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

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. β2M is excreted in increased amounts in the urine of patients with upper urinary tract infection and connective tissue diseases, such as rheumatoid arthritis and Sjogren’s syndrome. 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 and sarcoidosis. It has been shown that β2M may be superior to creatinine for estimating glomerular filtration rate (GFR). β2M is useful in diagnosing acute transplant rejection (TR). β2M increases in chronic renal failure (CRF) and decreases after renal transplant. In CRF, it parallels with the increase in serum creatinine (SCR). β2M increases in long-term haemodialysed patients. In these patients, DeltaK58-β2M, which is a cleaved product of β2M, is found which gives rise to amyloidosis, especially those who have been dialysed with cuprophone 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. It is also elevated in lymphoproliferative disorders. Very few comparative studies in our country are available on β2M...
was incubated for half an hour. Serum beta-2-microglobulin; ARF: acute renal failure; CRF: chronic renal failure; TR: transplant rejection; TS: transplant stable; NHC: normal healthy control.

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

<table>
<thead>
<tr>
<th>Patients group</th>
<th>Mean ± SD (range) β2M (µg/ml)</th>
<th>No. (%) of β2M (µg/ml) at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 2</td>
</tr>
<tr>
<td>ARF (23)</td>
<td>11.75 ± 2.90 (7.20-17.84)</td>
<td>0</td>
</tr>
<tr>
<td>CRF (22)</td>
<td>12.97 ± 3.83 (4.65-18.10)</td>
<td>0</td>
</tr>
<tr>
<td>TR (6)</td>
<td>9.55 ± 2.30 (6.35-12.10)</td>
<td>0</td>
</tr>
<tr>
<td>TS (7)</td>
<td>2.21 ± 1.01 (1.10-3.70)</td>
<td>4 (57.1)</td>
</tr>
<tr>
<td>NHC (28)</td>
<td>1.54 ± 0.59 (0.62-2.75)</td>
<td>22 (78.6)</td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Patients group</th>
<th>Mean ± SD (range) SCr (mg/dL)</th>
<th>No. (%) of SCr (mg/dL) at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.4-1.4</td>
</tr>
<tr>
<td>ARF (23)</td>
<td>6.17 ± 3.05 (2.20-14.70)</td>
<td>0</td>
</tr>
<tr>
<td>CRF (22)</td>
<td>7.56 ± 2.69 (3.0-14.20)</td>
<td>0</td>
</tr>
<tr>
<td>TR (6)</td>
<td>2.60 ± 0.54 (1.80-3.20)</td>
<td>0</td>
</tr>
<tr>
<td>TS (7)</td>
<td>1.45 ± 0.61 (0.80-2.60)</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>NHC (28)</td>
<td>0.78 ± 0.24 (0.40-1.20)</td>
<td>28 (100)</td>
</tr>
</tbody>
</table>


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. 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 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
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 CRF 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 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**

$\beta_2M$ is a 11.8 kD protein filtered by the glomerulus, reabsorbed and catabolised by the proximal tubule. It is not cleared efficiently by haemodialysis. 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). 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 $\beta_2M$ precedes the rise in SCr in 54% of patients with acute rejection with good initial function. 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.

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 µg/ml. 21.4% of normal healthy controls also had raised $\beta_2M$ between 2.1 and 10 µg/ml, but none of the normal healthy controls or TS cases had $\beta_2M$ 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 $\beta_2M$ in serum of patients with CRF, especially those on haemodialysis. 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 $\beta_2Ma$
We are required
Thirdly, being stable and
cases. Our study also suggests
superior test over
in
disease,
normal healthy controls showed
fibril
20%-40%
chromatography-mass spectrometry,
in
and
DeltaK58-132M.
in
recently, Corlin et al reported a structurally-
methylated and truncated \( \beta 2 \)M by immunoaffinity-liquid-
chromatography-mass spectrometry, which is called
\( \Delta \text{De} \text{K}58-\beta 2 \)M. This lysine
58-cleaved \( \beta 2 \)M was detected only in serum of haemodialysis patients in
20%-40% of cases, which was responsible for anyloid
fibril formation.\(^{11}\) In addition to the use of \( \beta 2 \)M in TR,
Mojiminiyi and Abdella reported that \( \beta 2 \)M 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 \( \beta 2 \)M levels were elevated. The rise of \( \beta 2 \)M 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 \( \beta 2 \)M in diagnosing ARF, CRF and TR
cases. Our study also suggests that in renal transplantation
cases, if \( \beta 2 \)M is less than 3.7 \( \mu \text{g/ml} \), it should be taken as
being stable and not at risk for rejection, unless and until
repeated examinations show a rising trend of \( \beta 2 \)M levels.
Thirdly, like SCR, \( \beta 2 \)M 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, Schmitting ZC, O'Connor TP. Serum beta 2
microglobulin monitoring in cyclosporine treated renal transplant
recipients. Transplantation 1999; 67:p82.
JP. Urinary beta-2-microglobulin in upper and lower urinary tract
3. Cooper E, Forbes M, Hammblin M. Serum \( \beta 2 \)-microglobulin and
C-reactive protein concentrations in viral infections. J Clin Pathol
4. Maury CPJ, Helve T, Sjobom C. Serum \( \beta \) microglobulin, sialic
acid and C-reactive protein in systemic lupus erythematosus.
Elevated serum beta-2 microglobulin levels and Clq-binding immune
weight proteins as markers for glomerular filtration rate. Clin
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
8. Pacheco-Silva A, Nishida SK, Silva MS. et al. Serum beta 2
microglobulin (beta 2M) following renal transplantation. Rev
9. Drneke TB. Beta2-microglobulin and amyloidosis. Nephrol Dial
Effect of beta 2-microglobulin on immunoglobulin production.
beta 2-microglobulin in serum from patients undergoing chronic
12. Iovanovic D, Krsivogevic P, Obadovic I, Durdevic V, Dukanovic
L. Serum cystatin C and beta-2-microglobulin as markers of
13. Child JA, Kushwaha MR. Serum beta 2-microglobulin in
lymphoproliferative and myeloproliferative diseases. Hematol
15. Skorecki K, Green J, Brenner BM. Chronic renal failure. In:
Harrison TR, ed. Principle of Internal Medicine, 16th ed, Vol II.
of beta 2-microglobulin (B2M/G) in blood serum of patients during
the early phase after kidney transplantation]. Pol Arch Med Wewn
1993; 99:254-9, Polish.
17. Motomiya Y, Ando Y, Haseoka K, et al. Circulating level of alpha2-
macroglobulin-beta2-microglobulin complex in hemodialysis
18. Raj DS, Ouwendyk M, Francisco R, Pierrotos A. Beta(2)-
microglobulin kinetics in nocturnal haemodialysis. Nephrol Dial
concentration in patients with chronic renal failure treated by
20. Mojmimnyi OA, Abdella N. Evaluation of cystatin C and beta 2
microglobulin as markers of renal function in patients with type 2