)	0.105±0.009	0.114±0.027	0.3
Connectivity density, normed by TV [1/mm^3] (VOX)	1.949±0.486	4.850±2.907	0.001
Trabecular separation = marrow thickness [mm] (VOX)	1.024±0.052	0.795±0.0280	0.02
Total Volume (Tmm^3) (TRI)	21.936±1.101	27.943±0.002	0.001
Bone Volume (TV mm^3) (TRI)	2.392±0.792	2.732±0.424	0.01
TRI-BS	41.725±13.101	60.761±17.74	0.001
TRI-BS/BV	17.711±1.635	21.951±4.025	0.001
Trabecular number [1/mm] (TRI)	0.747±0.181	1.087±0.318	0.008
Trabecular Thickness [1/mm] (TRI)	0.083±0.03	0.093±0.017	0.09
Trabecular Spacing	1.266±0.345	0.898±0.314	0.004

VOX = Based on counting voxels; TRI = based on Triangularization of surface.

Table 5. Comparison between control and osteoblast for structural indices of lumbar spine

Parameter	Control Group	Osteoblast Group	*P* Value
Total Volume (TV mm^3) (VOX)	21.251±5.980	29.592±4.317	0.001
Bone Volume (TV mm^3) (VOX)	2.446±0.783	4.367±2.363	0.001
BV/TV (Vox) Relative Bone Volume (%)	0.086±0.028	0.154±0.083	0.01
Trabecular Number [1/mm] (VOX)	0.996±0.002	1.553±0.651	0.01
Trabecular Thickness [1/mm] (VOX)	0.105±0.009	0.130±0.013	0.0002
Connectivity density, normed by TV [1/mm^3] (VOX)	1.949±0.486	6.371±4.910	0.001
Trabecular separation = marrow thickness [mm] (VOX)	1.024±0.052	0.727±0.385	0.03
Total Volume (Tmm^3) (TRI)	21.936±1.101	31.945±0.001	0.001
Bone Volume (TV mm^3) (TRI)	2.392±0.792	4.301±6.157	0.001
TRI-BS	41.725±13.101	113.65±15.90	0.001
TRI-BS/BV	17.711±1.635	19.852±7.217	0.01
Trabecular number [1/mm] (TRI)	0.747±0.181	1.390±0.0642	0.001
Trabecular Thickness [1/mm] (TRI)	0.083±0.03	0.117±0.011	0.0004
Trabecular Spacing	1.266±0.345	0.750±0.409	0.003

VOX = Based on counting voxels; TRI = based on Triangularization of surface.

ber of trabeculae is fewer and quantity of bone is less compared to MSCs treated, Osteoblasts and exosome treated rabbits. HRpQCT scans for Lumbar Spine for four Groups. In the control group, the number of trabeculae is fewer and quantity of bone is less compared to MSCs treated, Osteoblasts and exosome treated rabbits. The maximum bone quantity was observed in the osteoblasts group (**Figure 2**).

Discussion

This study shows a reversal of osteoporosis in rabbits using bone marrow-derived MSCs, osteoblasts, and osteoblast-derived exosomes. The effect was robust in the osteoblasts therapy when compared to the MSCs and osteoblast-derived exosomes. Exosome was the least effective in the reversal of osteoporosis. In the exosomes group, it appears that 100 μg protein extracted from the osteoblasts injected may not be enough for complete reversal of osteoporosis, as seen in the osteoblasts group.

Complications of osteoporosis in terms of fragility fractures are still causing a worldwide health care issue in the aging population [26, 27]. Oral medications for osteoporosis are very

Table 6. Comparison between control and exosome for structural indices of lumbar spine

Parameter	Control Group	Exosome Group	*P* value
Total Volume (TV mm^3) (VOX)	21.251±5.980	23.004±6.644	0.42
Bone Volume (TV mm^3) (VOX)	2.446±0.783	3.304±0.003	0.001
BV/TV (Vox) Relative Bone Volume (%)	0.086±0.028	0.117±0.033	0.5
Trabecular Number [1/mm] (VOX)	0.996±0.002	1.130±0.145	0.008
Trabecular Thickness [1/mm] (VOX)	0.105±0.009	0.132 ±0.002	0.001
Connectivity density, normed by TV [1/mm^3] (VOX)	1.949±0.486	4.127±0.125	0.001
Trabecular separation = marrow thickness [mm] (VOX)	1.024±0.052	0.889±0.129	0.01
Total Volume (Tmm^3) (TRI)	21.936±1.101	27.678±6.591	0.001
Bone Volume (TV mm^3) (TRI)	2.392±0.792	3.237±1.011	0.01
TRI-BS	41.725±13.101	63.451±0.935	0.001
TRI-BS/BV	17.711±1.635	19.605±0.257	0.1
Trabecular number [1/mm] (TRI)	0.747±0.181	1.135±0.017	0.001
Trabecular Thickness [1/mm] (TRI)	0.083±0.03	0.102±0.001	0.001
Trabecular Spacing	1.266±0.345	0.779±0.014	0.001

VOX = Based on counting voxels; TRI = based on Triangularization of surface.

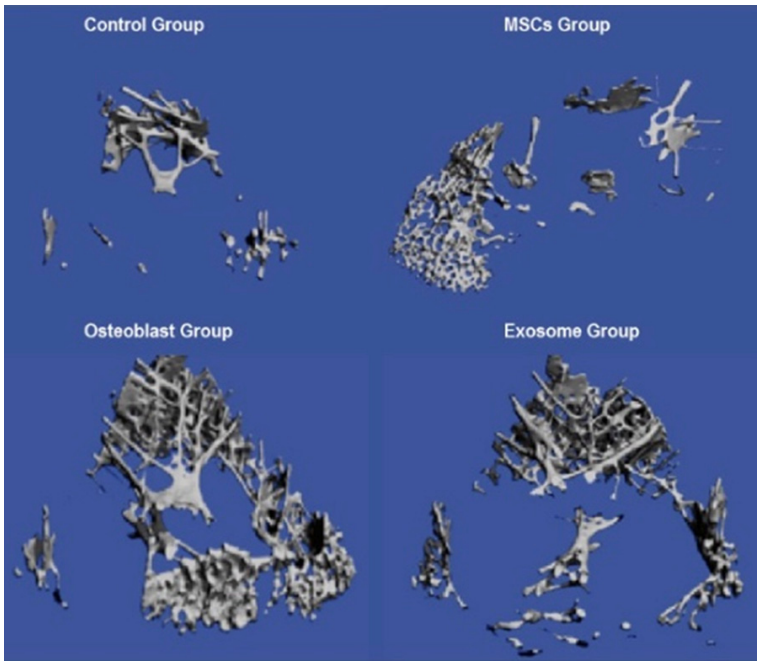

Figure 1. HRpQCT scans for Distal femur for four Groups. In the control group, the number of trabeculae was fewer and quantity of bone was less compared to MSCs treated, Osteoblasts and exosome treated rabbits. The maximum bone quantity was observed in the osteoblasts group.

effective, but the compliance is quite bad [28, 29]. Drugs do not work if they are not taken, and this leads to an increase in the incidence of fragility fractures [30, 31]. One way to prevent the escalating numbers of fragility fractures is to develop newer, safer, and less-expensive medications with minimal complications. The new approach to treat osteoporosis with bone marrow-derived osteoblast therapy in animals appears promising.

The US FDA issued in 1994 [32] and the 2016 [33] guidelines for preclinical and clinical evaluation of agents in the treatment and prevention of postmenopausal osteoporosis have suggested two animal models (e.g., a rodent (rat) and a non-rodent larger animal) in which the efficacy and safety can be demonstrated. This study has shown the effect of osteoblasts in rats [21, 22]. Rabbits were used in this study, and the effect was similar and positive.

This study was limited by the fact that a larger animal (i.e., sheep) should have been used to assess the reversal of osteoporosis because its bone loss with an estrogen deficiency is quite similar to that of women and is proven to be ideal [34, 35]. As strength, the study used a smaller mammal, and the researchers made sure that total osteoporosis was induced with OVX in addition to methyl prednisolone injections to block the extra ovarian estrogen. The present and previous studies in rats have confirmed

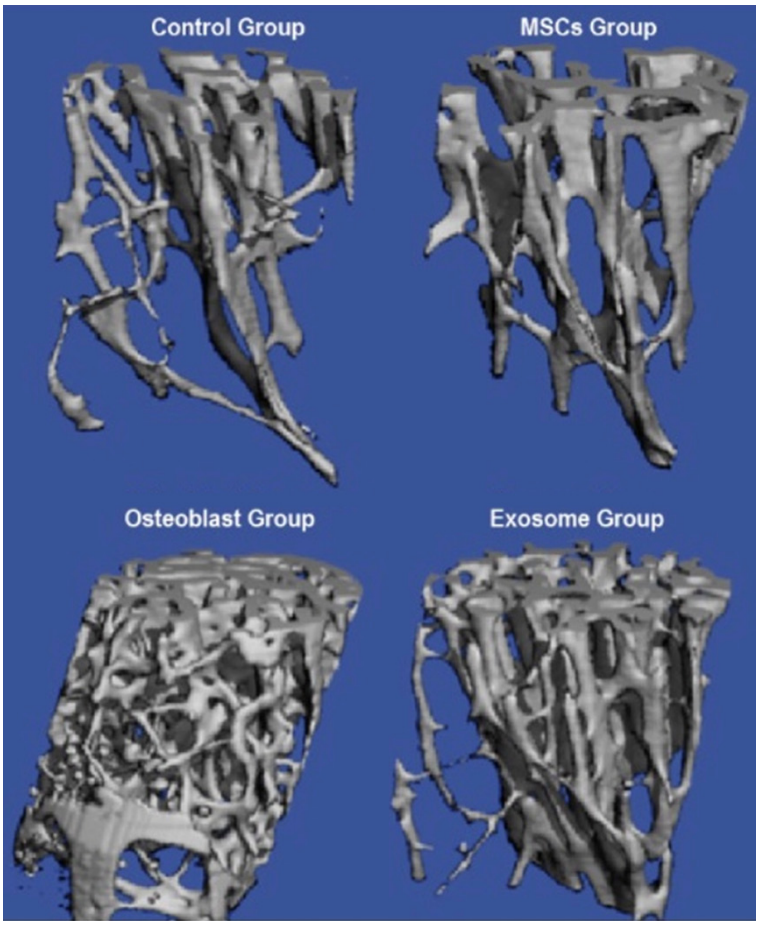

Figure 2. HRpQCT scans for Lumbar Spine for four Groups. In the control group the number of trabeculae are few in number and quantity of bone is less compared to MSCs treated, Osteoblasts and exosome treated rabbits. The maximum bone quantity was observed in the osteoblasts group.

that it is time to start thinking about monitoring bone mineral density and bone-turnover markers in phase I human trials in order to test the efficacy of autologous-derived osteoblasts.

Acknowledgements

The authors thank the Deanship of Scientific Research, Imam AbdulRahman Bin Faisal University, Dammam, Saudi Arabia fro providing the grant vide #2017-077.

Disclosure of conflict of interest

None.

Address correspondence to: Mir Sadat-Ali, College of Medicine, King Fahd Hospital of The University, POBOX 40071, AlKhobar 31952, Saudi Arabia. E-mail: drsadat@hotmail.com

References

[1] Rosen CJ. Restoring aging bones. Sci Am 2003; 288: 70-77.
[2] Marcus R. Post-menopausal osteoporosis. Best Prac Res Clin Obstet Gynaecol 2002; 16: 309-327.
[3] Fleisch H. Pathophysiology of osteoporosis. Bone Mineral 1993; 22 Suppl: S3-36.
[4] Nichols KJ. Evaluation of osteoporosis. J Am Osteopath Assoc 2000; 100 Suppl: S4-7.
[5] Chopra A. Osteoporosis: a new understanding of its impact and pathogenesis. J Am osteopath Assoc 2000; 100 Suppl: S1-4.
[6] Blume SW and Curtis JR. Medical costs of osteoporosis in the elderly Medicare population. Osteoporos Int 2011; 22: 1835-1844.
[7] Sadat-Ali M, Al-Dakheel DA, Azam MQ, Al-Bluwi MT, Al-Farhan MF, AlAmer HA, Al-Meer Z, Al-Mohimeed A, Tabash IK, Karry MO, Rassasy YM, Baragaba MA, Amer AS, AlJawder A, Al-Bouri KM, El-Tinay M, Badawi HA, Al-Othman AA, Tayara BK, Al-Faraidy MH and Amin AH. Reassessment of osteoporosis-related femoral fractures and economic burden in Saudi Arabia. Arch Osteoporos 2015; 10: 37.
[8] Sadat-Ali M, AlZamami JF, Al-Naimi SN, Al-Naimi DA, Al-Dakheel DA and AlTwejry AM. The Increasing Prevalence of Osteoporosis in Eastern Saudi Arabia. Annals of Afr Med (Accepted for Publications).
[9] Sadat-Ali M, Al-Habdan I, Al-Mulhim F and Yousef A. Bone mineral density among postmenopausal Saudi Arabian women. Saudi Med 2004; 25: 1623-1625.
[10] El-Desouki MI, Sherafzal MS and Othman SA. Comparison of bone mineral density with dual energy X-ray absorptiometry, quantitative ultrasound and single energy X-ray absorptiometry. Saudi Med J 2005; 26: 1346-1350.
[11] Ardawi MS, Maimany AA, Bahksh TM, Nasrat HA, Milaat WA and Al-Raddadi RM. Bone mineral density of the spine and femur in healthy Saudis. Osteoporosis Int 2005; 16: 43-55.
[12] Sadat-Ali M, Al-Habdan I and Marwah S. Bone mineral density measurements of distal radius

in Saudi Arabian females. Annals of Saudi Med 1996; 16: 414-416.
[13] Woodward A and Blakely T. The healthy country? A history of life and death in New Zealand (Auckland University Press) 2014.
[14] Cassel CK. Successful aging. How increased life expectancy and medical advances are changing geriatric care. Geriatrics 2001; 56: 35-39.
[15] Graham DY. What the gastroenterologists should know about the gastrointestinal safety profiles of bisphosphonates. Dig Dis Sci 2002; 47: 1665-1678.
[16] De Groen PC, Lubbe DF, Hirsch LJ, Daifotis A, Stephenson W, Freedholm D, Pryor-Tillotson S, Seleznick MJ, Pinkas H and Wang KK. Esophagitis associated with the use of alendronate. N Engl J Med 1996; 335: 1016-1021.
[17] Rupel K, Ottaviani G, Gobbo M, Contardo L, Tirelli G, Vescovi P, Di Lenarda R and Biasotto M. A systematic review of therapeutical approaches in bisphosphonates-related osteonecrosis of the jaw (BRONJ). Oral Oncol 2014; 50: 1049-57.
[18] Holzinger D, Seemann R, Matoni N, Ewers R, Millesi W and Wutzl A. Effect of dental implants on bisphosphonate-related osteonecrosis of the jaws. J Oral Maxillofac Surg 2014; 72: 1937.
[19] Kharazmi M and Hallberg P. Bisphosphonate-associated atypical femoral fractures and one-year mortality. Ups J Med Sci 2014; 18: 1-2.
[20] Bhadada SK, Sridhar S, Muthukrishnan J, Mithal A, Sharma DC, Bhansali A and Dhiman V. Predictors of atypical femoral fractures during long term bisphosphonate therapy: a case series & amp; review of literature. Indian J Med Res 2014; 140: 46-54.
[21] Sadat-Ali M, Al-Dakheel DA, AlMousa SA, AlAnii FM, Ebrahim WY, AlOmar HK, AlSayed HN, Acharya S and AlHawaj H. Stem-cell therapy for ovariectomy-induced osteoporosis in rats: a comparison of three treatment modalities. Stem Cells Cloning 2019; 12: 17-25.
[22] Sadat-Ali M, Al-Turki HA, Acharya S and Al-Dakheel DA. Bone marrow-derived osteoblasts in the management of ovariectomy induced osteoporosis in rats. J Stem Cells Regen Med 2018; 14: 63-68.
[23] Kiernan J, Hu S, Grynpas MD, Davies JE and Stanford WL. Systemic mesenchymal stromal cell transplantation prevents functional bone loss in a mouse model of age-related osteoporosis. Stem Cells Transl Med 2016; 5: 683-693.
[24] Ocarino Nde M, Boeloni JN, Jorgetti V, Gomes DA, Goes AM and Serakides R. Intra-bone marrow injection of mesenchymal stem cells improves the femur bone mass of osteoporotic female rats. Connect Tissue Res 2010; 51: 426-433.
[25] Piao H, Youn TJ, Kwon JS, Kim YH, Bae JW, Bora-Sohn, Kim D, Cho MC, Lee MM and Park YB. Effects of bone marrow derived mesenchymal stem cells transplantation in acutely infarcting myocardium. Eur J Heart Fail 2005; 7: 730-738.
[26] Burge R, Dawson-Hughes B, Solomon D, Wong JB, King A and Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. J Bone Miner Res 2007; 22: 465-475.
[27] Si L, Winzenberg TM, Jiang Q, Chen M and Palmer AJ. Projection of osteoporosis-related fractures and costs in China: 2010-2050. Osteoporos Int 2015; 26: 1929-1937.
[28] Yood RA, Emani S, Reed JI, Lewis BE, Charpentier M and Lydick E. Compliance with pharmacologic therapy for osteoporosis. Osteoporos Int 2003; 14: 965-968.
[29] Weycker D, Macarios D, Edelsberg J and Oster G. Compliance with osteoporosis drug therapy and risk of fracture. Osteoporos Int 2007; 18: 271-277.
[30] Rabenda V, Mertens R, Fabri V, Vanoverloop J, Sumkay F, Vannecke C, Deswaef A, Verpooten GA and Reginster JY. Adherence to bisphosphonates therapy and hip fracture risk in osteoporotic women. Osteoporos Int 2008; 19: 811-818.
[31] Caro JJ, Ishak KJ, Kf H, Raggio G and Naujoks C. The impact of compliance with osteoporosis therapies on fracture rates in actual practice. Osteoporos Int 2004; 15: 1003-1008.
[32] Thompson DD, Simmons HA, Pirie CM and Ke HZ. FDA guidelines and animal models for osteoporosis. Bone 1995; 17 Suppl: 125S-133S.
[33] http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm. Assessed December 2020.
[34] Chavassieux P, Garnero P, Duboeuf F, Vergnaud P, Brunner-Ferber F, Delmas PD and Meunier PJ. Effects of a new selective estrogen receptor modulator (MDL 103,323) on cancellous and cortical bone in ovariectomized ewes: a biochemical, histomorphometric, and densitometric study. J Bone Miner Res 2001; 16: 89-96.
[35] Hornby SB, Ford SL, Mase CA and Evans GP. Skeletal changes in the ovariectomised ewe and subsequent response to treatment with 17-beta oestradiol. Bone 1995; 17: 389S-394S.