Association of Serum Fetuin-A Levels with Allograft Outcome in Renal Transplant Recipients

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Purpose: To determine serum fetuin-A pattern after renal transplantation and its association with graft outcome.

Materials and Methods: In 41 renal transplant recipients, serum pretransplant fetuin-A levels and serum fetuin-A concentrations on days 7 and 30 after transplantation were measured using the enzyme-linked immunosorbent assay (ELISA) method. Also, the association between serum fetuin-A levels with clinical and laboratory parameters was evaluated.

Results: A significant decrease in serum fetuin-A levels was noted in the first week after transplantation ($P < .001$). Subsequently, it started to increase and surpass pretransplant values during the first month ($P < .001$). Pretransplant fetuin-A levels did not differ among patients with different diethylenetriamine pentaacetic acid (DTPA) results. In addition, serum fetuin-A levels did not significantly correlate with metabolic parameters.

Conclusion: In this prospective study there was no increase in serum fetuin-A levels during the first month and pretransplant fetuin-A levels are not predictive for allograft outcome in renal transplant recipients.

Keywords: kidney transplantation; postoperative complications; kidney failure; kidney function tests; prospective studies.

INTRODUCTION

Kidney transplantation is an important and effective treatment for most patients with end-stage renal disease, which confers a survival benefit compared to hemodialysis.[1] Improved technical approaches, new advances in immunosuppression, antibody definition and improvements in the overall care of the transplant recipient during the past decades, have significantly increased the short-term kidney allograft survival rate.[2,3] In spite of these advances, long-term allograft survival after the first year has not improved, and the length of kidney allograft survival is still shorter than that of the recipient's even with the use of immunosuppressive drugs in sufficient doses to prevent acute rejection.[2,3] Reasons for allograft failure are not well understood.[2] Renal allograft failure occurs by both immune and nonimmune mechanisms; for example, graft rejection, graft thrombosis, interstitial fibrosis and tubular atrophy (IF/TA), infection, chronic inflammation, nephrotoxicity from calcineurin inhibitors and calcification of the allografts.[2] Therefore, access to innovative risk-identifying strategies in order to increase the long-term allograft survival can be considered as the best target for studies.[4] In patients with end-stage renal disease (ESRD) and chronic kidney disease (CKD), the risk of subsequent cardiovascular events is higher than the age- and sex-matched populations with normal kidney function.[5] Recent observations have shown rapid progression of cardiovascular calcification in patients with CKD and ESRD.[5]

Cardiovascular calcification is a tightly regulated process affected by multiple mechanisms and also several serum proteins.[6] Although calcium and phosphate precipitation occur in several tissues due to multiple risk factors such as age or inflammation reactions, but some serum precipitation inhibitory proteins would not allow this process to proceed. A number of calcification inhibitors such as fetuin-A, have been identified in recent years.[5,6] Fetuin-A is a vertebrate plasma protein, belongs to the cystatin superfamily. Cystatins can inhibit cysteine peptidases and play key roles in inflammation.[7] Fetuin-A (molecular weight of about 60 kDa), also known as α-2 Heremans-Schmid glycoprotein (AHSG), is synthesized by hepatocytes and ubiquitously present in the serum.[8] Fetuin-A serum concentrations are relatively high (0.5 and 1.0 g/L) in the healthy population.[9] Fetuin-A has been recognized as a major serum-based inhibitor of vascular and soft-tissue calcification, limiting hydroxyapatite crystal formation and stabilizing calcium–phosphate in a complex, which enables its clearing by the phagocytic system.[9-11] In animal studies, fetuin A-deficient mice developed calcifications in soft-tissue and vessels, accelerated by a mineral-rich diet, as well as renal failure.[12] Fetuin-A is a multifunctional molecule with several biologic functions such as inhibition of calcification-inducing effects of transforming growth factor-β, inhibition

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of insulin receptor autophosphorylation, tyrosine kinase activity and protease.[8] Serum fetuin-A reacts as a negative acute phase glycoprotein and is down regulated in acute and chronic inflammatory states.[13] Some studies permit consideration of fetuin-A deficiency as a new mortality risk factor in patients with chronic kidney disease (CKD).[4,9] In hemodialysis patients, serum levels of fetuin-A is significantly lower than controls.[14] In addition, lower fetuin-A is associated with increased vascular calcification and inflammation and consequently high cardiovascular mortality.[9,14] Few data was available regarding effect of fetuin-A in renal transplant recipients. One study showed that fetuin-A levels increased after renal transplantation.[15] In another study, no association was found between fetuin-A levels and allograft rejection.[9] However, due to the reduction of serum fetuin-A levels during inflammation[16] and its inhibitory role in calcification and fibrosis,[8,17] fetuin-A serum levels may be considered as a predictor of allograft outcome and rejection episodes.[4] We assumed that serum fetuin-A levels would be related to the kidney function. Therefore, the aim of this study was to determine serum fetuin-A pattern after renal transplantation and to examine whether fetuin-A is associated with graft outcome.

MATERIALS AND METHODS

Study Subjects

In this single-center prospective study, 65 patients (48 males) who had undergone kidney transplantation were included. Of these patients, 41 were included in the final analysis and 24 were excluded due to incomplete data. Transplantations were performed in Sina Hospital, Iran, from October 2012 to May 2013. There were 30 (73%) deceased donor and 11 (27%) living donor. The following groups of recipients were excluded from the study: recipients of combined organs such as kidney/pancreas, children and patients with hepatitis B or C. The causes of renal failure were diabetic nephropathy (n = 7), hypertensive ischemic nephropathy (n = 16), reflux nephropathy (n = 10), nephrotic syndrome (n = 3), chronic nephrolithiasis (n = 3) and unknown etiology (n = 2). An informed consent was obtained from all patients prior to their inclusion in the study. This study was approved by the Medical Ethics Committee of the Tehran University of Medical Sciences. Patients' immunosuppressive protocols were based on calcineurin inhibitor (cyclosporine A, 6-7 mg/kg/day) along with mycophenolate mofetil hydrochloride (1200 mg/m^2). Additionally, all patients received prednisone prior to transplantation (methylprednisolone, 10 mg/kg prior to transplantation rapidly converting into prednisolone, tapering the dose over 12 weeks to the dose of 0.3 mg/kg); also, anti-thymocyte globulin (ATG rabbit, 1-1.5 mg/kg) was given to 5 patients with possible renal allograft rejection. Renal function was evaluated by measuring urine volume and serum creatinine before transplantation and on days 7 and 30 after transplantation (creatinine clearance was calculated using the Cockcroft/Gault formula). In order to evaluate renal perfusion and function, technetium-99 diethylenetriamine pentaacetic acid (99mTc-DTPA) scan was performed along with Doppler ultrasonography at the end of the first week after renal transplantation. Routine laboratory parameters were measured by standard clinical chemistry methods.

Serum Fetuin-A

Five milliliters of venous blood were withdrawn from every patient. Serum was collected after centrifugation at 2500 g for 20 min and stored at −80°C for subsequent analyses. Serum fetuin-A levels were measured using the enzyme-linked immunosorbent assay (ELISA, BioVendor Laboratory Medicine, Brno, Czech Republic) method according to manufacturer's instructions. The intra- and interassay coefficients of variation were both < 5.0%.

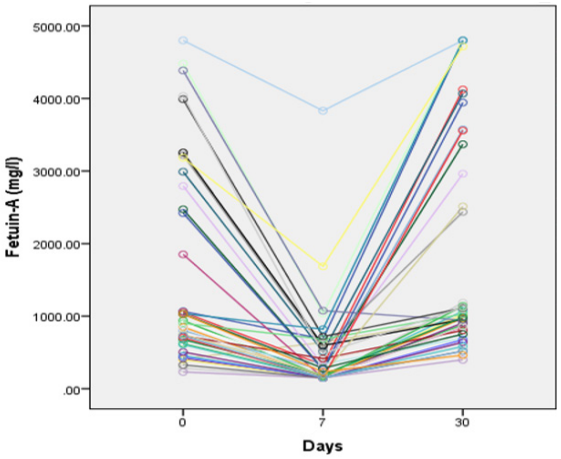

Figure 1. Individual course of fetuin-A levels from pretransplantation up to day 30 after kidney transplantation.

Statistical Analysis

Categorical variables are expressed as frequencies and percentages and continuous variables are reported as mean ± standard deviation (SD). Subgroups of DTPA were compared on serum fetuin-A at 7th and 30th days after transplantation using repeated measure analysis

Table. Demographic and clinical characteristics of study subjects.

Parameters	Values
Age, years	41.27 ± 12.6
Sex, male %	73.17
Body Mass Index, kg/m^2	27.48 ± 2
Smoking, %	51.2
Serum albumin, g/L	4.16 ± 0.69
Serum calcium, mg/dL	9.12 ± 0.79
Serum potassium, mmol/L	4.47 ± 0.53
Systolic blood pressure, mmHg	127.8 ± 21
Diastolic blood pressure, mmHg	79.76 ± 13.69
Serum fetuin levels, mg/L	
Before transplantation	1543 ± 1372.09
Day 7	465.99 ± 630.34
Day 30	1878.38 ± 1556.11
Serum creatinine levels, mg/dL	
Before transplantation	5.49 ± 2.06
Day 7	2.38 ± 1.5
Day 30	1.68 ± 0.8

All continuous data are expressed as mean ± SD.

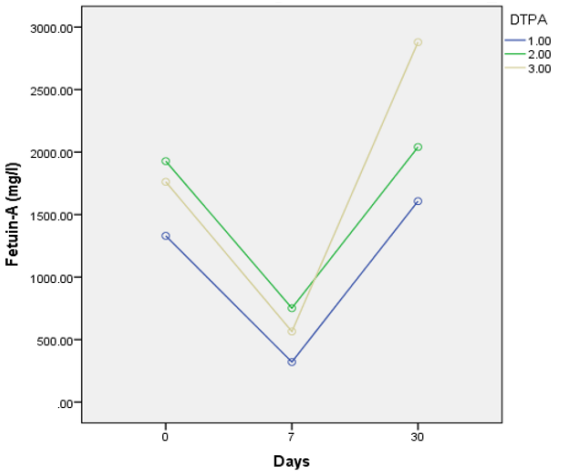

Figure 2. Changes in serum fetuin-A levels from pretransplantation up to day 30 after transplantation by tertile of diethylenetriamine pentaacetic acid (DTPA) groups.

of variance. To assess relationship between serum fetuin-A with age and serum creatinine levels the Pearson correlation coefficient was used at different time points.

RESULTS

Clinical and demographic characteristics of the patients are shown in **Table**. Based on DTPA scan results at the end of the first week after transplantation, the patients were divided into three subgroups. The first subgroup included 25 patients with fairly proper renal perfusion and function (DTPA-1). The second subgroup comprised of 11 patients with proper renal perfusion but decreased renal function and excretion (DTPA-2), and the third subgroup is composed of 5 patients who had decreased renal perfusion, function and excretion (DTPA-3). In the first week after transplantation, there was a significant decrease in serum fetuin-A levels ($P < .001$), it then started to increase and surpass pretransplant values on day 30 ($P < .001$) (**Figure 1**). Furthermore, pretransplant serum fetuin-A concentrations were correlated with serum fetuin-A levels on days 7 ($r = 0.638$, $P < .001$) and 30 ($r = 0.438$, $P < .004$). We evaluated serum levels of fetuin-A over time by tertile of DTPA subgroups (**Figure 2**). Subsequently, we found that changes in serum fetuin-A levels did not differ in patients with different DTPAs ($P = .541$). In order to investigate whether pretransplant levels of serum fetuin-A can be a predictor of renal transplant outcome, the mean changes of pretransplant levels of serum fetuin-A in DTPA subgroups were statistically analyzed and there was no statistically significant difference ($P = .461$). Also, we performed a correlation analysis in order to evaluate the correlation between serum fetuin-A levels and creatinine levels. There was no significant correlation between serum fetuin-A levels and creatinine. Finally, we assessed the role of age in fetuin-A levels and no relationship was observed (**Figure 3**). Moreover, no association was found between serum fetuin-A levels and gender ($P = .337$) and type of donors ($P = .987$).

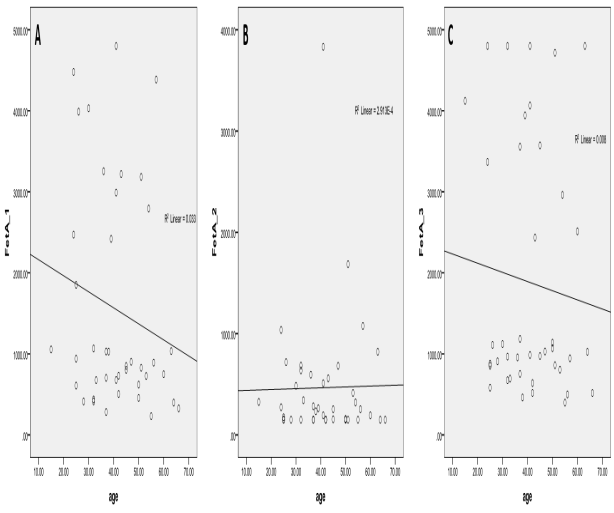

Figure 3. Relationship between serum fetuin-A levels and age. **A**) Before renal transplantation; **B**) one week after renal transplantation and **C**) one month after renal transplantation.

DISCUSSION

Renal allograft failure is the result of cumulative damage caused by various stressors and factors[18] such as IF/TA, chronic inflammation and calcification processes.[2] Fetuin-A is one of the most potent calcification inhibitor.[19] Since, soft tissue calcifications occurs in fetuin-A knockout mice,[12] and serum fetuin-A levels are reduced in uremic conditions, fetuin-A can be considered as an inhibitor of vascular calcification in healthy people as well as uremic patients.[20] Mori and colleagues showed that serum fetuin-A levels are reduced in patients with ESRD.[21] Similarly, it has been shown that low serum fetuin-A levels are independently associated with higher overall cardiovascular mortality in patients with renal failure.[22] One of the reasons indicating an association between low serum fetuin-A levels and increased mortality can be due to down regulation of fetuin-A in inflammation.[23] Previous studies have shown that renal transplantation leads to improved vascular function and increased serum levels of fetuin-A.[24] Hence, it can be expected that improvement in renal function is associated with increased serum levels of fetuin-A after kidney transplantation.[9] Therefore we hypothesized that serum fetuin-A concentrations increase by the time after renal transplantation. However, the results of our study did not demonstrate an increase of serum fetuin-A levels within the first post-transplant month. Moreover, in the first week after transplantation, we observed a significant decrease in serum fetuin-A levels, although it started to increase and surpass pretransplant values on day 30. Thus, there is an obvious disagreement between study results and study hypothesis. In line with our results, Urbanova and colleagues in a study on 30 deceased donor kidney recipients showed that serum fetuin-A concentrations decreased 2 weeks after kidney transplantation.[9] Similarly, Argani and colleagues observed lower serum fetuin-A concentrations in renal transplant (RT) patients than hemodialysis (HD) patients.[8] In contrast, Caglar and colleagues reported an increase in serum fetuin-A concentrations on days 30 and 90 after transplantation.[15] Moe and colleagues studied calcification inhibitors and reported an increase in serum fetuin-A levels after kidney transplantation in 11 patients with a well-functioning allograft.[25] Since serum fetuin-A is involved in inflammatory processes, and inflammation is asso-

ciated with allograft outcome in renal transplant recipients,[26,27] we expected that serum fetuin-A pretransplant serum levels are associated with subgroups of DTPA. However, we found no significant correlation between pretransplant serum fetuin-A levels and DTPA results. This might be due to the few number of subgroups. One of the most important factors for explaining the differences observed in serum fetuin-A behaviors may be systemic inflammation in the early post-transplant period.[28] Severe systemic inflammation may be caused by infection, surgical trauma and immune response against the graft in the early period after transplantation.[9] Previous studies have shown that the down regulation of fetuin-A in inflammation or trauma.[13,28] In addition, serum fetuin-A reacts as a negative acute phase glycoprotein and has a negative association with concentration of the tumor necrosis factor α, C-reactive protein (CRP) and inflammatory cytokines interleukin (IL)-1 β , IL-6,[4,28] supporting the hypothesis of inflammation-dependent down regulation of fetuin-A expression.[4] Hence, one possible explanation for our findings could be the difference in age recipients, which leads to a difference in the intensity of postoperative inflammation and stress. The mean age of patients in our study and in Urbanova and colleagues' study[9] was higher than Caglar and colleagues' study[15] (41.3, 50.2 and 25.2, respectively). On the other hand, it has been recently shown that serum fetuin-A levels may decrease after a single hemodialysis session, that supports the hypothesis of dialysis-induced inflammation. Therefore, serum fetuin-A levels can be affected by possible dialysis sessions.[29] Another reason to explain the observed differences may be due to pharmacological interventions (for example, treatment with calcium-phosphate medication) that affect the fetuin-A levels.[30] Coglar and colleagues showed that short-term sevelamer (phosphate binder) treatment significantly increases serum fetuin-A levels.[30] In this regard, we cannot ignore the possible impact of vitamin D preparations and the severity of glomerular dysfunction on the serum fetuin-A levels.

CONCLUSION

Our study demonstrated that fetuin-A levels decrease early after transplantation (day 7) and then start to increase in the first month. Fetuin-A down regulation early after transplantation may be caused by inflammation and trauma after surgery. However, further studies are needed to better clarify fetuin-A patterns after renal transplantation. In addition, we could not find any evidence to confirm the association between fetuin-A pretransplant serum levels and allograft outcome. Further investigations with larger sample sizes are required to elucidate the association between pretransplant serum fetuin-A levels and allograft outcome.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Veroux M, Corona D, Veroux P. Kidney transplantation: future challenges. Minerva Chirurgica. 2009;64:75-100.
2. El-Zoghby ZM, Stegall MD, Lager DJ, et al. Identifying specific causes of kidney allograft loss. Am J Transplant. 2009;9:527-35.
3. Matas AJ, Humar A, Gillingham KJ, et al. Five preventable causes of kidney graft loss in the 1990s: a single-center analysis. Kidney Int. 2002;62:704-14.
4. Roos M, Heinemann FM, Lindemann M, et al. Fetuin-A pretransplant serum levels, kidney allograft function and rejection episodes: a 3-year posttransplantation follow-up. Kidney Blood Press Res. 2011;34:328-33.
5. Ix JH, Chertow GM, Shlipak MG, Brandenburg VM, Ketteler M, Whooley MA. Fetuin-A and kidney function in persons with coronary artery disease: data from the Heart and Soul Study. Nephrol Dial Transplant. 2006;21:2144-251.
6. Ketteler M, Westenfeld R, Schlieper G, Brandenburg V, Floege J. "Missing" inhibitors of calcification: general aspects and implications in renal failure. Pediatr Nephrol. 2005;20:383-8.
7. Jahnen-Dechent W, Heiss A, Schafer C, Ketteler M. Fetuin-A regulation of calcified matrix metabolism. Circ Res. 2011;108:1494-509.
8. Argani H, Ghorbanihaghjo A, Panahi G, Rashtchizadeh N, Safa J, Meimand SM. Serum fetuin-A and Pentraxin3 in hemodialysis and renal transplant patients. Clin Biochem. 2012;45:775-9.
9. Urbanova M, Kalousova M, Zima T, Skibova J, Wohlfahrt P, Viklicky O. Fetuin-A early after renal transplantation. Kidney Blood Press Res. 2009;32:217-22.
10. Turkmen K, Gorgulu N, Uysal M, et al. Fetuin-A, inflammation, and coronary artery calcification in hemodialysis patients. Indian J Nephrol. 2011;21:90-4.
11. Hausler M, Schafer C, Osterwinter C, Jahnen-Dechent W. The physiologic development of fetuin-a serum concentrations in children. Pediatr Res. 2009;66:660-4.
12. Jahnen-Dechent W, Schinke T, Trindl A, et al. Cloning and targeted deletion of the mouse fetuin gene. J Biol Chem. 1997;272:31496-503.
13. Hermans MM, Brandenburg V, Ketteler M, Kooman JP, van der Sande FM, Boeschoten EW. Association of serum fetuin-A levels with mortality in dialysis patients. Kidney Int. 2007;72:202-7.
14. Ketteler M, Bongartz P, Westenfeld R, et al. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet. 2003;361:827-33.

15. Caglar K, Yilmaz MI, Saglam M, et al. Endothelial dysfunction and fetuin A levels before and after kidney transplantation. Transplantation. 2007;14:392-7.
16. Marechal C, Schlieper G, Nguyen P, et al. Serum fetuin-A levels are associated with vascular calcifications and predict cardiovascular events in renal transplant recipients. Clin J Am Soc Nephrol. 2011;6:974-85.
17. Verma-Gandhu M, Peterson MR, Peterson TC. Effect of fetuin, a TGF beta antagonist and pentoxifylline, a cytokine antagonist on hepatic stellate cell function and fibrotic parameters in fibrosis. Eur J Pharmacol. 2007;572:220-7.
18. Nankivell BJ, Chapmann BJ. Chronic allograft nephropathy: current concepts and future directions. Transplantation. 2006;81:643-54.
19. Jung JY, Hwang YH, Lee SW, et al. Factors associated with aortic stiffness and its change over time in peritoneal dialysis patients. Nephrol Dial Transplant. 2010;25:4041-8.
20. Cozzolino M, Mazzaferro S, Pugliese F, Brancaccio D. Vascular calcification and uremia: what do we know? Am J Nephrol. 2008;28:339-46.
21. Mori K, Ikari Y, Jono S, et al. Fetuin-A is associated with calcified coronary artery disease. Coron Artery Dis. 2010;21:281-5.
22. Moe SM, Chen NX. Inflammation and vascular calcification (review). Blood Purif. 2005;23:64-71.
23. Wang AY, Woo J, Lam CW, et al. Associations of serum fetuin-A with malnutrition, inflammation, atherosclerosis and valvular calcification syndrome and outcome in peritoneal dialysis patients. Nephrol Dial Transplant. 2005;20:1676-85.
24. Van Summeren MJ, Hameleers JM, Schurgers LJ, et al: Circulating calcification inhibitors and vascular properties in children after renal transplantation. Pediatr Nephrol. 2008;23:985-93.
25. Moe SM, Reslerova M, Ketteler M, et al. Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). Kidney Int. 2005;67:2295-304.
26. Goldfarb-Rumyantzev AS, Naiman N. Genetic predictors of acute renal transplant rejection. Nephrol Dial Transplant. 2010;25:1039-47.
27. Ombrellino M, Wang H, Yang H, et al. Fetuin, a negative acute phase protein, attenuates TNF synthesis and the innate inflammatory response to carrageenan. Shock. 2001;15:181-5.
28. Lebreton JP, Joisel F, Raoult JP, Lannuzel B, Rogez JP, Humbert G. Serum concentration of human α2 - HS glycoprotein during inflammatory process. Evidence that α2 -HS glycoprotein is a negative acute-phase reactant. J Clin Invest. 1979;64:1118-29.
29. Ciaccio M, Bivona G, Di Sciacca R, et al. Changes in serum fetuin-A and inflammatory markers levels in end-stage renal disease (ESRD): effect of a single session haemodialysis. Clin Chem Lab Med. 2008;46:212-4.
30. Caglar K, Yilmaz MI, Saglam M, et al. Short-term treatment with sevelamer increases serum fetuin-a concentration and improves endothelial dysfunction in chronic kidney disease stage 4 patients. Clin J Am Soc Nephrol. 2008;3:61-8.