

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

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## ABSTRACT

**Introduction:** Beta-2-microglobulin ( $\beta$ 2M) 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  $\beta$ 2M in acute renal failure (ARF), chronic renal failure (CRF), renal transplant rejection (TR) and renal transplantation stable (TS) cases, and correlation of  $\beta$ 2M 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  $\beta$ 2M was noted (12.97 +/- 3.83  $\mu$ g/ml) in CRF, and all cases had elevated  $\beta$ 2M of which 81.8 percent of cases had  $\beta$ 2M above 10  $\mu$ g/ml. In ARF, all cases had elevated  $\beta$ 2M and 78.3 percent patients had a value more than 10  $\mu$ g/ml with a mean value of 11.75 +/- 2.09  $\mu$ g/ml. TR cases also had elevated  $\beta$ 2M but 50 percent had mild elevation (less than 10  $\mu$ g/ml) and 50 percent had marked elevation (more than 10  $\mu$ g/ml). 42.8 percent of TS patients also had mild elevation of  $\beta$ 2M in the range 2.10–3.70  $\mu$ g/ml. Interestingly, in normal healthy controls, 21.4 percent of patients had mild elevation of  $\beta$ 2M of 2.1–2.75  $\mu$ g/ml, while 78.6 percent of cases had a normal range of  $\beta$ 2M (less than 2  $\mu$ g/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  $\beta$ 2M 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

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## INTRODUCTION

Serum beta-2-microglobulin ( $\beta$ 2M) 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.  $\beta$ 2M is found on the surface of all nucleated cells.  $\beta$ 2M is filtered by the glomerulus, absorbed and catabolised by the proximal tubules.<sup>(1)</sup>  $\beta$ 2M is excreted in increased amounts in the urine of patients with upper urinary tract infection<sup>(2)</sup> and connective tissue diseases, such as rheumatoid arthritis and Sjogren's syndrome.<sup>(3)</sup> Elevated serum concentrations in the presence of normal glomerular filtration rate suggest increased  $\beta$ 2M production or release. The  $\beta$ 2M levels change in relation to disease activity, such as systemic lupus erythematosus<sup>(4)</sup> and sarcoidosis.<sup>(5)</sup> It has been shown that  $\beta$ 2M may be superior to creatinine for estimating glomerular filtration rate (GFR).<sup>(6)</sup>  $\beta$ 2M is useful in diagnosing acute transplant rejection (TR).<sup>(7,8)</sup>  $\beta$ 2M increases in chronic renal failure (CRF)<sup>(9,10)</sup> and decreases after renal transplant. In CRF, it parallels with the increase in serum creatinine (SCr).  $\beta$ 2M increases in long-term haemodialysed patients.<sup>(7)</sup> In these patients, DeltaK58- $\beta$ 2M, which is a cleaved product of  $\beta$ 2M, is found which gives rise to amyloidosis,<sup>(11)</sup> especially those who have been dialysed with cuprophane membrane and polysulfone membrane dialyser. Besides CRF and acute TR, elevated  $\beta$ 2M have also been reported in viral infection due to increased major histocompatibility complex expression.<sup>(12)</sup> It is also elevated in lymphoproliferative disorders.<sup>(13)</sup> Very few comparative studies in our country are available on  $\beta$ 2M

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

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

$\beta$ 2M: 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 $\pm$ 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 $\pm$ 3.05 (2.20–14.70)	0	9 (39.1)	14 (60.9)
CRF (22)	7.56 $\pm$ 2.69 (3.0–14.20)	0	4 (18.2)	18 (81.8)
TR (6)	2.60 $\pm$ 0.54 (1.80–3.30)	0	6 (100.0)	0
TS (7)	1.45 $\pm$ 0.61 (0.80–2.60)	5 (71.4)	2 (28.6)	0
NHC (28)	0.78 $\pm$ 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  $\beta$ 2M 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.<sup>(14,15)</sup> 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  $\beta$ 2M 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  $\beta$ 2M was assayed using sandwich ELISA (UBI Magiwels, USA), supplied by Avadh Scientific, Lucknow, India. Brief method was as follows: 100  $\mu$ L of reference standards and 100  $\mu$ L 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  $\mu$ L of enzyme conjugate was dispensed and again incubated for half an hour. The

wells were rinsed five times using a wash buffer. 100  $\mu$ L of solution A and 100  $\mu$ L of solution B were added to each well and incubated for ten minutes. Reaction was stopped by adding 50  $\mu$ L 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  $\pm$  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  $\beta$ 2M less than 2  $\mu$ g/ml, while 21.4% (6/28) normal healthy controls had a mildly elevated  $\beta$ 2M. Maximum limit was 2.75  $\mu$ g/ml. In ARF cases, 78.3% (18/23) had a severe rise of > 10  $\mu$ g/ml and 21.7% (5/23) had a mild rise of up to 10  $\mu$ g/ml. None of the ARF patients had  $\beta$ 2M < 2  $\mu$ g/ml. In CRF cases, about 81.8% (18/22) of patients had a marked rise (> 10  $\mu$ g/ml) and 18.2% (4/22) had a mild rise of up to 10  $\mu$ g/ml. None of the patients in this group had normal  $\beta$ 2M. 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  $\beta$ 2M (Table I). A rise of the mean value of  $\beta$ 2M 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 $\beta$ 2M)
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;  $\beta$ 2M: 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  $\beta$ 2M for transplant rejection and stable cases.**

	Sensitivity (%)	Specificity (%)
SCr (cut-off 2.60 mg/dL)	100.0	40.0
$\beta$ 2M (cut-off 3.7 $\mu$ g/ml)	100.0	42.5

of  $\beta$ 2M 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).  $\beta$ 2M can differentiate TR from TS cases as it was statistically significant, but it cannot differentiate ARF from CRF cases (Table III).

Contrary to  $\beta$ 2M, 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).  $\beta$ 2M 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  $\mu$ g/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

$\beta$ 2M is a 11.8 kD protein filtered by the glomerulus, reabsorbed and catabolised by the proximal tubule. It is not cleared efficiently by haemodialysis.<sup>(1)</sup> The main importance of  $\beta$ 2M comes in detection of renal TR. Roberts and Lewis in 1979 found  $\beta$ 2M to be elevated in both acute and chronic TR. They also noted that a rise of  $\beta$ 2M preceded a rise in SCr, and a sustained rise of urine  $\beta$ 2M 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  $\beta$ 2M (less than 3.7 mg/L).<sup>(8)</sup> 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  $\beta$ 2M, while patients with acute rejection and cyclosporine toxicity with ATN had elevated  $\beta$ 2M.

Lange et al studied 88 kidney transplant patients and found that  $\beta$ 2M 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  $\beta$ 2M precedes the rise in SCr in 54% of patients with acute rejection with good initial function.<sup>(1)</sup> Burak et al studied  $\beta$ 2M 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  $\beta$ 2M. Patients with good function had a decline in  $\beta$ 2M parallel with SCr after kidney transplant, while in patients with ARF after transplantation, both  $\beta$ 2M and SCr lowering were delayed.<sup>(16)</sup>

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  $\beta$ 2M, and the remaining 57.1% (4/7) of TS cases of renal transplant had a value < 2  $\mu$ g/ml. 21.4% of normal healthy controls also had raised  $\beta$ 2M between 2.1 and 10  $\mu$ g/ml, but none of the normal healthy controls or TS cases had  $\beta$ 2M above 10  $\mu$ g/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  $\beta$ 2M in serum of patients with CRF, especially those on haemodialysis.<sup>(9,17-19)</sup> Motomiya et al studied 137 patients with haemodialysis and 11 prehaemodialysis patients with CRF by immunoblotting method for alpha-2-macroglobulin ( $\beta$ 2Ma) and  $\beta$ 2M complex. They found that only two out of 11 prehaemodialysis patients and 95 out of 137 (69.3%) haemodialysis patients had the  $\beta$ 2Ma

and  $\beta$ 2M complex. This complex was more in patients who had multiple dialysis, while it was significantly lower in haemofiltration patients. These authors proposed that  $\beta$ 2Ma and  $\beta$ 2M complex may be responsible for amyloid formation.<sup>(17)</sup>

Recently, Corlin et al reported a structurally-modified and truncated  $\beta$ 2M by immunoaffinity-liquid-chromatography-mass spectrometry, which is called DeltaK58- $\beta$ 2M. This lysine 58-cleaved  $\beta$ 2M was detected only in serum of haemodialysis patients in 20%–40% of cases, which was responsible for amyloid fibril formation.<sup>(11)</sup> In addition to the use of  $\beta$ 2M in TR, Mojiminiyi and Abdella reported that  $\beta$ 2M may be superior to SCr in estimating the GFR,<sup>(20)</sup> but our study found otherwise, because although 21.4% (6/28) of normal healthy controls showed no evidence of any renal disease, their  $\beta$ 2M levels were elevated. The rise of  $\beta$ 2M in a normal healthy person may be due to activation of the immune system from a subclinical chronic infection which is still unknown.<sup>(12)</sup>

Our study concludes that SCr is a simple, cheaper, and superior test over  $\beta$ 2M in diagnosing ARF, CRF and TR cases. Our study also suggests that in renal transplantation cases, if  $\beta$ 2M is less than 3.7  $\mu$ g/ml, it should be taken as being stable and not at risk for rejection, unless and until repeated examinations show a rising trend of  $\beta$ 2M levels. Thirdly, like SCr,  $\beta$ 2M also cannot differentiate ARF from CRF. However, further studies with a larger sample size are required to validate the results.

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