A preliminary study on the significant value of beta-2-microglobulin over serum creatinine in renal transplant rejection and renal failure

Sonkar G K, Usha, Singh R G

ABSTRACT

Introduction: Beta-2-microglobulin (β2M) is a light chain of HLA class I molecule, which is filtered by glomerulus, reabsorbed and catabolised by proximal tubule. It is one of the markers of transplant rejection. The aim of the present study was to find out the level of β2M in acute renal failure (ARF), chronic renal failure (CRF), renal transplant rejection (TR) and renal transplantation stable (TS) cases, and correlation of β2M with serum creatinine (SCr) in assessing renal failure.

Methods: 23 patients with ARF, 22 patients with CRF, six cases of TR, seven patients with TS, and 28 normal healthy controls were studied within a one-year period.

Results: Highest mean value of β2M was noted (12.97 +/− 3.83 µg/ml) in CRF, and all cases had elevated β2M of which 81.8 percent of cases had β2M above 10 µg/ml. In ARF, all cases had elevated β2M and 78.3 percent patients had a value more than 10 µg/ml with a mean value of 11.75 +/− 2.09 µg/ml. TR cases also had elevated β2M but 50 percent had mild elevation (less than 10 µg/ml) and 50 percent had marked elevation (more than 10 µg/ml). 42.8 percent of TS patients also had mild elevation of β2M in the range 2.10–3.70 µg/ml. Interestingly, in normal healthy controls, 21.4 percent of patients had mild elevation of β2M of 2.1–2.75 µg/ml, while 78.6 percent of cases had a normal range of β2M (less than 2 µg/ml). All normal healthy controls and 71.4 percent of TS cases had normal SCr (less than 1.4 mg/dL). All cases of CRF and TR cases, and 28.6 percent of TS cases had elevated SCr. 81.8 percent of cases with CRF and 60.9 percent of cases with ARF had a marked rise of serum creatinine above 5 mg/dL.

Conclusion: Our study showed that β2M is not superior over SCr for renal failure and TR cases, because it is also elevated in 21.4 percent of normal controls and 42.8 percent of TS cases. SCr is a cheaper, simpler and comparatively good test to assess renal failure and TR.

Keywords: acute renal failure, beta-2-microglobulin, chronic renal failure, renal transplant rejection, serum creatinine

Singapore Med J 2008; 49(10): 786-789

INTRODUCTION

Serum beta-2-microglobulin (β2M) was first isolated in 1968 from the urine of patients with Wilson's disease and cadmium poisoning. It has been identified as a low molecular weight protein of 11800 Da. It forms a light chain of class I HLA antigen. It has a 100 amino acid length and is non-covalently associated with a heavy chain of HLA antigens. β2M is found on the surface of all nucleated cells. β2M is filtered by the glomerulus, absorbed and catabolised by the proximal tubules.[1] β2M is excreted in increased amounts in the urine of patients with upper urinary tract infection[2] and connective tissue diseases, such as rheumatoid arthritis and Sjogren's syndrome.[3] Elevated serum concentrations in the presence of normal glomerular filtration rate suggest increased β2M production or release. The β2M levels change in relation to disease activity, such as systemic lupus erythematosus[4] and sarcoidosis.[5] It has been shown that β2M may be superior to creatinine for estimating glomerular filtration rate (GFR).[6] β2M is useful in diagnosing acute transplant rejection (TR).[7,8] β2M increases in chronic renal failure (CRF)[9,10] and decreases after renal transplant. In CRF, it parallels with the increase in serum creatinine (SCr). β2M increases in long-term haemodialysed patients.[7] In these patients, DeltaK58-β2M, which is a cleaved product of β2M, is found which gives rise to amyloidosis,[11] especially those who have been dialysed with cuprophane membrane and polysulfone membrane dialyser. Besides CRF and acute TR, elevated β2M have also been reported in viral infection due to increased major histocompatibility complex expression.[12] It is also elevated in lymphoproliferative disorders.[13] Very few comparative studies in our country are available on β2M

Division of Immunopathology, Department of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

Sonkar GK, BSc, MSc
Senior Research Fellow

UGC Advanced Immunodiagnostic Training and Research Centre

Usha, MD, FICN, FICAI
Professor and Head

Department of Nephrology

Singh RG, MD, DM, MNAMS
Professor and Head

Correspondence to:
Prof Usha
Tel: (91) 542 230 9542
Fax: (91) 542 236 756
Email: usha_ugcitrc@rediffmail.com

Table I. Serum beta-2-microglobulin in renal failure, renal transplant and normal healthy control cases.

Patients group (no. of cases)	Mean ± SD (range) β2M (μg/ml)	No. (%) of β2M (μg/ml) at:		
		< 2	2.1–10	>10
ARF (23)	11.75 ± 2.90 (7.20–17.84)	0	5 (21.7)	18 (78.3)
CRF (22)	12.97 ± 3.83 (4.65–18.10)	0	4 (18.2)	18 (81.8)
TR (6)	9.55 ± 2.30 (6.35–12.10)	0	3 (50.0)	3 (50.0)
TS (7)	2.21 ± 1.01 (1.10–3.70)	4 (57.1)	3 (42.8)	0
NHC (28)	1.54 ± 0.59 (0.62–2.75)	22 (78.6)	6 (21.4)	0

β2M: serum beta-2-microglobulin; ARF: acute renal failure; CRF: chronic renal failure; TR: transplant rejection; TS: transplant stable; NHC: normal healthy control.

Table II. Serum creatinine in renal failure, renal transplant and normal healthy control cases.

Patients group (no. of cases)	Mean ± SD (range) SCr (mg/dL)	No. (%) of SCr (mg/dL) at:		
		0.4–1.4	1.41–5.0	> 5.0
ARF (23)	6.17 ± 3.05 (2.20–14.70)	0	9 (39.1)	14 (60.9)
CRF (22)	7.56 ± 2.69 (3.0–14.20)	0	4 (18.2)	18 (81.8)
TR (6)	2.60 ± 0.54 (1.80–3.30)	0	6 (100.0)	0
TS (7)	1.45 ± 0.61 (0.80–2.60)	5 (71.4)	2 (28.6)	0
NHC (28)	0.78 ± 0.24 (0.40–1.20)	28 (100)	0	0

SCr: serum creatinine; ARF: acute renal failure; CRF: chronic renal failure; TR: transplant rejection; TS: transplant stable; NHC: normal healthy control.

in ARF, CRF, and transplant cases and its comparison with SCr. Hence, the aim of the present study was to observe the level of β2M in acute renal failure (ARF), CRF, TR and renal transplantation stable (TS) cases and its utility over SCr.

METHODS

A total of 86 cases, including 23 patients with ARF, 22 patients with CRF, six patients of TR, seven cases of TS and 28 normal healthy controls, were included in this study from August 2005 to July 2006. These cases were taken from the in- and outpatient Department of Nephrology of Sir Sunderlal Hospital, Banaras Hindu University, Varanasi, India. Patients of ARF and CRF were diagnosed by standard criteria.[14,15] Stable transplant cases were clinically proven and had stable SCr, while rejection cases were histologically proven. In post-transplant cases, SCr was assayed every month for one year, and their mean value was taken, while serum β2M was assayed at a mean value of 3.03 months post-transplant during follow-up. In cases of ARF and CRF, blood samples were taken once they were clinically proven and before undergoing dialysis.

Serum β2M was assayed using sandwich ELISA (UBI Magiwels, USA), supplied by Avadh Scientific, Lucknow, India. Brief method was as follows: 100 μL of reference standards and 100 μL of 1:100 diluted samples were dispensed in the respective coated wells and incubated for half an hour. The wells were rinsed for five times using a wash buffer. 100 μL of enzyme conjugate was dispensed and again incubated for half an hour. The wells were rinsed five times using a wash buffer. 100 μL of solution A and 100 μL of solution B were added to each well and incubated for ten minutes. Reaction was stopped by adding 50 μL of stop solution and the absorbance was read at 450 nm. SCr was done by Jaffe's alkaline picrate method, the kit was supplied by Tulip Diagnostics (P) Ltd, Goa, India. The study was approved by the local ethics committee and informed consent was obtained from all patients enrolled into this study. For statistical analysis, values are given as mean ± SD. Analysis was performed using Mann-Whitney test done on the Statistical Package for Social Sciences for windows version 11.0 (SPSS Inc, Chicago, IL, USA) computer statistics programme. A p-value of less than 0.05 was considered to be significant.

RESULTS

We found that 78.6% (22/28) normal healthy controls, who were blood donors, had β2M less than 2 μg/ml, while 21.4% (6/28) normal healthy controls had a mildly elevated β2M. Maximum limit was 2.75 μg/ml. In ARF cases, 78.3% (18/23) had a severe rise of > 10 μg/ml and 21.7% (5/23) had a mild rise of up to 10 μg/ml. None of the ARF patients had β2M < 2 μg/ml. In CRF cases, about 81.8% (18/22) of patients had a marked rise (> 10 μg/ml) and 18.2% (4/22) had a mild rise of up to 10 μg/ml. None of the patients in this group had normal β2M. In TR cases, 50% (3/6) patients had a mild and 50% (3/6) had marked rise, while 42.8% (3/7) TS cases had a mild rise of β2M (Table I). A rise of the mean value of β2M in ARF cases as compared to TS cases and healthy controls were statistically significant ($p < 0.05$). Similarly, a rise

Table III. Statistical analysis of serum beta-2-microglobulin and serum creatinine in different groups.

Groups	p-value (in cases measured for SCr)	p-value (in cases measured for β2M)
ARF vs. CRF	NS	NS
ARF vs. TR	$p < 0.05$	NS
ARF vs. TS	$p < 0.05$	$p < 0.05$
ARF vs. NHC	$p < 0.05$	$p < 0.05$
CRF vs. TR	$p < 0.05$	NS
CRF vs. TS	$p < 0.05$	$p < 0.05$
CRF vs. NHC	$p < 0.05$	$p < 0.05$
TR vs. TS	$p < 0.05$	$p < 0.05$
TR vs. NHC	$p < 0.05$	$p < 0.05$
TS vs. NHC	$p < 0.05$	$p < 0.05$

SCr: serum creatinine; β2M: serum beta-2-microglobulin; ARF: acute renal failure; CRF: chronic renal failure; TR: transplant rejection; TS: transplant stable; NHC: normal healthy control; NS: non-significant; $p < 0.05$: significant.

Table IV. Diagnostic accuracy of SCr and β2M for transplant rejection and stable cases.

	Sensitivity (%)	Specificity (%)
SCr (cut-off 2.60 mg/dL)	100.0	40.0
β2M (cut-off 3.7 μg/ml)	100.0	42.5

of β2M in CRF cases in comparison to TS and healthy controls was also statistically significant ($p < 0.05$), but in comparison to TR cases, it was non-significant ($p > 0.05$). β2M can differentiate TR from TS cases as it was statistically significant, but it cannot differentiate ARF from CRF cases (Table III).

Contrary to β2M, SCr was within normal limits (0.4–1.4 mg/dL) in all the healthy controls. All CRF and TR cases had elevated SCr. In TS cases, 28.6% of patients had a mild rise of SCr between 1.41 and 5.0 mg/dL. Contrary to ARF cases, the majority of patients (81.8%) in CPF cases had a higher level of SCr (> 5 mg/dL) (Table II). A rise of SCr in ARF, CRF and renal transplant cases with or without rejection were statistically significant ($p < 0.05$) (Table III). β2M was found to be 100% sensitive and 42.5% specific for diagnosis of renal TR and TS cases when the cut-off level was taken as 3.7 μg/ml, while SCr showed 100% sensitivity and only 40% specificity when the cut-off level was taken as 2.60 mg/dL for differentiation of stable and rejection cases (Table IV).

DISCUSSION

β2M is a 11.8 kD protein filtered by the glomerulus, reabsorbed and catabolised by the proximal tubule. It is not cleared efficiently by haemodialysis.[1] The main importance of β2M comes in detection of renal TR. Roberts and Lewis in 1979 found β2M to be elevated in both acute and chronic TR. They also noted that a rise of β2M preceded a rise in SCr, and a sustained rise of urine β2M resulted in graft loss. Pacheco-Silva et al studied 20 patients with renal transplant, of which eight patients with immediate good renal function had lower β2M (less than 3.7 mg/L).[8] Sensitivity for diagnosing acute rejection was only 87.5% and specificity was 46%. They noted that patients with simple acute tubular necrosis (ATN) had low β2M, while patients with acute rejection and cyclosporine toxicity with ATN had elevated β2M.

Lange et al studied 88 kidney transplant patients and found that β2M is an early marker of acute rejection, and this is particularly useful in kidney recipients with delayed graft function in whom SCr levels remain elevated. They noticed that a rise of serum of β2M precedes the rise in SCr in 54% of patients with acute rejection with good initial function.[1] Burak et al studied β2M in 25 uraemic patients and 12 controls. Patients were examined after 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 days after transplant for β2M. Patients with good function had a decline in β2M parallel with SCr after kidney transplant, while in patients with ARF after transplantation, both β2M and SCr lowering were delayed.[16]

Contrary to these studies, we have found that all cases of acute or chronic TR and 42.8% (3/7) of TS cases had raised β2M, and the remaining 57.1% (4/7) of TS cases of renal transplant had a value < 2 μg/ml. 21.4% of normal healthy controls also had raised β2M between 2.1 and 10 μg/ml, but none of the normal healthy controls or TS cases had β2M above 10 μg/ml. Contrary to this, all cases of ARF, CRF and TR had raised SCr and only 28.6% (2/7) of TS cases had a mild rise of SCr between 1.41 and 5.0 mg/dL. None of the normal healthy controls had SCr above 1.20 mg/dL. There are several reports which have found elevated β2M in serum of patients with CRF, especially those on haemodialysis.[9,17-19] Motomiya et al studied 137 patients with haemodialysis and 11 prehaemodialysis patients with CRF by immunoblotting method for alpha-2-macroglobulin (β2Ma) and β2M complex. They found that only two out of 11 prehaemodialysis patients and 95 out of 137 (69.3%) haemodialysis patients had the β2Ma

and β2M complex. This complex was more in patients who had multiple dialysis, while it was significantly lower in haemofiltration patients. These authors proposed that β2Ma and β2M complex may be responsible for amyloid formation.[17]

Recently, Corlin et al reported a structurally-modified and truncated β2M by immunoaffinity-liquid-chromatography-mass spectrometry, which is called DeltaK58-β2M. This lysine 58-cleaved β2M was detected only in serum of haemodialysis patients in 20%–40% of cases, which was responsible for amyloid fibril formation.[11] In addition to the use of β2M in TR, Mojiminiyi and Abdella reported that β2M may be superior to SCr in estimating the GFR,[20] but our study found otherwise, because although 21.4% (6/28) of normal healthy controls showed no evidence of any renal disease, their β2M levels were elevated. The rise of β2M in a normal healthy person may be due to activation of the immune system from a subclinical chronic infection which is still unknown.[12]

Our study concludes that SCr is a simple, cheaper, and superior test over β2M in diagnosing ARF, CRF and TR cases. Our study also suggests that in renal transplantation cases, if β2M is less than 3.7 μg/ml, it should be taken as being stable and not at risk for rejection, unless and until repeated examinations show a rising trend of β2M levels. Thirdly, like SCr, β2M also cannot differentiate ARF from CRF. However, further studies with a larger sample size are required to validate the results.

ACKNOWLEDGEMENT

We are thankful to UGC Advanced Immunodiagnostic Training and Research Centre, Department of Pathology, Institute of Medical Sciences, Banaras Hindu University, India, for financial support.

REFERENCES

1. Lange DP, Schmittling ZC, O'Connor TP. Serum beta 2 microglobulin monitoring in cyclosporine treated renal transplant recipients. Transplantation 1999; 67:pS82.
2. Schardijn G, Statius van Eps LW, Swaak AJ, Kager JC, Persijn JP. Urinary beta 2-microglobulin in upper and lower urinary tract infections. Lancet 1979; 1:805-7.
3. Cooper E, Forbes M, Hammbling M. Serum β2-microglobulin and C-reactive protein concentrations in viral infections. J Clin Pathol 1984; 37:1140-3.
4. Maury CPJ, Helve T, Sjoblom C. Serum β microglobulin, sialic acid and C-reactive protein in systemic lupus erythematosus. Rheumatol Int 1982; 2:145-9.
5. Mornex JF, Revillard JP, Vincent C, Deteix P, Brune J. Elevated serum beta-2 microglobulin levels and C1q-binding immune complexes in sarcoidosis. Biomedicine 1979; 31:210-3.
6. Woitas RP, Stoffel-Wagner B, Poege U, et al. Low-molecular weight proteins as markers for glomerular filtration rate. Clin Chem 2001; 47:2179-80.
7. Backman L, Ringden O, Bjorkhem I, Lindback B. Increased serum beta 2 microglobulin during rejection, cyclosporine-induced nephrotoxicity, and cytomegalovirus infection in renal transplant recipients. Transplantation 1986; 42:368-71.
8. Pacheco-Silva A, Nishida SK, Silva MS, et al. [Serum beta 2 microglobulin (beta 2M) following renal transplantation]. Rev Assoc Med Bras 1994; 40:172-8. Portugese
9. Drueke TB. Beta2-microglobulin and amyloidosis. Nephrol Dial Transplant 2000; 15 Suppl 1:17-24.
10. Paczek L, Czarkowska B, Schaefer L, Schaefer RM, Heidland A. Effect of beta 2-microglobulin on immunoglobulin production. Immunol Lett 1992; 33:87-91.
11. Corlin DB, Sen JW, Ladefoged S, et al. Quantification of cleaved beta 2-microglobulin in serum from patients undergoing chronic hemodialysis. Clin Chem 2005; 51:1177-84.
12. Jovanovic D, Krstivojevic P, Obradovic I, Durdevic V, Dukanovic L. Serum cystatin C and beta2-microglobulin as markers of glomerular filtration rate. Renal Fail 2003; 25:123-33.
13. Child JA, Kushwaha MR. Serum beta 2-microglobulin in lymphoproliferative and myeloproliferative diseases. Hematol Oncol 1984; 2:391-401.
14. Brady HR, Brenner BM, Clarkson MR, Lieberthal W. Acute renal failure. In: Brenner BM, ed. The Kidney, 6th ed. Philadelphia: Saunders, 2000:1201-62.
15. Skorecki K, Green J, Brenner BM. Chronic renal failure. In: Harrison TR, ed. Principle of Internal Medicine, 16th ed, Vol II. New York: McGraw Hill, 2005: 1653-63.
16. Burak W, Grzeszczak W, Kochanska-Dziurowicz A, et al. [Levels of beta-2-microglobulin (B2MG) in blood serum of patients during the early phase after kidney transplantation]. Pol Arch Med Wewn 1993; 90:254-9. Polish.
17. Motomiya Y, Ando Y, Haraoka K, et al. Circulating level of alpha2-macroglobulin-beta2-microglobulin complex in hemodialysis patients. Kidney Int 2003; 64:2244-52.
18. Raj DS, Ouwendyk M, Francoeur R, Pierratos A. Beta(2)-microglobulin kinetics in nocturnal haemodialysis. Nephrol Dial Transplant 2000; 15:58-64.
19. Krol Z, Morawska Z. [The analysis of plasma beta 2-microglobulin concentration in patients with chronic renal failure treated by hemodialysis]. Pediatr Pol 1996; 71:121-5. Polish
20. Mojiminiyi OA, Abdella N. Evaluation of cystatin C and beta-2 microglobulin as markers of renal function in patients with type 2 diabetes mellitus. J Diabetes Complications 2003; 17:160-8.