A Prospective Evaluation of Vascular Calcification Markers in Renal Transplantation

Renal Transplantasyonda Vasküler Kalsifikasyon Belirteçlerinin Prospektif Değerlendirmesi

Bilge Karatoy Erdem* **Vural Taner Yılmaz**** **Gültekin Süleymanlar****
Ayhan Dinckan*** **Halide Akbas***

* Akdeniz Üniversitesi Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, Antalya, Türkiye
** Akdeniz Üniversitesi Tıp Fakültesi, Nefroloji Bilim Dalı, Antalya, Türkiye
*** Akdeniz Üniversitesi Tıp Fakültesi, Genel Cerrahi Anabilim Dalı, Antalya, Türkiye

Başvuru Tarihi: 16 Ekim 2020 **Kabul Tarihi:** 05 Aralık 2020

ABSTRACT

Aim: The evaluation of biomarkers linked with inhibition and activation of vascular calcification in pre and post renal transplant period in renal transplant recipients was aimed in this research.

Patients and Methods: 35 recipients who had undergone living-donor kidney transplantation were included. Plasma 1,25-dihydroxyvitamin D3, serum 25-hydroxyvitamin D3, calcium, phosphorus, inorganic pyrophosphate (PPi), osteoprotegerin (OPG), alpha-2 heremans-schmid glycoprotein (Fetuin-A), alkaline phosphatase (ALP), bone morphogenic protein-2 (BMP-2), creatinine, parathyroid hormone (PTH) levels were analyzed immediately before and 6 months after transplantation. Statistical analysis was done using SPSS version 20.0.

Results: This research reported that vitamin D deficiency continues independently of renal function in renal transplant recipients. Despite the low levels of vitamin D, its active form was increased with the improvement of renal function. It also observed that PPi and Fetuin-A levels increase, OPG and ALP levels decrease with the improvement of graft function.

Conclusion: Serum biomarkers could serve as important indicators of vascular calcification in renal transplant recipients. The increase in serum levels of OPG and ALP, which are thought to play a role as activators in the calcification process in patients with Chronic Kidney Disease may be beneficial in early detection of calcification. Further larger studies are required to evaluate time-dependent changes after renal transplantation.

Key Words: *renal transplant, vascular calcification, biomarkers, Fetuin A, PPi*

Bilge Karatoy Erdem	0000-0003-3594-0759
Vural Taner Yılmaz	0000-0002-1313-8856
Gültekin Süleymanlar	0000-0001-7935-6402
Ayhan Dinckan	AAO-2633-2020
Halide Akbas	0000-0003-2191-1789

Yazışma adresi: Halide S. Akbaş
Akdeniz Üniversitesi Tıp Fakültesi, Biyokimya Anabilim Dalı,
Antalya, Türkiye
E-posta: halideakbas@akdeniz.edu.tr

ÖZET

Amaç: Bu araştırmada renal transplant alıcılarında, renal transplant öncesi ve sonrası dönemde vasküler kalsifikasyonun inhibisyonu ve aktivasyonu ile ilişkili biyobelirteçlerin değerlendirilmesi amaçlanmıştır.

Hastalar ve Yöntemler: Canlı donörden renal transplantasyon yapılan 35 hasta araştırmaya dahil edildi. Plazma 1,25-dihidroksivitamin D3, serum 25-hidroksivitamin D, kalsiyum, fosfor, inorganik pirofosfat (PPi), osteoprotegerin (OPG), alfa-2 heremans-schmid glikoprotein (Fetuin-A), alkalen fosfataz (ALP), kemik morfojenik protein-2 (BMP-2), kreatinin, paratiroid hormon (PTH) seviyeleri, transplantasyondan hemen önce ve 6 ay sonra analiz edildi. İstatistiksel analizler için SPSS 20.0 programı kullanıldı.

Bulgular: Bu araştırma renal transplant alıcılarında D vitamini eksikliğinin greft fonksiyonundan bağımsız olarak devam ettiğini bildirmiştir. Düşük D vitamini seviyelerine rağmen, greft fonksiyonunun düzelmesiyle aktif D vitamini formu artmıştır. Ayrıca greft fonksiyonunun düzelmesiyle PPi ve Fetuin-A düzeylerinin arttığı, OPG ve ALP düzeylerinin ise azaldığı gözlemlenmiştir.

Sonuç: Serum biyobelirteçleri, renal transplant alıcılarında vasküler kalsifikasyonun önemli göstergeleri olarak hizmet edebilir. Kronik böbrek hastalarında kalsifikasyon sürecinde aktivatör olarak rol oynadığı düşünülen serum OPG ve ALP düzeylerindeki artış, kalsifikasyonun erken tespitinde faydalı olabilir. Renal transplantasyondan sonra zamana bağlı değişiklikleri değerlendirmek için daha büyük ölçekli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: renal transplantasyon, vasküler kalsifikasyon, biyobelirteçler, Fetuin A, PPi

INTRODUCTION

Renal transplantation is an ideal treatment for end-stage renal failure. However, it suffers from certain limitations of it own including adverse effects such as increased incidence of infectious diseases, tumor development due to the necessity of a lifelong immunosuppressive therapy and graft function failure.

A literature review revealed that cardiovascular diseases (CVD) are the leading cause of mortality in renal transplant recipients. Mineral-bone disorders and the subsequent vascular calcification, are the leading factors contributing towards cardiovascular morbidity and mortality in chronic kidney disease (CKD) population (1, 2). Secondary hyperparathyroidism remains the upmost important contributor related to vascular calcification in CKD patients. However, there exists subtantial gap in present day understanding of the exact mechanism of the uremic calcification process. In previous years, vascular calcification was thought to be associated with serum calcium-phosphorus balance, but recent studies have shown that many minerals (such as citrate, magnesium) and specific proteins also play a role in the pathogenesis of vascular calcification (3). Activators and inhibitors involved in this pathogenesis regulate the process by virtue of complex mechanisms. Bone morphogenic protein-2 (BMP-2) and Alkaline Phosphatase (ALP) are considered as activators; whereas, Osteoprotegerin (OPG), Alpha-2 Heremans-Schmid Glycoprotein (Fetuin-A) and inorganic Pyrophosphate (PPi) are considered as inhibitors of this process. Furthermore, ALP, Osteopontin (OPN), Bone Sialoprotein and proteins such as type I collagen have been found to be associated with calcification in vascular smooth muscle cells (4). There exists scarce literatüre in context of vascular calcifications in renal transplant recipients. Some authors have reported a trend toward normalization of calcification inhibitors (Fetuin A, matrix Gla protein and OPG) serum levels after transplantation (5).

Vitamin D has been linked with the acceleration of the vascular calcification phenomenon and its levels has been shown to correlate well with the vascular calcification intensity (6). In addition, uremia also stimulates proinflammatory cytokine release. The cytokines such as TNF-α (tumor necrosis factor-α) lead to matrix mineralization in vascular muscle cells of

experimental animals by increasing ALP activity (6).

Previous studies regarding the vitamin D analogues have shown conflicting effects on vascular calcification and cardiovascular organization (7). Additionally, vitamin D has been proven as potent preventer of vascular calcification by inhibiting BMP-2 expression (8).

As the progression of vascular calcification in renal transplant recipients is not homogeneous and is influenced by several factors, some of which still not widely studied till date. The aim of this study was to evaluate the relationship between calcification activators and inhibitors before and 6 months after renal transplantation.

PATIENTS AND METHODS

Adult renal transplant recipients, registered at the kidney transplantation unit, at Akdeniz University Medical Faculty Organ Transplantation Center between March 2013-March 2015, were included in this single-center prospective study. The study was conducted in accordance with the ethical standards of our Institutional Ethics Committee (Approval number: 461) and with the Helsinki Declaration, and all patients provided written informed consent. Patients who had transplantation from a cadaveric donor, malignancy, combined (pancreas or liver) transplantation, graft failure related to surgical causes were excluded. The kidney graft recipients were treated according to our center protocol which include Tacrolimus, mycophenolate mofetil, basiliximab/ evorolimus/ sirolimus. Blood samples were collected before and 6 months after surgery. Estimated Glomerular filtration rates (eGFR) were calculated by "Chronic Kidney Disease Epidemiology" (CKD-EPI) Formula implementing the values of patients serum creatinine (10).

All blood samples were centrifuged and stored at -80 °C until analysis. Plasma 1,25-dihydroxyvitamin D3 levels and serum 25-hydroxyvitamin D3, calcium, phosphorus, PPi, OPG, Fetuin A, ALP (tissue non-specific alkaline phosphatase), BMP-2 levels were analyzed.

1,25-dihydroxyvitamin D3 Analysis by LC-MS/MS

1,25-dihydroxyvitamin D3 measurements were completed by using a triple quadrupole LC-MS/MS instrument. A commercial kit (1,25(OH)2D3/D2 ImmuTube LC/MS/MS Kit, Immundiagnostik AG, Catalog No. KM1000, Bensheim, Germany) was used. This assay is based on the quantitative measurement of 1,25-dihydroxyvitamin D3 after extraction steps. A LCMS-8040 triple quadrupole tandem mass spectrometer (Shimadzu Corporation, Japan) combined with ultra fast liquid chromatography (LC-20 AD UFLC XR, Shimadzu Corporation, Japan) was used by multiple reaction monitoring (MRM) method with positive electrospray ionization (ESI).

Two different solvents were used for the mobile phase, Mobil Phase A and Mobil Phase B, and 1,25(OH)2D3-d6 with isotope labeling was used as the internal standard. A multiple-reaction monitoring (MRM) transition and internal standard data were obtained for each analyte. m/z values were found to be 399.11/134.58 for 1,25(OH)2D3; 405.21/ 134.68 for 1,25(OH)2D3-d6 (internal standard). A column of Raptor™ ARC-18 (100 × 2.1 mm, 2.7 μm particle size) was used for chromatographic separation. The flow rate of the device was 0.3 mL /min, the column temperature was 45 °C and the injection volume was 50 μL, gradient program solvent B was 10% (0.01-2.49 min), 100% (2.5-3.5 min), 10% (3.51-6 min). Analysis and column cleaning time was 6 min for each sample.

25-Hydroxyvitamin D, Calcium and Phosphorus Analysis

Serum 25(OH)D levels were measured by the electrochemiluminescence immunoassay (ECLIA) method on the COBAS E-602 autoanalyzer using the Vitamin D Total commercial kit (Roche Diagnostics, Germany).

Serum calcium and phosphorus levels were measured by using colorimetric commercial

kits in the COBAS 8000 autoanalyzer (Roche Diagnostics, Germany).

PPi Analysis

Serum PPi levels were measured by fluorometer (BioTek, SynergyMx, USA) using fluorometric ABCAM Pyrophosphate Assay kit (Cat. No: ab112155).

OPG, Fetuin-A, ALP and BMP-2 Analysis

Serum OPG levels were measured using YEHUA Human Osteoprotegerin (OPG) ELISA kit (Cat. No. YHB2218 Hu). Serum fetuin-A levels were measured using QUANTIKINE ELISA Human Fetuin A Immunassay kit (Cat. No. DFTA00). Serum ALP levels were measured using YEHUA Human Alkaline Phosphatase (ALP) ELISA kit (Cat.No: YHB0138Hu). Serum BMP-2 levels were measured using YEHUA Human Bone Morphogenic Protein 2 (BMP-2) ELISA kit (Cat. No. YHB0498Hu).

Statistical analysis

All statistical analysis were done using SPSS version 20.0. Kolmogorov-Smirnov test was performed to assess deviation from normal distribution. Quantitative variables were summarized as mean and standard deviation (SD), or as median. Student t test or the Mann–Whitney's U test was used for comparison of continuous variables between groups. Differences in the levels of 1,25-dihydroxyvitamin D3, 25-hydroxyvitamin D3, calcium, phosphorus, PPi, OPG, Fetuin A, ALP and BMP-2 between the groups were examined before and after transplantation. Pearson correlation analysis was performed for the parameters fit the normal distribution, and Spearman Rank correlation analysis was performed for the parameters not fit the normal distribution. A p value of 0.05 was considered significant.

RESULTS

A total of 35 patients were registered for renal transplant during the study period. After excluding patients (n=5) who had transplantation from a cadaveric donor, malignancy, combined (pancreas or liver) transplantation, graft failure related to surgical cuses, 30 patients were included in the final study analysis. Age of patients was 40.30±12.86 years (mean±SD, age range: 19-70). There were 20 (66.7%) male and 10 (33.3%) female patients. 12 patients received hemodialysis (HD), 5 patients received peritoneal dialysis (PD) treatment before transplantation. The preemptive kidney transplantation was carried out for 13 patients.

Table 2 summarizes the serum creatinine, phosphorus, calcium, PTH, 25(OH)D, 1,25 $(OH)_2$ D_3, ALP, PPi, OPG, BMP-2, Fetuin A levels and eGFR values in patients before and after transplantation. $1,25(OH)_2D_3$ and 25(OH)D levels showed a significant increase due to the improvement of kidney function after transplantation ($p \leq 0.0001$, $p=0.0003$ respectively) (Table 2). Serum creatinine, phosphorus and ALP levels significantly decreased after transplantation, while calcium levels increased. Serum PTH levels were significantly lower after transplantation, but still above the reference ranges. We did not find any significant difference BMP-2 levels before and after transplantation. On the other hand, significant differences were observed for PPi, OPG and Fetuin A levels before and after transplantation. The percentage of recipents with vitamin D deficiency (<15 ng/mL) was 87 % before transplantation, which exhibited a downward trend to 73 % six months after transplantation. Furthermore, vitamin D insufficiency (15-30 ng/mL) was detected in 13 % before transplantation and in 27 % after 6 months.

Table 1. Demographic features of all patients.
Tablo 1. Tüm hastaların demografik özellikleri.

	All patients
Recipient age (year)	40.30±12.85
Donor age (year)	41.43±12.82
Gender (F/M) (%)	10/20 (66.7/33.3)
BMI (kg/m^2)	23.74± 4.76
Type of Renal Replacement Therapy	n(%)
PD (%)	5 (17)
HD (%)	12 (40)
Preemptive (%)	13 (43)
The Etiologies of End Stage Renal Disease	n(%)
Glomerulonephritis (%)	5 (16.67)
Diabetes Mellitus (DM) (%)	4 (13.33)
Hypertension (%)	3 (10)
Vesico Urethral Reflux (%)	3 (10)
Polycystic Kidney Disease (%)	3 (10)
Etiology unknown (%)	9 (30)
Other (%)	3 (10)
Total (%)	**30 (100)**

Results are expressed as numbers and percentages.
PD, peritoneal dialysis; HD, hemodialysis.

Correlation analyzes were performed in order to evaluate the relation between parameters before and after transplantation. All significant correlations are depicted in Table 3.

DISCUSSION

Vascular calcifications have been revealed to be strongly associated with cardiovascular events in patients with renal transplantation. The mechanisms underlying vascular calcifications are multifactorial and incompletely understood. The aim of our study was to reveal possible interactions of vascular calcification activators and inhibitors in the early post-transplantation period. Furthermore, the relationship between vitamin D status and vascular calcification activators (BMP-2, ALP) and inhibitors (PPI, OPG and Fetuin-A) were investigated.

Vitamin D deficiency is associated with vascular calcification in patients with CKD. Wang et al. found that the frequency of vascular calcification was significantly higher in hemodialysis patients with low 25-hydroxyvitamin D levels in comparison to patients with normal levels. They also reported that 25-hydroxyvitamin D levels were negatively correlated with the calcification score (11). A long-term administration of vitamin D to patients with CKD was found to induce vascular calcification. On the other hand, there is increasing evidence suggesting that calcitriol increases vitamin D receptor (VDR) expression in vascular smooth muscle cells. VDR also modulates cell proliferation and differentiation. Calcitriol has been also shown to induce of osteopontin (OPN) expression, which is a local inhibitor of vascular calcification, in vascular smooth muscle cells. It was also reported that calcitriol may lead to calcification by increasing the RANKL / OPG ratio (6).

In our study, both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D3 levels were increased significantly after transplantation in line with previous studies (12). It can be expected that improvement in renal function after transplantation is associated with this increment.

In a study by Rennenberg et al. OPG elevation was associated with coronary artery calcification progression 1 year after transplantation (4). However it is still uncertainty whether OPG is a marker of atherosclerosis or an active component of atherosclerotic process. Most studies have shown that cardiovascular events are inversely related to serum RANKL and positively related to serum OPG (13). Serum OPG levels decreased after transplantation in

our study (p=0.007). These data could support that high OPG levels may be a predictor for cardiovascular events in CKD.

Dalfino et al. reported that BMP-2 is negatively associated with renal function in a study conducted in CKD patients and also they found a moderately positive correlation between BMP-2 and vascular stiffness in CKD patients (14). In our study, glomerular filtration rate improved by renal transplantation did not have a significant effect on BMP-2 (Table 2). On the other hand, a strong positive correlation between BMP-2 and OPG was found in our study which may suggest that BMP-2 could be a component of the calcification process in both periods of transplantation (Table 3). Further investigations with larger sample size are required to elucidate the role of serum BMP-2 levels in renal transplant patients.

Table 2. Comparison of the parameters of renal transplant recipients measured before and 6 months after transplantation.
Tablo 2. Renal transplant alıcılarının nakil öncesi ve nakil sonrası 6. ayda ölçülen parametrelerinin karşılaştırılması.

Parameters	Pre-transplant	Post-transplant	*p*
$1,25(OH)_2D_3$ (pg/mL)	43.70±14.15	68.48±18.35	**≤0.0001**
25(OH)D (ng/mL)	5.80±4.28	10.96±5.50	**0.0003**
Ca (mg/dL)	8.38±0.87	9.08±0.50	**≤0.0001**
P (mg/dL)	4.78±1.10	2.90±0.62	**≤0.0001**
CaxP	39.89±9.88	26.38±6.26	**≤0.0001**
PTH (pg/mL)	424.04±399.30	116.59±82.30	**≤0.0001**
Creatinine (mg/dL)	8.94±2.85	1.21±0.29	**≤0.0001**
eGFR (mL/min)	6.80±2.55	73.17±20.10	**≤0.0001**
ALP (U/L)	112.63±40.45	90.77±23.42	**0.011**
PPi (μM)	1.27±0.50	1.79±1.23	**0.03**
OPG (ng/mL)	3.10±0.85	2.30±1.31	**0.007**
Fetuin A (ng/mL)	792.80±119.55	897.69±155.05	**0.006**
BMP-2 (ng/mL)	3.158±0.76	3.239±0.61	0.333

p<0.05 was accepted as significant and marked as bold.
Data are given as mean±standard deviation.

Table 3. Significant correlations of parameters measured before and after renal transplantation.
Tablo 3. Renal transplantasyon öncesi ve sonrasında ölçülen parametrelerin anlamlı korelasyonları.

PRE-TRANSPLANT CORRELATIONS (r)								
	$1,25(OH)_2D_3$	**25(OH)D**	**Fetuin-A**	**PPi**	**OPG**	**BMP-2**	**ALP**	**Creatinine**
$1,25(OH)_2D_3$		0.317	-0.422					0.509
25(OH)D	0.317			0.390	0.317	0.397	-0.339	0.286
Fetuin-A	-0.422			-0.478	-0.500	-0.444	0.485	-0.721
PPi		0.390	-0.478		0.715	0.716	-0.781	0.701
OPG		0.317	-0.500	0.715		0.827	-0.853	0.516
BMP-2		0.397	-0.444	0.716	0.827		-0.837	0.500
ALP		-0.339	0.485	-0.781	-0.853	-0.837		0.643
Creatinine	0.509	0.286	-0.721	0.701	0.516	0.500	-0.643	
POST-TRANSPLANT CORRELATIONS (r)								
	$1,25(OH)_2D_3$	**25(OH)D**	**Fetuin-A**	**PPi**	**OPG**	**BMP-2**	**ALP**	**Creatinine**
$1,25(OH)_2D_3$								0.755
25(OH)D				0.603	0.513	0.465	-0.626	0.306
Fetuin-A				-0.395	-0.397	-0.335	0.548	-0.819
PPi		0.603	-0.395		0.721	0.667	-0.799	0.721
OPG		0.513	-0.397	0.721		0.865	-0.781	0.696
BMP-2		0.465	-0.335	0.667	0.865		-0.714	0.639
ALP		-0.626	0.548	-0.799	-0.781	-0.714		-0.813
Creatinine	0.755	0.306	-0.819	0.721	0.696	0.639	-0.813	

p<0.05 was accepted as significant.

Additionally, serum Fetuin-A reacts as a negative acute phase glycoprotein is down-regulated acute and chronic inflammatory states. In CKD patients, serum Fetuin-A levels are significantly lower than in controls and are associated with increase vascular calcification and inflammation and consequently high cardiovascular mortality (15). Few data are avaliable regarding effect of Fetuin-A in renal transplant recipients. Study of Mehrsai et al. showed that Fetuin-A levels increased after transplantation (16). Lorenzen et al. found that serum Fetuin A levels exhibited specific time-dependent changes after transplantation, but no significant change in patients with graft calcification (17). It can be expected that improvement in renal function after kidney transplantation is associated with increased serum levels of Fetuin A. Therefore we hypothesized that serum fetuin A concentrations increase with time after renal transplantation and our results are in line with this hypothesis ($p=0.006$). In addition, there was strong inverse correlation with fetuin and creatinine in both periods of transplantation (Table 3).

A comparios of circulating PPi levels and vascular calcification in CKD patients revealed that plasma PPi is negatively associated with vascular calcification in CKD (18). In our study, plasma PPi levels are increased after transplantation ($p=0.03$). However, there was a significant correlation between plasma PPi and serum BMP-2 and OPG levels in both periods of transplantation (Table 3). Considering that uremic vascular calcification has multifactorial etiology, it may be thought that the effect of increase in plasma PPi levels may be a defense mechanism against increased risk of calcification.

ALP is considered a sign of bone metabolism in individuals with CKD. Tissue non-specific ALP inactivates PPi, the endogenous inhibitor of hydroxy apatite formation that causes medial calcification. In our study, there was a strong negative correlation between ALP and PPI in both periods of transplantation. these findings also support that ALP inhibits PPi. In our study, serum ALP levels were significantly lower after transplantation, whereas PPi levels after transplantation showed a significant increase. which may indicate that the risk of calcification is reduced.

Thus to conclude, our results revealed that in renal transplant recipients, vitamin D deficiency continues independently of renal function. Despite the low levels of vitamin D, its active form was increased with the improvement of renal function. According to this study, it may be possible to think that PPi, Fetuin A and vitamin D as inhibitors; OPG and ALP as activators in the calcification process. The change in these parameters, which is seen with improvement of the graft function, may suggest that decreases the risk of calcification after transplantation.

Addition to these results, it observed that serum/plasma vitamin D, PPi and Fetuin-A levels increase, OPG and ALP levels decrease with the improvement of graft function. The increase in serum levels of OPG and ALP, which are thought to play a role as activators in the calcification process in CKD patients, may be beneficial in early detection of calcification.

In summary, we observed that in renal transplant recipients, the important markers of vascular calcification risk were significantly associated with the improvement of graft function except BMP-2. The present study has several limitations; for example, relatively small sample size may be insufficient to allow exploration of the potential relationships between parameters and vascular calcification risk. Further larger studies are required to evaluate time-dependent changes after renal transplantation.

CONCLUSION

Detection of vascular calcification is complex and asymptomatic in early stages of CKD. Therefore, selected biomarkers would be valuable and clinically important in non-invasive monitoring of early signs of vascular calcification owing to the significant differences observed in this study. Larger scale studies in this context are required to

support our findings and improve CKD outcomes.

Disclosure Statement

Coauthors report that they have no conflicts of interest.

Acknowledgements

This study is supported by Turkish Scientific and Technological Research Center (TUBITAK), project no. 2014S608.

REFERENCES

1. Derici U, Nahas M. Vascular Calcifications in Uremia: Old Concepts and New Insights. Seminars in Dialysis 2006; 19(1):60-68.
2. Ahmed S, Jafri L, Khan AH. Evaluation of CKD-EPI Pakistan'equation for estimated glomerular filtration rate (eGFR): a comparison of eGFR prediction equations in Pakistani population. Journal of the College of Physicians and Surgeons Pakistan 2017;27(7):414.
3. Kakani E, Elyamny M, Ayach T, El-Husseini A. Pathogenesis and management of vascular calcification in CKD and dialysis patients. Seminars in Dialysis 2019; 00:1–9.
4. Rennenberg RJ, Schurgers LJ, Kroon AA, Stehouwer CD. Arterial calcifications. J Cell Mol Med 2010; 14:2203-10.
5. Viegas C, Araújo N, Marreiros C, Simes D. The interplay between mineral metabolism, vascular calcification and inflammation in Chronic Kidney Disease (CKD): challenging old concepts with new facts. Aging (Albany NY) 2019; 11(12):4274-4299.
6. Mizobuchi M, Towler D, Slatopolsky E. Vascular Calcification: The Killer of Patients with Chronic Kidney Disease. J Am Soc Nephrol 2009; 20:1453–1464.
7. Drüeke T, Massy Z. Role of Vitamin D in Vascular Calcification: Bad or Good Guy? Nephrol Dial Transplant 2012; 27:1704-1707.
8. Hou Y, Liu W, Zheng C, Zheng J, Yen T, Lu K. Role of Vitamin D in Uremic Vascular Calcification. BioMed Research International 2017; Special Issue, Article ID 2803579.
9. Chau K, Martinez G, Elder G. Vascular Calcification in Patients Undergoing Kidney and Simultaneous Pancreas-Kidney Transplantation. Nephrology 2014; 19:275-281.
10. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease 2013;(3):1.
11. Wang F, Wu S, Ruan Y, Wang L. Correlation of serum 25-hydroxyvitamin D level with vascular calcification in hemodialysis patients. Int J Clin Exp Med 2015; 8(9): 15745-15751.
12. Karatoy Erdem B, Yılmaz VT, Suleymanlar G, Ozcan F, Baykal A, Akbas H. The relationship between vitamin D status and graft function in renal transplant recipients. Int J Med Biochem 2018;1 (1):1-5.
13. Melaku L, Mossie A. Molecular mediators and controlling mechanism of vascular calcification. International Journal of Clinical and Experimental Physiology 2017; 4(1): 3-14.
14. Dalfino G, Simone S, Porreca S, Cosola C, Balestra C, Manno C, et al. Bone morphogenetic protein-2 may represent the molecular link between oxidative stress and vascular stiffness in cronic kidney disease. Atherosclerosis 2010; 211:418-423.
15. Mann A, Makkar V, Mann S, Dhamija P, Soundarajan P. Fetuin-A and vascular calcification in Indian end-stage renal disease population. Indian J Nephrol 2016; 26(1):33-8.
16. Mehrsai A, Pourmand G, Tehrani H, Keyhan H, Rahmati RM, Ahmadi A, et al. Association of Serum Fetuin-A Levels with Allograft Outcome in Renal Transplant Recipients. Urol J 2015; 12(3):2182-2186.
17. Lorenzen JM, Martino F, Scheffner I, Bröcker V, Leitolf H, Haller H, et al. Fetuin, Matrix-Gla Protein and Osteopontin in Calcification of Renal Allografts. Plos One 2012; 7(12):e52039.
18. Azpiazu D, Gonzalo S, González-Parra E, Egido J, Villa-Bellosta R. Role of pyrophosphate in vascular calcification in chronic kidney disease. Nefrologia 2018; 38(3):250-257.