BONE METABOLISM AND TURNOVER RATES IN DIABETIC KIDNEY DISEASE- A CROSS-SECTIONAL OBSERVATIONAL STUDY

K. V. Rajalakshmi[1], P. Boopathi Rajan[2], T. Mohanasundaram[3], M. Bathragiri[4], G. Gurunamasivayam[5]

[1]Professor and HOD, Department of Medicine, Kilpauk Medical College, Chennai.
[2]Assistant Professor, Department of Medicine, Kilpauk Medical College, Chennai.
[3]Assistant Professor, Department of Medicine, Kilpauk Medical College, Chennai.
[4]Assistant Professor, Department of Medicine, Kilpauk Medical College, Chennai.
[5]Assistant Professor, Department of Medicine, Kilpauk Medical College, Chennai.

ABSTRACT

BACKGROUND

With the increasing trend in the diabetic population and the diabetic nephropathy, it is necessary for us to look into the bone metabolism in the diabetics with CKD. This study aims to describe the bone metabolism and the bone turnover rates in diabetic kidney disease and to see if there is a difference between diabetic and non-diabetic CKD in the bone turnover rates.

MATERIALS AND METHODS

We did a cross-sectional observational study between January and June 2016 at the Govt. Kilpauk Medical College with 90 CKD patients. Of them 44 were diabetics with a mean age of 56.18 +/- 8.88 and 46 were non-diabetics with a mean age of 52.19 +/- 14.55. Patients were analysed for various biochemical parameters. The data were analysed using SPSS (Statistical Package for Social Sciences) ver 16.01.

RESULTS

82% of our diabetic CKD patients have diabetic retinopathy. Diabetic retinopathy is not associated with abnormalities in bone turnover (odds ratio= 1). For the diabetic CKD (n= 44), the prevalence of hypoparathyroidism is 4.5%, hyperparathyroidism is 59%, hyperparathyroidism more than twice the upper limit of normal is 36.36%, hypocalcaemia is 15.9%, hypercalcaemia is 4.5%, hypophosphataemia is 20.45% and vitamin D deficiency is 95.45%. The most common bone turnover abnormality observed in diabetics in our study is the mixed turnover state (30%) followed by osteomalacia (21%), whereas in non-diabetics, high turnover state (26%) is the most common abnormality, but there is no statistically significant difference between the two (p= 0.81). Low turnover state in diabetics and non-diabetics in our study are 7% and 9% respectively.

CONCLUSION

Thus, we conclude that a mixed bone turnover state followed by osteomalacia is commoner in diabetic CKD than other patterns. Diabetic retinopathy is not predictive of the bone turnover state. Diabetic and non-diabetic CKD need not be considered as separate entities as far as bone metabolism is considered.

KEYWORDS

Bone Specific Alkaline Phosphatase, Bone Turnover, Chronic Kidney Disease, Diabetic Kidney Disease, Diabetic Retinopathy.

HOW TO CITE THIS ARTICLE: Rajalakshmi KV, Rajan PB, Mohanasundaram T, et al. Bone metabolism and turnover rates in diabetic kidney disease- a cross-sectional observational study. J. Evolution Med. Dent. Sci. 2018;7(08):957-961, DOI: 10.14260/jemds/2018/219

BACKGROUND

It is well known that chronic kidney disease is associated with bone disease. With the increasing trend in the diabetic population and the diabetic nephropathy, it is necessary for us to look into the bone metabolism in the diabetics with CKD, so that we can predict the fracture risk as well as extra-skeletal calcification. The effects of diabetes in bone turnover are not clear. Some studies say that bone formation rate is not affected by diabetes per se.[1-6] Other studies say that BAP, the bone turnover marker is increased in type 2 diabetes mellitus.[7-9]

'Financial or Other Competing Interest': None.
Submission 13-12-2017, Peer Review 05-02-2018,
Acceptance 12-02-2018, Published 19-02-2018.
Corresponding Author:
Dr. P. Boopathi Rajan,
Assistant Professor of Medicine,
Government Kilpauk Medical College,
Poonamallee High Road, Kilpauk, Chennai-600010.
E-mail: rajanmd1994@gmail.com
DOI: 10.14260/jemds/2018/219

This study aims to describe the effects as observed in the south Indian population.

Aims and Objectives-

1. To describe the bone metabolism and the bone turnover rates in diabetic kidney disease.
2. To see if there is a difference between diabetic and non-diabetic CKD in the bone turnover rates.

MATERIALS AND METHODS

This is a cross-sectional observational study conducted from January 2016 to June 2016 in Govt. Kilpauk Medical College, Chennai, Tamilnadu. A total of 90 CKD patients attending the Nephrology Department, Govt. Kilpauk Medical College, Chennai were included in the study. Of them 44 were diabetics and 46 were non-diabetics.

We included stage 3-5 pre-dialytic CKD patients and for those whom dialysis had been done for less than a month. Patients already on dialysis who underwent renal transplantation, those with known parathyroid abnormalities, liver disease and rickets and those on anti-

epileptics were excluded. None of the patients were on vitamin D supplementation. But all of them were on calcium supplementation.

Ethics committee approval from the Institutional Ethics Committee was obtained prior to the study. Questionnaire regarding symptoms of CKD-MBD is given to all the patients in our study. Those who are having one or more of the following such as non-specific aches in lower back, hips and legs that increase on weight bearing, gradual onset muscle weakness, bone pains that are deep dull aching pains that cannot be often localised by the patient, fractures occurring to trivial injuries are considered as symptomatic. Ophthalmic fundus examination is done.

After obtaining consent from the patient with aseptic precautions, about 8 mL of venous blood is obtained from the median cubital vein without applying tourniquet, at 10 am to 12 pm. Blood collection is done like this because factors like central vein blood or peripheral blood, winter season or summer season, nocturnal or daylight will affect the PTH concentration.[10] 3 mL of blood is collected in K2-EDTA tube for separating plasma. 5 mL of blood is collected in Red topped standard serum separating tube. After allowing the sample to clot at room temperature for about one hour, samples are centrifuged and serum and plasma are separately aspirated using Pasteur pipettes. Samples are separately stored in 1 mL Eppendorf tubes.

Thus, 4 aliquots are allotted for every patient. Serum and plasma samples are stored at -20°C ice lined refrigerator. All samples are analysed within 3 months of collection. Plasma samples are analysed for 25-OH Vitamin D and iPTH. Serum samples are analysed for all other biochemical parameters.

Vitamin D

The kit used in this study is ms-05894913 190 by Roche Diagnostics and was carried out on Cobas e411 ECLIA analyser measures 25-OH vitamin D. The stability of vitamin D in K2 EDTA plasma at -20^{0}C is for 24 weeks. The measuring range is 3.00 - 70.0 ng/mL. The shelf life of unopened kit is upto expiry date; opened and at 2 – 8^{0}C is 8 weeks; On Cobas e411 analyser is 3 weeks; < 30 ng/mL is considered as Vitamin D deficiency.

Parathormone

The iPTH assay is carried out using the 2 site Electrochemiluminescence Immunoassay. The kit used is ms-11972103122 on Cobas e 411 analyser. The antibodies used in this test reacts at N-terminal 1-37 amino acids at 26 - 32 amino acid epitope and C-terminal 38-84 amino acids at 37 - 42 amino acid epitope. Measuring range is 1.20 – 5000 pg/mL. The stability of PTH at -20^{0}C in plasma is for 6 months. This method has no cross reactivity with PTH 1-37, PTHrP 1-86. The shelf life of unopened kit at 2 – 8^{0}C is up to expiry date; opened and at 2 – 8^{0}C is 12 weeks; on the analyser is 8 weeks. The normal value of iPTH is 15 - 65 pg/mL.

Bone Specific Alkaline Phosphatase

This assay was carried out by ELISA in microtitre strip method with MicroVue BAP ELISA kit-046001 from Quidel Corporation, San Diego, USA in Robonik Readwell Touch ELISA plate analyser and Robonik Washwell Plate ELISA washer. The stability of the enzyme in serum is 5 days at 2 - 8^{o}C; twelve months at -20^{0}C; 36 months at -80^{0}C. The normal value of BAP is Female age 25 - 44= 11.6 - 29.6U/L; Female age > 44= 14.2 - 42.7 U/L; Male age > 25= 15 - 41.3 U/L.

Calcium

This assay is done using the Calcium Gen 2 kit- 05061482 190 from Roche and analysed using Cobas C 311 analyser. The assay is based on the change in absorbance of 376 nm wavelength light that is measured photometrically. Stability of the serum calcium at -20^{0}C is 8 months. The range of measurement of this method is 0.8 - 20.1 mg/dL. The measured parameter is the serum total calcium. It is corrected according to the serum albumin to calculate corrected calcium.

Phosphorus

This assay is done using Inorganic Phosphate ver. 2 kit-03183793 122 from Roche, on Cobas C 311 analyser. The assay is based on Molybdate UV principle, which measures the change in absorbance of the Ammonium phosphomolybdate which subsequently reduces to molybdenum. The stability of the phosphate ions in serum stored at -20^{0}C is 1 year.

Urea

This assay is done using the UreaL kit- 04460715 190 from Roche, on Cobas C 311 analyser. The assay is based on the Kinetic test principle with Urease and Glutamate dehydrogenase enzymes. The resulting NADH concentration is measured photometrically and is directly proportionate to the concentration of urea. The stability of urea in serum stored at -20^{0}C is 1 year.

Creatinine

This assay is done using the CREJ2 kit- 04810716 190 from Roche, on Cobas C 311 analyser. The assay is based on kinetic colorimetric principle using Jaffe's method that forms a Yellow orange complex that is measured photometrically. The serum stability for creatinine at -20^{0}C is 3 months, the shortest of all parameters in our study. Hence, all analyses are completed within three months of sample collection. Patients are staged according to eGFR calculation using CKD-EPI equation.

Magnesium

This assay is done using Robonik Prietest Touch photocolorimeter machine based on Xylidyl blue method. The normal range by this method is 1.6 to 2.8 mg/dL.

eGFR

Calculation done by CKD- EPI equation.

Statistical Analysis

The data were analysed using SPSS (Statistical Package for Social Sciences) Ver 16.0. Student t-test was used for testing the significance of all the variables, mean and standard deviation in groups. Chi-square test was used to compare proportions. All the statistical results were considered significant at P value ≤ 0.05.

RESULTS

We analysed in our study 44 diabetics with CKD and 46 non-diabetics with CKD. The demographics of the 90 patients are as follows.

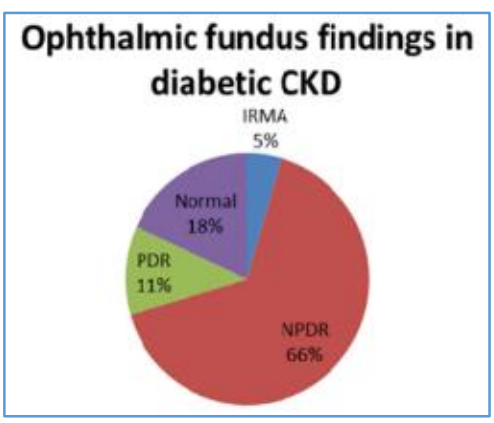
Parameter	Diabetic	Non-Diabetic
Age yrs.	56.18 +/- 8.88	52.19 +/- 14.55
M:F	18:26	25:21
Urea mg/dL	84.5(50.5, 108)	63 (51.2, 103.2)
Creatinine mg/dL	3(2.1, 4.6)	2.5 (1.8, 3.85)
eGFR mL/min/1.73 m2	20.34 +/- 11.35	25.5 +/- 14.18
Calcium mg/dL	9.2 (8.7, 9.47)	9.2 (8.7, 9.4)
Phosphorus mg/dL	3.8 (3.4, 4.5)	3.9 (2.9, 4.5)
Magnesium mg/dL	2.15 (1.7, 3.7)	2.15 (1.6, 3.1)
iPTH pg/mL	75.35 (45.35, 179.1)	95.9 (54.1, 179.2)
Vitamin D ng/mL	15.7 (10.9, 18.9)	17.65 (9.9, 24.8)
BAP U/L	28.72 (18.95, 40.7)	27.05 (20.1, 37.3)

Table 1

Table 1: Demographic profile of 90 CKD patients. Age, creatinine and eGFR are presented as mean with standard deviation. Urea, calcium, phosphorus, magnesium, iPTH, vitamin D and BAP are expressed as median with 1st and 3rd Quartiles in parentheses.

eGFR- Estimated Glomerular Filtration Rate; iPTH- Intact parathormone; BAP- Bone Specific Alkaline Phosphatase.

The clinical and biochemical parameters describing the bone metabolism in diabetic CKD from our analysis are as follows-



Picture 1. Ophthalmic Fundus findings in Diabetic CKD. IRMA- Intraretinal Microvascular Abnormalities; NPDR- Non-Proliferative Diabetic Retinopathy; PDR- Proliferative Diabetic Retinopathy.

Diabetic CKD							
	Stage 3		Stage 4		Stage 5		P
	n	%	n	%	n	%	
Hypocalcaemia	0	0	4	21.03	3	17.64	0.749
Normocalcaemia	8	100	15	78.97	14	82.35	
Hypercalcaemia	0	0	0	0	0	0	
Hypophosphataemia	1	12.5	1	5.26	0	0	0.143
Normophosphataemia	6	75	16	84.21	10	58.82	
Hyperphosphataemia	1	12.5	2	10.52	7	41.17	
Hypomagnesaemia	1	12.5	3	15.78	4	23.52	0.542
Normomagnesaemia	2	25	6	31.57	8	47.05	
Hypermagnesaemia	5	62.5	10	52.63	5	29.41	
Vitamin D deficiency	8	100	17	89.47	17	100	<0.001
Hypoparathyroidism	2	25	0	0	1	5.88	<0.001
Hyperparathyroidism < 2 x	1	12.5	7	36.84	2	11.76	
Hyperparathyroidism > 2 x	2	25	1	5.26	13	76.47	
Normoparathyroidism	3	37.5	11	57.89	1	5.88	
Low turnover	0	0	3	15.78	0	0	0.106
Normal turnover	8	100	11	57.89	14	82.35	
High turnover	0	0	5	26.31	3	17.64	

Table 2. Biochemical Parameters of Bone Metabolism in Diabetic CKD in Various Stages

The biochemical parameters describing the bone metabolism in non-diabetic CKD are as follows.

Non-Diabetic CKD							
	Stage 3		Stage 4		Stage 5		P
	N	%	N	%	N	%	
Hypocalcaemia	1	5.56	2	11.11	4	40	0.063
Normocalcaemia	17	94.44	14	77.78	5	50	
Hypercalcaemia	0	0	2	11.11	1	10	
Hypophosphataemia	4	22.22	2	11.11	0	0	0.001
Normophosphataemia	14	77.78	12	66.67	3	30	
Hyperphosphataemia	0	0	4	22.22	7	70	
Hypomagnesaemia	4	22.22	2	11.11	2	20	0.416
Normomagnesaemia	5	27.78	11	61.11	4	40	
Hypermagnesaemia	9	50	5	27.78	4	40	
Vitamin D deficiency	17	94.44	13	72.22	10	100	<0.001
Hypoparathyroidism	2	11.11	1	5.56	0	0	0.748
Hyperparathyroidism < 2 x	7	38.89	7	38.89	1	10	
Hyperparathyroidism > 2 x	4	22.22	6	33.33	8	80	
Normo-parathyroidism	5	27.78	4	22.22	1	10	
Low turnover	2	11.11	1	5.56	1	10	0.429
Normal turnover	14	77.78	11	61.11	5	50	
High turnover	2	11.11	6	33.33	4	40	

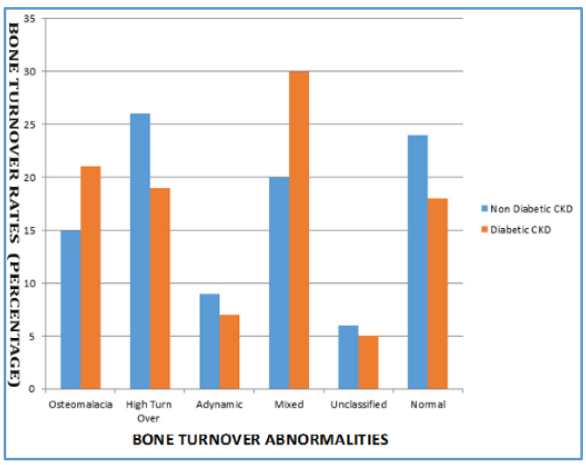
Table 3. Biochemical Parameters of Non-Diabetic CKD in Various Stages

With the bone turnover marker, Bone specific Alkaline Phosphatase, bone turnover rates are measured in our study. We considered the various bone turnover abnormalities in our study as follows-

Bone Turnover	BAP (Female Age 25-44= 11.6 - 29.6 U/L Female Age >44= 14.2 - 42.7 U/L Male Age > 25= 15 - 41.3 U/L)	iPTH (15 - 65 pg/mL)	Vit D (Deficiency= < 30 ng/mL)
Normal	Normal	Normal or elevated, but less than twice the upper limit	Deficient
Adynamic bone disease	Low	Low	Deficient
High turnover bone disease	High	Elevated or normal	
Mild turnover disease	Normal	Elevated more than twice the upper limit	Deficient
Osteomalacia	Normal	Normal	Deficient
Unclassified	Normal	Low	Deficient

Table 4. MBD Classes according to the Biochemical Parameters alone. MBD- Mineral Bone Disease; BAP- Bone Specific Alkaline Phosphatase; iPTH- Intact Parathormone; Vit D- Vitamin D.

The pattern of bone turnover rates (percentage) in diabetic kidney disease and non-diabetic CKD are as follows-



Bone turnover patterns of diabetic and non-diabetic CKD do not differ statistically (p= 0.81034).

Biochemical parameters of bone metabolism in diabetic vs. non-diabetic CKD are as follows-

	Diabetic (N= 44)		Non-Diabetic (N= 46)		P value
	N	%	N	%	
Hypocalcaemia	7	15.9	6	13	0.867
Normo calcaemia	35	79.5	37	80.4	
Hyper calcaemia	2	4.5	3	6.5	
Hypopho sphataemia	2	4.5	6	13	0.341
Normopho sphataemia	33	73.3	30	65	
Hyperpho sphataemia	9	20.4	10	21.7	
Hypomagnesaemia	5	11.4	8	17.4	0.518
Normomagnesaemia	19	43.2	22	47.8	
Hypermagnesaemia	20	45.4	16	34.8	
Vitamin D deficiency	42	95.4	40	86.9	0.897
Hypoparathyroidism	2	4.5	3	6.5	0.447
Hyperparathyroidism < 2 x	10	22.7	15	32.6	
Hyperparathyroidism > 2 x	16	36.3	18	39.1	
Normo parathyroidism	16	36.3	10	21.7	
Low turnover	3	6.8	4	8.6	0.594
Normal turnover	33	73.3	30	65	
High turnover	8	18.2	12	26	

Table 5. Comparison between the Prevalence of Diabetic and Non-Diabetic CKD Metabolic Parameters. P value is derived from Chi-Square Test.

DISCUSSION

We studied 44 diabetics with CKD and a mean age of 56.18 years +/- 8.88 and 46 non-diabetics with CKD and a mean age of 52.19 years +/- 14.55. In our study, there is equal distribution between males and females (p= 0.202). For the diabetic patients, the duration of diabetes ranges from 3 to 20 years with a median of 10 years. The most common fundus finding for them is the Non-Proliferative Diabetic Retinopathy (p= < 0.001). 82% of our diabetic CKD patients have diabetic retinopathy. According to our study, the presence or absence of diabetic retinopathy is not associated with abnormalities in bone turnover (odds ratio= 1; p= 1.00). The presence or absence of diabetes also does not significantly alter the bone metabolism in CKD. This is in accordance with other studies.[11]

The bone turnover abnormalities are associated with histomorphometric changes in the bone. Without a bone biopsy, it is difficult to confirm the turnover abnormalities. Since bone biopsy is very painful procedure and is not feasible in our setup, we proceeded with biochemical parameters alone. The most common bone turnover abnormality observed in diabetics in our study is the mixed turnover state (30%) followed by osteomalacia (21%), whereas in non-diabetics high turnover state (26%) is the most common abnormality, but there is no statistically significant difference between the two (p= 0.81). Low turnover state in diabetics and non-diabetics in our study are 7% and 9% respectively. Ours is the first Indian study to describe the bone turnover abnormalities based on biochemical parameters alone.

In our study, for the diabetic CKD (n= 44) the prevalence of hypoparathyroidism is 4.5%, hyperparathyroidism is 59%, hyperparathyroidism more than twice the upper limit of normal is 36.36%, hypocalcaemia is 15.9%, hypercalcaemia is 4.5%, hyperphosphataemia is 20.45% and vitamin D deficiency is 95.45%. In other Indian study, the prevalence are hypocalcaemia, hyperphosphataemia, hyperparathyroidism (> 150 pg/mL) and 25(OH) D levels <30 ng/mL was 66.3%, 59%, 89.3% and 74.7% respectively.[12] Another Indian study shows the prevalence of hypocalcaemia (56.41% vs. 54.95%), hyperphosphataemia (64.10% vs. 70.27%) and hyperparathyroidism (84.62% vs. 88.29%) was not different between patients with CKD 4 and 5D.[13]

In our study there is no significant difference in the calcium, phosphorus, magnesium, iPTH, vitamin D and BAP metabolism between the diabetics and the non-diabetics. This is in accordance with some studies,[11] but they found that there is elevation in the osteoclast activity marker osteocalcin compared to non-diabetics which in our study is there is no difference in BAP. Another study says that BAP is low among diabetics.[14,15] Another study says that iPTH levels and BAP levels are low in diabetics with CKD.[16-20]

CONCLUSION

Thus, we conclude that a mixed bone turnover state followed by osteomalacia is commoner in diabetic CKD than other patterns. Diabetic retinopathy is not predictive of the bone turnover state. Diabetic and non-diabetic CKD need not be considered as separate entities as far as bone metabolism is considered.

REFERENCES

[1] Verhaeghe J, Van Herck E, Visser WJ, et al. Bone and mineral metabolism in BB rats with long-term diabetes: decreased bone turnover and osteoporosis. Diabetes 1990;39(4):477-82.

[2] Pedrazzoni M, Ciotti G, Pioli G, et al. Osteocalcin levels in diabetic subjects. Calcified Tissue International 1989;45(6):331-6.

[3] Sayinalp S, Gedik O, Koray Z. Increasing serum osteocalcin after glycemic control in diabetic men. Calcified Tissue International 1995;57(6):422-5.
[4] Okazaki R, Totsuka Y, Hamano K, et al. Metabolic improvement of poorly controlled noninsulin-dependent diabetes mellitus decreases bone turnover. The Journal of Clinical Endocrinology & Metabolism 1997;82(9):2915-20.
[5] Akin O, Göl K, Aktürk M, et al. Evaluation of bone turnover in postmenopausal patients with type 2 diabetes mellitus using biochemical markers and bone mineral density measurements. Gynecological Endocrinology 2003;17(1):19-29.
[6] Rosato MT, Schneider SH, Shapses SA. Bone turnover and insulin-like growth factor I levels increase after improved glycemic control in noninsulin-dependent diabetes mellitus. Calcified Tissue International 1998;63(2):107-11.
[7] Bouillon R, Bex M, Van Herck E, et al. Influence of age, sex and insulin on osteoblast function: osteoblast dysfunction in diabetes mellitus. The Journal of Clinical Endocrinology & Metabolism 1995;80(4):1194-202.
[8] Štěpán J, Havranek T, Formankova J, et al. Bone isoenzyme of serum alkaline phosphatase in diabetes mellitus. Clinica Chimica Acta 1980;105(1):75-81.
[9] Gallacher SJ, Fenner JA, Fisher BM, et al. An evaluation of bone density and turnover in premenopausal women with type 1 diabetes mellitus. Diabetic Medicine 1993;10(2):129-33.
[10] Hanon EA, Sturgeon CM, Lamb EJ. Sampling and storage conditions influencing measurement of parathyroid hormone in blood samples: a systematic review. Clin Chem Lab Med 2013;51(10):1925-41.
[11] Isaia GC, Ardissone P, Di Stefano M, et al. Bone metabolism in type 2 diabetes mellitus. Acta Diabetologica 1999;36(1-2):35-8.
[12] Valson AT, Sundaram M, David VG, et al. Profile of incident chronic kidney disease related-mineral bone disorders in chronic kidney disease stage 4 and 5: a hospital based cross-sectional survey. Indian Journal of Nephrology 2014;24(2):97-107.
[13] Ghosh B, Brojen T, Banerjee S, et al. The high prevalence of chronic kidney disease-mineral bone disorders: a hospital-based cross-sectional study. Indian Journal of Nephrology 2012;22(4):285-91.
[14] Lumachi F, Camozzi V, Tombolan V, et al. Bone mineral density, osteocalcin and bone-specific alkaline phosphatase in patients with insulin-dependent diabetes mellitus. Annals of the New York Academy of Sciences 2009;1173 (Suppl 1):E64-7.
[15] Oz SG, Guven GS, Kilicarslan A, et al. Evaluation of bone metabolism and bone mass in patients with type-2 diabetes mellitus. Journal of the National Medical Association 2006;98(10):1598-604.
[16] Rubin MR, Patsch JM. Assessment of bone turnover and bone quality in type 2 diabetic bone disease: current concepts and future directions. Bone Research 2016;4:16001.
[17] Dobnig H, Piswanger-Sölkner JC, Roth M, et al. Type 2 diabetes mellitus in nursing home patients: effects on bone turnover, bone mass and fracture risk. The Journal of Clinical Endocrinology & Metabolism 2006;91(9):3355-63.
[18] Ardawi MS, Akhbar DH, Alshaikh A, et al. Increased serum sclerostin and decreased serum IGF-1 are associated with vertebral fractures among postmenopausal women with type-2 diabetes. Bone 2013;56(2):355-62.
[19] Yamamoto M, Yamaguchi T, Nawata K, et al. Decreased PTH levels accompanied by low bone formation are associated with vertebral fractures in postmenopausal women with type 2 diabetes. The Journal of Clinical Endocrinology & Metabolism 2012;97(4):1277-84.
[20] Gaudio A, Privitera F, Battaglia K, et al. Sclerostin levels associated with inhibition of the Wnt/β-catenin signaling and reduced bone turnover in type 2 diabetes mellitus. The Journal of Clinical Endocrinology & Metabolism 2012;97(10):3744-50.