ORIGINAL ARTICLE

Serum Beta2-Microglobulin as a Biomarker in Early Stages of Chronic Kidney Disease

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Abstract:

Background: Chronic Kidney Disease (CKD) is a global health problem with rising incidence. Serum Creatinine (SCr) is insensitive to moderate reductions in Glomerular Filtration Rate (GFR). Low molecular weight proteins like Beta2-Microglobulin (BMG) are cleared by the plasma through glomerular filtration. Hence serum concentrations increase progressively with reduction of GFR. *Aim and Objectives:* To correlate serum concentrations of BMG with creatinine and estimated GFR (eGFR) in patients with early stages of CKD. *Material and Methods*: Seventy four adults in early stages of CKD were included based on eGFR, calculated using the 4 variable Modification of Diet in Renal Disease (MDRD) equation and albumin creatinine ratio. They were divided into four groups based on the stages of CKD. SCr was measured using Jaffes reaction with Rosche Hitachi P800 autoanalyser and serum BMG was measured using Calbiotech ELISA kit and compared using one way ANOVA, followed by post hoc Tukey's test and Pearson's correlation tests with SPSS version 16 software. *Results:* Levels of serum BMG were significantly elevated in all groups, ($p<0.01$) while SCr levels were in normal range in patients with eGFR >60 ml/min/ 1.73m^2. Both BMG ($r=-0.792$) and SCr ($r=-0.913$) increased with reduction of eGFR ($p<0.01$). Correlation with eGFR in Stage 1 CKD showed serum BMG ($r=-0.824$, $p<0.01$) and SCr ($r=-0.362$) and in Stage 2 CKD, BMG ($r=-0.705$ $p<0.01$) and SCr ($r=-0.609$, $p<0.01$). *Conclusion*: Serum beta2-microglobulin is elevated in asymptomatic patients with normal creatinine, thereby demonstrating its reliability in detecting early stages of CKD.

Keywords: Chronic Kidney Disease, Creatinine, Serum Beta2-Microglobulin, Glomerular Filtration Rate

Introduction:

Chronic Kidney Disease (CKD) is an emerging health hazard with a global concern [1]. CKD is a spectrum consisting of different pathological processes which are associated with abnormal kidney function and a progressive decline in Glomerular Filtration Rate (GFR) [2]. Diabetic nephropathy is India's leading cause of CKD. It is often silent until advanced stages and the occurrence of kidney failure is associated with poor outcomes and high cost, especially in the developing countries, as therapy is life-long. Early stage of renal impairment is obscure and detection of proteinuria is the only evidence for subsequent intervention [3].

Serum Creatinine (SCr) the most routinely used marker for kidney dysfunction is often detected in normal range in the early stage of kidney dysfunction thus making diagnosis difficult. Effect of age, gender, ethnicity, nutritional status,

and body muscle mass on the serum levels of creatinine are proven beyond doubt [4]. The major restraint of SCr is its reduced sensitivity as a pointer of early impairment of GFR as it requires at least 50% loss of the glomerular function for its elevation beyond the reference limits [5]. SCr when combined with estimated Glomerular Filtration Rate (eGFR) detects kidney disease in its early stages more reliably than the SCr test alone. The most widely used substantiated method to estimate GFR is the MDRD (Modification of Diet in Renal Disease) equation [6].

Low Molecular Weight Proteins (LMWP) have been proposed to substitute SCr since they can be readily spotted in clinically silent stages of CKD [7]. Serum Beta2-Microglobulin (BMG) is a low molecular weight protein of 11800 Da present on the surface of all nucleated cells. It is filtered by the glomerulus, absorbed and catabolised by the proximal tubules [8]. CKD is a major risk factor for Cardiovascular Disease (CVD) even in its early stages [9]. Therefore, detection and treatment of CKD in its early stages is not only effective in slowing the progression toward kidney failure but also reduces cardiac morbidity and mortality. This study was aimed at studying the usefulness of BMG in detecting early stages of CKD in comparison to SCr.

Material and Methods:

A cross sectional study was undertaken with 74 patients of both sexes, aged >18 years, who were included on the basis of eGFR and Albumin-Creatinine Ratio (ACR) in a tertiary care hospital. Serum creatinine levels, tested in the central laboratory, were used to determine eGFR based on the MDRD four variable equation. Sample size was calculated by following formula:

$$n = \frac{(\sigma)^2 (Z\ \alpha)^2}{(d)^2}$$ [9].

The sample size obtained after calculation was 68. Patients with eGFR ⩾ 60 ml/min/1.73 m^2 were screened for kidney damage with urine ACR with a random spot urine sample. Patients with ACR ⩾ 30mg/g were enrolled into the study as

Stage 1 CKD if eGFR >90 ml/min/1.73 m^2 and Stage 2 CKD if eGFR 60-90 ml/min/1.73 m^2. Patients with eGFR 30-59 ml/min/1.73 m^2 were further divided as Stage 3A CKD if eGFR was 45-59 ml/min/ 1.73 m^2 and Stage 3B CKD with eGFR 30-44 ml/min/1.73 m^2. Patients with eGFR <30 ml/min/1.73 m^2, acute renal failure, uncontrolled hyperglycemia, recurrent stone formation, malignancy and acute febrile illness were excluded from the study. Informed consent was obtained before including the patients into the study and the serum sample obtained was stored at -20°C until analysis. This study was approved by the Institutional Ethics Committee.

Coexisting morbid conditions in the patients were listed and anthropometric measurements such as height and weight were recorded as per the questionnaire. Body Mass Index (BMI) was calculated using the formula weight (kg)/height (m^2). Serum and urine creatinine were measured using kinetic Jaffes reaction, whereas urine albumin was measured with immunoturbidimetry method using Rosche kits on Rosche Hitachi P800 autoanalyser. Kinetic Jaffes reaction method used in our laboratory was traceable to a reference method based on Isotope Dilution-Mass Spectrometry (IDMS). Serum BMG was measured using Calbiotech ELISA kit. Serum urea, uric acid,

haemoglobin values were obtained from the laboratory database.

Statistical Analysis:

Statistical package SPSS version 16 was used for analyzing data. Continuous variables were presented as mean ± Standard Deviation (SD) and categorical variables as percentage. One way ANOVA and post hoc Tukey's test was used to determine the variation of the different analytes amongst the four groups. Pearson's correlation was used to check for association of various analytes and eGFR. p value <0.05 was considered to be significant.

Results:

The study population comprised of 46 men and 28 women with a mean age of 57.6 years (range 34–86 years). The mean eGFR was 66.7 ± 23.8 mL/min/1.73 m^2. The study population was divided into four groups according to their eGFR, there were 15 patients in Stage 1 (eGFR >90mL/min/1.73m^2), 25 patients in Stage 2 (eGFR 60–89 mL/min/1.73m^2), 16 patients in stage 3A (eGFR 45–59 mL/min/1.73m^2) and 18 patients in stage 3B (eGFR 30–44 mL/min/1.73m^2). The physical characteristics and distribution of risk factors is displayed in Table 1.

Table 1: Physical Characteristics and Risk Factor Distribution of the Study Population

Variables	Stage 1	Stage 2	Stage 3A	Stage 3B	*p*
Subjects (N)	15	25	16	18	
Male (%)	53.3	72	75	44.4	0.168
Diabetic (%)	93.3	88	100	88.9	0.536
Hypertensive (%)	46.7	84	62.5	61.1	0.098
Age (yrs)	49 ±7.2	54± 11.4	59±11.2	67±13.5$^{\phi\#}$	<0.001
Height (cm)	160.9 ± 7.5	164.7 ± 7.6	162.1 ± 8.2	155.4 ± 7.2	<0.01
Weight (Kg)	74.4 ± 6.9	78.7 ± 9.4	78 ± 7.7	68.1 ± 8.3	<0.001
BMI (kg/m^2)	28.7 ± 2.2	28.9 ± 2.9	29.7 ± 3.2	28.3 ± 2.6	0.535
SBP (mmHg)	135 ± 13	143 ± 12	145 ± 13	146 ± 17	0.150
DBP (mmHg)	78 ± 8	88 ± 8*	90 ± 8$^{\$}$	91 ± 10$^{\phi}$	<0.001

*SBP- systolic blood pressure, DBP- diastolic blood pressure Statistical analysis is presented as mean±SD, p value <0.05 denotes significant variation in mean of all four groups as calculated by one way ANOVA. The post hoc Tukey's test is denoted by symbols as: statistical significance stage 1 & stage 2 *; stage 1 & stage 3$^{\$}$; stage 1 & stage 4$^{\phi}$; stage 2 & stage 4$^{\#}$*

Table 2: Biochemical Parameters of the Study Population

Variables	Stage 1	Stage 2	Stage 3A	Stage 3B	*p*
Subjects (N)	15	25	16	18	
Creatinine (mg/dl)	0.7 ± 0.1	1 ± 0.2*	1.4 ± 0.2$^{\$\infty}$	1.7 ± 0.2$^{\phi\varphi\#}$	<0.001
eGFR (ml/min/1.73 m^2)	100.4 ± 7.7	76.6 ± 8*	52.3 ±4.5$^{\$\infty}$	37.4 ±4.9$^{\phi\varphi\#}$	<0.001
Beta$_2$-microglobulin (µg/ml)	1.5 ± 0.4	2.5 ± 0.7*	3.2 ±0.7 $^{\$\infty}$	4.9 ±1.6 $^{\phi\varphi\#}$	<0.001
Urea (mg/dl)	23.2 ± 5.4	25.8 ± 3.5	29.5 ± 8.2	43.9 ± 22$^{\phi\varphi\#}$	<0.001
Uric acid (mg/dl)	4.7 ± 0.9	6.2 ± 1.3	6.4 ± 1.3$^{\$}$	7.5 ± 1.7$^{\phi\varphi\#}$	<0.001
Haemoglobin (g/dl)	13.3 ± 1.3	13 ± 0.9	12.3 ± 1.2	11.1± 0.9$^{\phi\varphi\#}$	<0.001

Statistical analysis is presented as mean±SD, p value <0.05 denotes significant variation in mean of all four groups as calculated by one way ANOVA. The post hoc Tukeys test is denoted by symbols as: statistical significance stage 1 & stage 2; stage 1 & stage 3$^{\$}$; stage 1 & stage 4$^{\phi}$; stage 2 & stage 3$^{\infty}$; stage 3 & stage 4$^{\varphi}$; stage 2 & stage 4$^{\#}$*

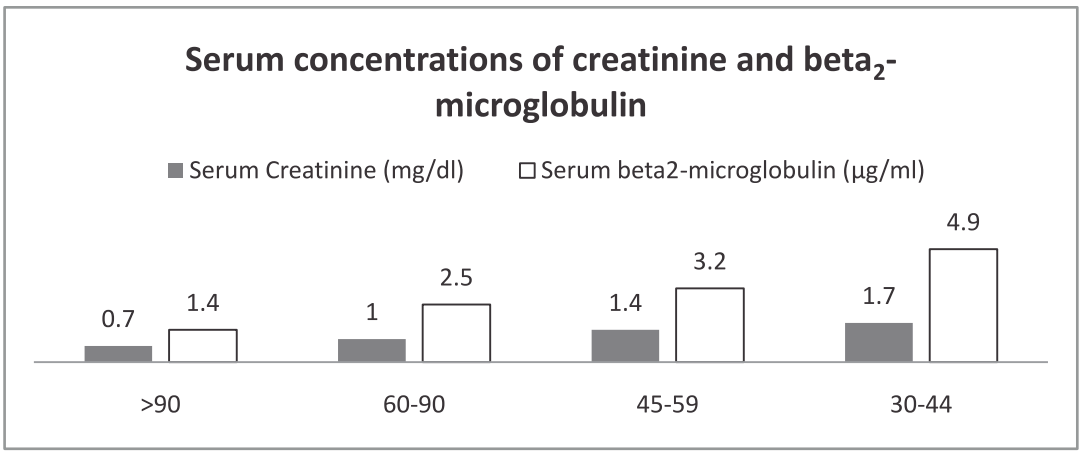

Fig. 1: Serum Concentrations of Creatinine and Beta2-Microglobulinare shown in the 4 Groups of Study Participants Based on their eGFR, Displayed on the X Axis

The biochemical parameters of the study population are presented as mean and standard deviation in Table 2. The post hoc Tukey's test is done and the significance is shown in the Tables 1 and 2. Fig. 1 shows the distribution of the mean concentrations of SCr and serum BMG in the four groups. SCr elevation beyond its reference range is seen only in patients with stage 3 CKD whereas serum BMG levels elevate in initial stages of CKD. Table 3 displays the inter-correlation of BMG, SCr and eGFR in all study subjects. Significant negative correlation is seen between serum BMG and eGFR calculated using MDRD equation.

Table 3: Correlation across Various Groups among BMG, SCr and eGFR

Variables	Stage 1	Stage 2	Stage 3A	Stage 3B	All groups
BMG vs SCr	0.194	0.252	0.597*	0.516*	0.776#
BMG vs eGFR	-0.824#	-0.705#	-0.925#	-0.557*	-0.792#
SCr vs eGFR	-0.362	-0.609#	-0.621#	-0.213	-0.913#

BMG: beta$_2$-microglobulin, eGFR: estimated glomerular filtration rate, SCr: serum creatinine
** signifies $p < 0.05$ and # signifies $p < 0.01$*

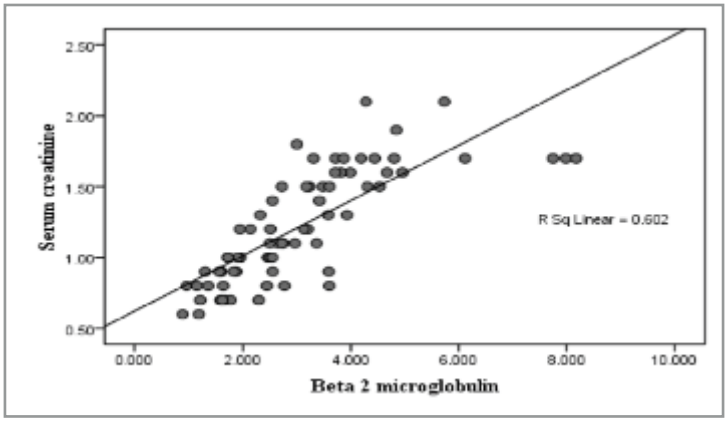

Fig. 2: Correlation of Beta 2 Microglobulin with Serum Creatinine in Early Stages of CKD

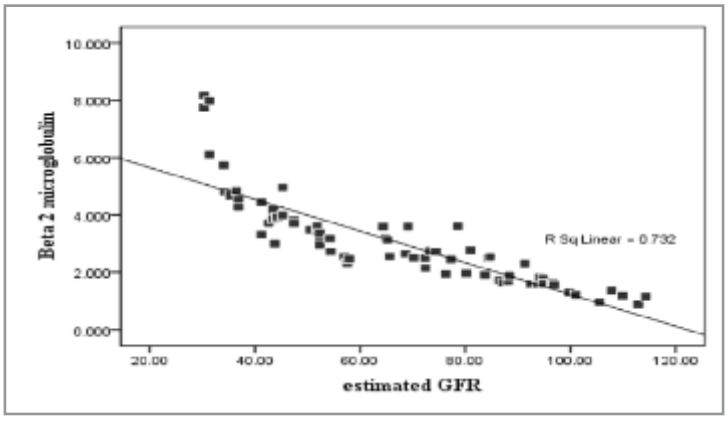

Fig. 3: Correlation of Beta 2 Microglobulin with eGFR in Early Stages of CKD

Discussion:

This cross-sectional study carried out in early stage CKD patients showed that BMG is positively correlated with SCr (Fig. 2) and negatively correlated with eGFR (Fig. 3) across all groups. BMG and SCr were found to be elevated in conjunction with the severity of the disease. In the first two stages of CKD, BMG correlated with eGFR better than SCr. Levels of BMG were significantly elevated in all groups, ($p<0.01$) while SCr levels were in normal range in patients with eGFR >60 ml/min/1.73 m^2 (Fig. 1). In our study, we found BMG levels elevated early in the course of CKD than SCr, which is in agreement with previous study [8]. Serum urea and uric acid being the other markers of renal function also showed an increase with the progression of CKD.

SCr elevation is coupled with considerable reduction in the filtration ability of the kidneys. It surpasses the upper limit of reference range only when patients reach stages ⩾ 3b (GFR 30-44 ml/min/1.73 m^2) [10]. Reduced muscle mass in the elderly population maintains SCr within the laboratory reference limit, masking the diminution of renal function that tends to occur with increased age [11]. Therefore, patients with reduced GFR will be considered normal if only SCr were to be relied on. Failure to detect patients in the initial stages of renal impairment can become fatal as it withholds timely therapeutic intervention [12]. SCr thus has been reported to be unproductive in diagnosing CKD unless combined with eGFR [5].

Scr has been found to be insensitive in detecting early renal impairment in the creatinine blind area [13]. BMG, a LMWP, component of MHC class one, is fully catabolized by the proximal tubular cells [8]. Hence in normal subjects its concentration in the urine as well as serum is negligible. Early kidney damage is associated with reduced GFR which in turn will lead to elevation of serum levels of the LMWP. Gradual decrease in GFR is coupled with progression of renal destruction. Concentration of BMG is found to be negatively correlated with eGFR in all stages of CKD, thus symbolizing its importance in prediction of renal damage in early stages of CKD. In our study it was found to be an independent variable that influences the progression of CKD.

There are conflicting opinions regarding the equation that predicts eGFR accurately. Few studies predict Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation as being the most accurate and precise [14-15]. It was found to be more suitable in the Asian population for calculating eGFR [16]. CKD EPI equation is one more formula similar to MDRD equation to estimate GFR, both use the same variables but they differ in the coefficients, resulting in minor difference in the eGFR so calculated. A study by Kumar *et al.* placed MDRD equation to be more accurate than CKD-EPI 2009 and Cockcroft-Gault formulae in estimating GFR [17]. Few nephrologists are of the opinion that opine the usage of MDRD 4 variable equation for calculating eGFR in the Indian population is rational until we have our own validated eGFR equation for clinical and epidemiological use [18]. Thus, it can be summed up that BMG is a potential indicator of reduction of renal functions and can possibly predict renal insufficiency earlier than SCr. A biomarker like BMG helps in timely diagnosis of CKD in its early

stage, thereby aids in appropriate therapy which can prevent or delay further complications.

Drawback of this study is utilizing eGFR instead of gold standard measurement of glomerular filtration rate, such as inulin clearance. The choice of using 4 variable MDRD formula was primarily done to avoid subjecting patients to cumbersome procedure of measuring GFR as well as financial constraint. The drawbacks of BMG as an endogenous biomarker for renal function are its elevation in non-renal conditions such as inflammation, steroid therapy and lymphoproliferative disease [19]. This necessitates further studies to assess the effect of non-renal conditions on BMG levels.

CKD is a leading cause of cardiovascular mortality [20]. Dialysis is mandatory and expensive, the financial burden of treatment of CKD is associated with development of chronic depression [21]. With the growing pandemic of cardiometabolic diseases in our country, this study emphasizes the importance of early detection of CKD.

Conclusion:

Serum beta2-microglobulin was elevated in asymptomatic patients with normal creatinine, thereby demonstrating its reliability in detecting early stages of CKD. Serum BMG may possibly substitute SCr in diagnosing early stages of CKD and facilitate appropriate therapeutic interventions.

Acknowledgements:

This study was funded by MAHE Post Graduate Research Grant. The authors are thankful to the physicians and to the patients for extending cooperation to the study.

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How to cite this article:
Shenoy MT, Rukmini MS, Manjrekar PA, Hegde A, Akshatha LN. Serum Beta2-Microglobulin as a Biomarker in Early Stages of Chronic Kidney Disease. *J Krishna Inst Med Sci Univ* 2021;10(3):21-28.

Submitted: *18-Feb-2021* ***Accepted:*** *01-Jun-2021* ***Published:*** *01-Jul-2021*