ORIGINAL ARTICLE

Is Serum Transforming Growth Factor beta-1 Superior to Serum Creatinine for assessing Renal Failure and Renal Transplant Rejection

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Abstract

A sustained overexpression of Transforming Growth Factor beta1 (TGF beta1), a cytokine has been implicated in the pathogenesis of fibrosis of kidney leading to end stage . The main aim of present study was to find the utility of TGF beta1 and serum creatinine in differentiating chronic renal failure (CRF) from acute renal failure (ARF), renal transplant rejection (Tx Rej) and stable renal transplant (Tx Stb) and to study has attempted histopathological correlation of rejection cases with TGF beta1 and serum creatinine. TGF beta1 was determined by using ELISA and serum creatinine was done by autoanalyser. In normal healthy controls (NHC), in majority of cases (80.0%) TGF beta1 was below 25 ng/ml while in 6.0% cases it was upto 34 ng/ml. Rise of TGF beta1 was significant in CRF patients as compared to ARF and NHC ($p<0.05$) .In rejection cases, TGF beta1 level was significantly raised as compared to NHC and stable graft cases ($p<0.05$). In rejection cases, it was raised above 40 ng/ml in only 50% cases. In two cases inspite of more than 70% glomerular fibrosis, the patient had TGF beta1 level of only 5 ng/ml and in other three cases of acute cellular rejection the level was 70, 35 and 28 ng/ml respectively.Contrary to it serum creatinine was raised above 2 mg/dl in all cases of transplant rejection but in stable transplant cases in majority (70.6%) it was below 1.5 mg/dl and in 5 cases it was between 1.5 – 1.9 mg/dl.Thus the study suggests that TGF beta1 may not be a good marker for chronic transplant rejection, as it does not correlate well with glomerular fibrosis, probably it is more associated with interstitial inflammation but it can differentiate CRF from ARF if cut off of 40 ng/ml is taken.

Key Words

Transplant Rejection, Renal Failure, Transforming Growth Factor beta-1, Cytokine

Introduction

Transforming growth factor beta is a signalling molecule with multiple actions. It is produced by variety of immune and nonimmune cells - monocytes, lymphocytes, renal tubular cells, vascular endothelium and respiratory system epithelium (1). The structure of transforming growth factor beta1 (TGF beta1) was the first to be recognized among its different isoforms (2). TGF beta1 belongs to a family of dimeric 25 kDa polypeptide synthesized by many different cells (3). Gene of transforming growth factor beta (TGF beta) is located on chromosome 19 (19q13.2 , 13.1). The three isoforms of TGF beta (TGF beta1, TGF beta2, TGF beta3) plays critical roles in growth regulation , and development of cells . Each isoform is encoded by a unique gene on different chromosome. TGF beta is released as an inactive precursor, in a complex bound with a latency - associated peptide (LAP) (4) and requires activation for exerting biological response.There are three known classes of

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TGF beta receptors (TGF R) , but only two of these, TGF RI and TGF RII are involved in signal pathways (5). Fibroblasts are stimulated by TGF beta to produce matrix proteins such as collagen and fibronectin (6). The TGF -betas are involved in wound repair processes by reducing inflammatory reactions and then in healing of fibrosis (7). It induces fibrosis by inhibiting collagenase and metalloproteinase. It inhibits T and B cell proliferation as well as to maturation and activation of macrophages. It further inhibits activity of natural killer cells (NK cells) and lymphokine activated killer cells and blocks production of cytokines (2). It acts on podocyte and mesangial cells and acts as mediator of glomerulosclerosis (8-10). Several studies (11,12) have found elevated TGF beta1 in glomerular fibrosis and vasculopathy and chronic transplant rejection (13-16). Only few studies are available on TGF beta1 in acute renal failure (ARF), and chronic renal failure (CRF).

Hence, the present study was doen to find out the utilility of TGF beta1 in differentiating ARF from CRF and correlation of TGF beta1 and serum creatinine in various types of transplant rejection with histopathological finding.

Material and Methods

Total 30 cases of chronic renal failure (CRF), 26 cases of acute renal failure (ARF), 10 cases of renal transplant rejection , 17 cases of stable renal transplant and 30 cases of normal healthy control were taken between the period of December 2006 to December 2007. Cases were collected from the indoor and outdoor Department of Nephrology of Sir Sunderlal Hospital, Banaras Hindu University .In all cases clinical details were recorded on preformed proforma in each case. Diagnosis of CRF and ARF was done by standard criteria. In post transplant cases, 17 cases were stable and 10 cases showed progressive decline of renal function. In all cases serum creatinine (SCr.) was done by autoanalyser and TGF beta1 was assayed using enzyme linked immunosorbent assay (ELISA) of Bender Med System, Austria, supplied by OSB Agencies , New Delhi. Assay of serum TGF beta1 in CRF and ARF were done at the time of their diagnosis and in case of stable transplant cases it was assayed during the patient follow up and in cases of rejection, sample was taken at the time just before biopsy. Paraffin embedded histological slides of rejection cases were stained with haematoxylin and eosin (H&E), Acid fuschin orange G (AFOG) and Periodic Acid Stain (PAS) and histological gradings were done based on Banff 2003 classification (17).

The principle of this in brief is as follows-An anti TGF beta1 coating antibody is adsorbed onto microwells. TGF beta1 present in the sample or standard binds to antibodies adsorbed to the microwells ; a HRP-conjugate monoclonal anti - TGF beta1 antibody is added and binds to TGF beta1 captured by the first antibody. Following incubation, unbound enzyme conjugated anti TGF beta1 is removed during a wash step and substrate solution reactive with HRP is added to the wells. A coloured product is formed in proportion to the amount of TGF beta1 present in the sample. The reaction is terminated by addition of acid and absorbance was measured at 450nm. A standard curve is prepared from TGF beta1 standard dilutions absorbance and TGF beta1 sample concentration was determined by graph and multiplied by dilution factor of sample.

Statistical Analysis

Values were recorded as Mean ± SD. Where applicable analysis was performed using Student 't' test on SPSS 10.0 computer statistics programme. P values less than 0.05 were considered significant

Results

In normal healthy control 80.0% (24/30) had TGF beta1 level below 25 ng/ml while in rest 6 cases upper limit was 36 ng/ml. Mean value of serum TGF beta1 in the NHC was 16.63 ± 8.33 ng/ml and range varied from 6 to 36 ng/ml (Table 1). In ARF patients none of the patients had TGF beta1 below 15 ng/ml and 84.6% patients had value less than or equal to 40 ng/ml and only 15.4% patients had value above 40 ng/ml. Mean value of TGF beta1 in ARF was 33.19 ± 8.33 ng/ml (*Table-1*). The TGF beta1 level in CRF patients were markedly raised. All the cases had value above 40 ng/ml. The mean value recorded was 75.26 ± 28.43 ng/ml (*Table-1*). Rise of TGF beta1 in CRF patient as compared to NHC and ARF cases was statistically significant ($P<0.05$).

In another 17 stable cases of transplantation, mean TGF beta1 level was found to be 22.82 ± 6.04 ng/ml with range from 11 - 32 ng/ml. TGF beta1 level was significantly raised ($P<0.05$) in rejection cases as compared to stable cases (*Table 1*) and also there was significant difference in the level of serum creatine in both these groups (*Table 3*). The TGF beta1 level did not correlated with glomerular fibrosis as in two rejection cases inspite of 70% glomerular hyalinization and moderate interstitial hyalinization TGF beta1 was only 5.0 ng/ml (*Table 2*).

In transplant rejection cases 3 were diagnosed as acute

Table 1 Serum TGF beta1 Level in Renal Failure, Rejection and Normal Controls

Groups (No. of cases)	TGF β1 Range (ng/ml)	TGF β1(ng/ml)			Mean ± SD (ng/ml)	P
		<25 No. %	25 – 40 No. %	>40 No. %		
A – CRF (30)	30 – 165	0 0.0	1 03.3	29 96.6	75.26 ± 28.43	A vs B – S A vs C – S A vs D – S A vs E – S B vs C – S B vs D – NS B vs E – S C vs D – S C vs E – S D vs E – S
B – ARF (26)	15 – 45	4 15.4	18 69.2	4 15.4	33.19 ± 8.33	
C – Tx Stb (17)	11 – 32	10 58.8	7 41.2	0 0.0	22.82 ± 6.04	
D – Tx Rej (10)	5 – 78	2 20.0	3 30.0	5 50.0	42.80 ± 27.49	
E – NHC (30)	6 – 36	24 80.0	6 20.0	0 0.0	16.63 ± 8.33	

Table 2 Serum TGF beta1 and SCr Level with Histological Findings in Rejection Cases

Case No.	TGF β1 (ng/ml)	SCr (mg/dl)	Grading of Histological findings in rejection cases using Banff criteria'03	Grade
1.	70.0	2.9	Acute cellular rejection with tubulitis.	ACR- I B
2.	05.0	3.3	Chronic sclerosing allograft nephropathy.	CAN- II
3.	05.0	2.9	HUS with mild focal tubular atrophy and interstitial fibrosis.	CAN- I
4.	25.0	3.6	Chronic rejection with nephritis with tubercular pyelonephritis and polyoma virus infection.	CAN- I
5.	55.0	2.5	Chronic sclerosing glomerular nephritis with MPGN, polyoma, patchy MNC infiltrate, elastosis, arteriosclerosis, thyroidisation.	CAN- I
6.	35.0	2.7	Focal ATN, focal interstitial nephritis with old HUS	ACR- I A
7.	50.0	4.2	Membranous glomerular nephritis with fibrin and lipid thrombi.	CAN- I B
8.	28.0	3.5	Ureteritis, pyelitis with focal ATN and focal interstitial nephritis.	ACR- I B
9.	77.0	2.4	Mild to moderate interstitial fibrosis with tubular atrophy.	CAN- II
10.	78.0	2.9	Chronic rejection (Biopsy not done).	

Table 3 SCr Level in RenalFailure, Rejection and Normal Controls

Groups (No. of cases)	SCr Range (mg/dl)	SCr (mg/dl)			Mean ± SD (mg/dl)	P
		<1.5 No. %	1.5 – 2.0 No. %	>2.0 No. %		
A – CRF (30)	3.0 – 10.8	0 0.0	0 0.0	30 100.0	6.68 ± 1.68	A vs B A vs C A vs D A vs E B vs C B vs D – B vs E C vs D C vs E D vs E – S
B – ARF (26)	2.0 – 9.1	0 0.0	0 0.0	26 0.0	4.10 ± 1.85	
C – Tx Stb (17)	0.8 – 1.9	12 70.6	5 29.4	0 0.0	1.27 ± 0.31	
D - Tx Rej (10)	2.4 – 4.2	0 0.0	0 0.0	10 100.0	3.09 ± 0.55	
E– NHC (30)	0.5 – 1.3	30 100.0	0 0.0	0 0.0	0.89 ± 0.23	

Note :For both Table 1 and 3: TGF â1 – Transorming growth factor â1, CRF – Chronic renal failure, ARF – Acute renal failure, Tx Stb – Transplant stable, Tx Rej – Transplant rejection, NHC – Normal healthy control, S – Significant (P<0.05), NS – Non significant,.

cellular rejection. They had TGF beta1 as 70, 35 and 28 ng/ml. Five of these cases were diagnosed as chronic rejection with interstitial nephritis and fibrosis. Out of this 40% (2/5) cases had TGF beta1 as 5 ng/ml each while rest had value of 25, 55 and 77 ng/ml. One case had membranous glomerulonephritis with hemolytic uremic syndrome (HUS) with TGF beta1 of 50 ng/ml while another case had features of chronic HUS with interstitial nephritis with TGF beta1 level of 35 ng/ml (*Table 2*).

Serum creatinine (SCr) in normal healthy persons was less than 1.5 mg/dl. In ARF in 50% cases it was between 2 to 3 mg/dl and rest 50% cases it varied from 3.1 to 9 mg/dl. In CRF patients SCr was above 4 mg/dl and in all cases it varied from 4.1 to 10.8 mg/dl (*Table 3*). In stable transplant cases only in 3 cases mild rise of SCr was noted (1.6, 1.8 and 1.9 mg/dl) and rest 12 cases it was within the normal range (0.8 - 1.5 mg/dl). Contrary to it in transplant rejection all cases had SCr above 2.4 mg/dl with range between 2.4 - 4.2 mg/dl (*Table 3*).

Discussion

The diagnosis of transplant rejection by non-invasive means has been a goal for many years because kidney biopsies are invasive, costly, and can be associated with morbidity and even death from bleeding. Chronic transplant nephropathy is characterized by interstitial fibrosis, tubular atrophy, fibrous intimal thickening of blood vessels, proliferation of myofibroblast and glomerular hyalinization. Low grade chronic inflammation triggers the endothelial and parenchymal injury and causes expression of adhesion molecules, favouring the cell accumulation, myofibroblast proliferation, transformation, migration, increased release of cytokines, growth factors and enzymes which ultimately lead to scar formation . TGF beta1 is one of the cytokine which has implicated for extracellular matrix deposition and degradation and it's expression is related with fibrotic diseases (18,8,19).

In our study we found slightly higher level of TGF beta1 in normal healthy persons. In majority (83.3%) it was upto 25 ng/ml or below it but 5 patients (16.7%) had value between 25.1 to 36 ng/ml. In literature normal value of TGF beta1 is variable in different series. In one study (20) mean value of TGF beta1 in normal healthy control was reported 3.8 ± 2.9 ng/ml while in other study (21) reported it was between 7 to 8 ng/ml. In ARF majority (84.6%) patients had TGF beta1 level below 40 ng/ml and only 15.4% patients had raised value (above 40 ng/ml). Contrary to it in CRF all patients had TGF beta1 above 40 ng/ml.In transplant rejection cases 50% (5/10) patients had TGF beta1 value above 40 ng/ml and rest had value below 40 ng/ml. TGF beta1 (25, 35 and 70 ng/ml) was also raised in acute rejection, while two cases of severe chronic transplant rejection showing interstitial and glomerular fibrosis were found to have normal TGF beta1. Similarly to our study, other workers (13) also noted a high level of TGF beta1 in acute and chronic rejection of the kidney but they did not found significant relation of TGF beta1 with decline in renal function and fibrosis (22).

The molecular study done in USA also supports our findings. They noted increased expression of TGF beta1 in tubule and interstitium in both acute and chronic rejection while healthy kidney tissue was either negative or weakly positive and they suggested that it is a good marker for rejection (13). Another study done at Netherland (16) reported that mRNA of TGF beta1 in stable post transplant cases who did not develop rejection was 3.4 times than and those who gradually developed chronic rejection. Study conducted in U.K (14) measured the level of TGF beta1 mRNA at different duration after transplantation and noted that the highest level of mRNA TGF beta1 was found 2 weeks after transplantation when histologically maximum number of inflammatory cells are present and by 12 weeks , TGF beta1 expression in the graft is reduced . So, this suggest that increased number of cells are responsible for TGF beta1 secretion. Study of TGF beta1 was done by ELISA technique (23) in 43 normal healthy controls, 11 patients with membranous nephropathy (MN), 17 transplant recipients with stable renal function, 27 patients with acute cellular rejection, 7 patients with chronic vascular rejection, and 10 patients with acute tubular necrosis/cyclosporine toxicity. Contrary to our study, they did not detect TGF beta1 in plasma of normal healthy person but similar to our study, they found significant increase of TGF beta1 in all transplant recipients and rejection cases. They did not find any correlation of TGF beta1 with renal function , serum creatinine, kidney donor age, recipient age, duration of transplantation or cyclosporine level. They also studied the TGF beta1 in urine of healthy controls and found that all the urine sample of both groups had TGF beta1 between 1 to 35 ng/ml. Interestingly, in their study also, in 20 transplant patient urines tested, 2 were negative, 18 were positive but within the range determined for the healthy controls suggesting that urine TGF beta1 estimation is not of any value in diagnosis of graft rejection.To the best of our knowledge no study is available on serum TGF beta1 level in acute and chronic renal failure. In our study in ARF 84.6% had TGF beta1 level below 40 ng/ml while only 15.4% had value above 40 ng/ml. Contrary to it in CRF all cases had TGF beta1 level above 40 ng/ml. From our study, we presume that in chronic renal failure, along with tubular atrophy , glomerular hyalinization, interstitial fibrosis and plenty

of mononuclear cell (MNC) infiltration are present, while in acute renal failure severe interstitial inflammation is not the feature. That is why because of cellular infiltration , TGF beta1 were higher in chronic renal failure cases. Contrary to TGF beta1, SCr level was normal in majority (82.4%) stable transplant cases. Only in 17.6% cases there was mild rise from 1.6 to 1.9 mg/dl. In transplant rejection cases SCr was raised in all the cases above 2 mg/dl.Few recent reports also suggested that producer genotype of profibrogenetic TGF-beta 1, proinlammatory TNF-alpha and IL-6 might be a risk factor for CAN nephropathy devlopers (Chronic allografts) (24). Similarly ,in another report TGFbeta1 along with platelet derived growth factor has a role in allograft arteriosclerosis and graft failures(25).

Conclusion

Thus our study concludes that serum TGF beta1 is neither a good marker for transplant rejection nor it correlates well with fibrosis. SCr is still a simple and sensitive marker for renal transplant rejection. The cut off value of TGF beta1 above 40ng/ml can be used to distinguish ARF from CRF.

References

1. Pozzeto U, Citterio F, Romagnoli J, *et al*. Effects of immunosuppressive regimens on plasma levels of transforming growth factor-beta 1 in renal transplant recipients. *Transplant Proc* 2002; 34: 1740-42.
2. Chambaz EM, Souchelnitskiy S, Pellerin S, *et al*. Transforming growth factors-betas: a multifunctional cytokine family. Implication in the regulation of adrenocortical cell endocrine functions. *Horm Res* 1996; 45: 222-26.
3. Kropf J, Schurek JO, Wollner A, Gressner AM. Immunological measurement of transforming growth factor-beta 1 (TGF-beta1) in blood; assay development and comparison. *Clin Chem* 1997; 43: 1965-74.
4. Waltenberger J, Miyazono K, Funa K, *et al.* Transforming growth factor-beta and organ transplantation. *Transplant Proc* 1993; 25: 2038-40.
5. Massague J. TGF-beta signal transduction. *Annu Rev Biochem* 1998; 67: 753-91.
6. Ignotz RA, Endo T, Massague J. Regulation of fibronectin and type I collagen mRNA levels by transforming growth factor-beta. *J Biol Chem* 1987; 262: 6443-46.
7. Lawrence DA. Transforming growth factor-beta: a general review. *Eur Cytokine Netw* 1996;7: 363-74.
8. Kopp J, Factor V, Mozes M. Transgenic mice with increased plasma levels of TGF-beta 1 develop progressive renal disease. *Lab Invest* 1996; 74: 991-03
9. Ziyadeh FN, Hoffman BB, Han DC *et al*. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice. *Proc Natl Acad Sci USA* 2000; 97: 8015-20
10. Zeisberg M, Ericksen MB, Hamano Y, *et al*. Differential expression of type IV collagen isoforms in rat glomerular endothelial and mesangial cells.*Biochem Biophys Res Commun* 2002; 295: 401-07
11. Goumenos DS, Tsakas S, El Nahas AM, *et al.* Transforming growth factor- beta (1) in the kidney and urine of patients with glomerular disease and proteinuria. *Nephrol Dial Transplant* 2002 ; 17: 2145-52
12. De Muro P, Faedda R, Fresu P *et al*. Urinary transforming growth factor beta 1 in various types of nephropathy. *Pharmacol Res*2004; 49: 293-98
13. Shihab FS, Yamamoto T, Nast CC, *et al.* Transforming growth factor-beta and matrix protein expression in acute and chronic rejection of human renal allografts. *J Am Soc Nephrol* 1995; 6: 286-94.
14. Little DM, Haynes LD, Alam T, *et al.* Does transforming growth factor beta 1 play a role in the pathogenesis of chronic allograft rejection? *Transpl Int* 1999; 12: 393-01.
15. Boratynska M. Urine excretion of transforming growth factor-beta1 in chronic allograft nephropathy. *Ann Transplant* 1999; 4: 23-28.
16. Eikmans M, Sijpkens YW, Baelde HJ, *et al*. High transforming growth factor-beta and extracellular matrix mRNA response in renal allografts during early acute rejection is associated with absence of chronic rejection. *Transplantation* 2002; 73: 573-79.
17. Racusen LC, Colvinb RB, Solezc K *et al*. Antibody-Mediated Rejection Criteria - an Addition to the Banff '97 Classification of Renal Allograft Rejection. *Am J Transplant* 2003; 3: 708-14
18. Yamamoto T, Noble NA, Miller DE, Border WA. Sustained expression of TGF-beta 1 underlies development of progressive kidney fibrosis. *Kidney Int* 1994; 45: 916 - 27.
19. Awad M, El-Gamel A, Hasleton P, *et al.* Genotypic variation in the transforming growth factor-beta 1 gene: Association with Transforming Growth Factor-beta 1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation* 1998; 66: 1014-20
20. Wunderlich H, Steiner T, Kosmehl H, *et al.* Increased transforming growth factor beta 1 level in patients with renal cell carcinoma: a tumor-specific marker ? *Urol Int* 1998; 60: 205-07
21. Cuhaci B, Kumar MS, Bloom RD *et al*. Transforming growth factor-beta levels in human allograft chronic fibrosis correlate with rate of decline in renal function. *Transplantation* 1999; 68: 785-90.
22. Junker U, Haufe CC, Nuske K *et al.* Elevated plasma TGF-beta 1 in renal diseases: cause or consequence ? *Cytokine* 2000; 12: 1084-91
23. Coupes BM, Newstead CG, Short CD, Brenchley PE. Transforming growth factor beta 1 in renal allograft recipients. *Transplantation* 1994; 57:1727-31.
24. Nikolova PN. Cytokine gene polymorphism in Kidney transplantation-Impact of TGF beta 1, TNF-alpha7 IL-6 on graft outcome.*Transpl Immunol* 2008; 18(4): 344-48
25. Pazzetto U. Balance of TGF beta 1 and PDGF-BB expression with KIdney allograft rejection. *Ann Clin Biochem* 2008; 45 (2): 213-14.