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ORIGINAL ARTICLE

The usefulness of serum beta-2 microglobulin as a biomarker for evaluating renal function decline in type II diabetes mellitus

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ABSTRACT

Background and objective: Serum beta-2 microglobulin (β2M) has been used as a useful clinical marker of chronic kidney dysfunction. The current study aims to evaluate the diagnostic accuracy of β2M for the early detection of diabetic nephropathy among Iraqi patients with type II diabetes mellitus.
Methods: The study included 84 participants divided into four groups, three of them were type II diabetics and the fourth is the healthy individuals' (control) group. The diabetic' subgroups were named according to the Micral test as: normoalbuminuria (21 patients), microalbuminuria (21 patients), and macroalbuminuria group (21 patients). The control group included 21, age- and sex-matched, healthy participants. Biochemical markers for diabetes mellitus as well as β2M were determined for each participant and then were analyzed statistically.
Results: The serum β2M of normoalbuminuria group was (2.86$\pm$0.95 μg/mL), microalbuminuria group was (5.06$\pm$1.97 μg/mL) and macroalbuminuria group (3.6$\pm$1.59 μg/mL). The results showed significant increase ($p< 0.05$) in the β2M level of microalbuminuria group when compared with that of normoalbuminuria and macroalbuminuria groups. In addition, a highly significant increase ($p< 0.01$) in β2M concentration was observed in microalbuminuria group when compared with that of the control group.
Conclusions: β2M can be used as a useful biomarker for the early detection of diabetic nephropathy.

Keywords beta-2 microglobulin, diabetic nephropathy, diabetes mellitus, macroalbuminuria

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INTRODUCTION

Beta-2 microglobulin (β2M) is a protein (11,800 Dalton) found in almost all nucleated cells and most body fluids, including serum, urine, and synovial fluid. In humans, it is

encoded by the *B2M* gene that located on the chromosome 15 and consists of 99 amino acids with one disulfide bond.[1,2] The abnormal level of β2M in blood or urine is mostly associated with certain diseases, such as some acute and chronic inflammations, liver or renal dysfunctions, some viral infections, and several malignancies.[3] The serum β2M level is inversely correlated with the glomerular filtration rate (GFR), as one of the classic low-molecular-weight indicators of kidney function.[4] At the same time, several non-renal factors, such as systolic blood pressure, gender, total cholesterol, inflammation, and smoking have an effect on serum β2M concentration.[5] About 99% of β2M is eliminated by glomerular filtration and mostly it reabsorbed by the proximal tubules and less than 1% of the filtered amount is excreted in the urine.[6] Thus, in individuals with chronic kidney disease and especially end-stage renal disease, β2M is increased in the blood.[7] In addition, its levels were significantly and positively correlated with the end stage renal disease.[8] In general, the plasma β2M elevation is mostly resultant from the decreased glomerular filtration rate or increased rate of its synthesis.[9]

The β2M is the most efficient test for the detection of proximal tubular dysfunction. However, urinary β2M determination is important for following-up renal transplantation patients.[10] Renal filtration and reabsorption disorders can be detected by abnormal urinary β2M values. Because of its small size, β2M passes through the glomerular membrane and reabsorbed by specific receptors in the proximal tubules. Its concentrations increase due to the decreased ability to reabsorb this protein. When the glomeruli are damaged, they become unable to filter out β2M, and this leads to elevated blood concentration, while tubular injury leads to decreased reabsorption of β2M, leading to elevated urinary concentrations.[11] Till now, there is no research study showing the role of serum β2M as a clinical predictor for kidney dysfunction in Iraqi patients with type II diabetes mellitus. Thus, the current study was designed to investigate the diagnostic accuracy of β2M for the early detection of diabetic nephropathy among Iraqi patients with type II diabetes mellitus.

MATERIALS AND METHODS

Study design and subjects

This case-control study was conducted at the National Diabetes Center for Treatment and Research of Mustansiriyah University (Baghdad, Iraq). Eighty-four participants included in the study and divided into four groups, three of which included type II diabetes mellitus patients diagnosed depending on the case history, levels of fasting blood sugar (FBS) and HbA_1c. They were divided, according to the Micral test (urine albumin/creatinine ratio), into three groups classified as: normalbuminuria (21 participants), 21 microalbuminuria (21 participants), and 21 macroalbuminuria (21 participants). The fourth group included 21 apparently healthy subjects, in term of non-diabetes, with matched gender and age. Patients with type 1 diabetes, gestational diabetes, chronic disease, cardiovascular and other complications of diabetes mellitus, patients on insulin therapy, smoker patients were excluded.

Ten milliliters of fasting blood taken from each participant. Four milliliters were placed in EDTA tube for the measurement of glycated hemoglobin (HbA_1c). The remaining 6 milliliters of blood were placed into gel-containing anticoagulant-free tubes and allowed to clot at room temperature for 2 hours to be analyzed later. The urine sample of each participant was also collected for the Micral test.[12]

Biochemical parameters

Serum β2M and insulin were determined quantitatively by the enzyme-linked immunosorbent assay (ELISA) *in vitro*, using commercial kits ELISA manufactured by Demeditec, Germany. The renal profile, blood sugar and HbA_1 *c* were assessed by using cobas c311 (Roche, Germany). Insulin resistance was measured by HOMA-IR while beta cell function estimated by HOMA-β as shown in the following equations:

$$HOMA - IR = \frac{\left(FBS_{(mmol\backslash L)} \times FI_{(\mu IU/mL)}\right)}{22.5}$$

$$HOMA - \beta = \left(\frac{20 \times FI_{(\mu IU/mL)}}{FBS_{(mmol\backslash L)} - 3.5}\right)\%$$

Statistical analysis

Statistical Package for Social Sciences, SPSS v19 (IBM, IL, USA) was used for data analysis. The quantitative variables (measured parameters) were expressed as means±standard deviation (SD) by using analysis of variation (ANOVA) to compare between means, a *p*-value of $\leq$0.05 was considered statistically significant. In addition, receiver operating characteristics (ROC) curve was used to evaluate the marker accuracy as predictor. The best cut-off point of β2M, sensitivity, and specificity were also calculated.

RESULTS

The results of age and BMI presented in Table 1 showed no significant differences (p>0.05) between studied groups. The mean value of age were 54.59 years, 56.52 years, 58.4 year and 54.5 years for control, normoaluminuria, microaluminuria, and macroaluminuria, respectively. The BMI results of all groups indicated that all participants were obese (BMI>30 kg/m^2).

The mean±SD of serum β2M of normoalbuminuria group was (2.86±0.95 μg/mL), microalbuminuria group was (5.06±1.97 μg/mL) and macroalbuminuria group (3.6±1.59 μg/mL). The comparison results indicated significant increase (p< 0.05) of β2M in microalbuminuria group in comparison to that of normoalbuminurea and macroalbuminuria groups. Also, highly significant increase of β2M was observed in microalbuminuria group

in comparison to that of control group. The profile of blood sugar for studied groups indicated highly significant differences ($p< 0.01$) in FBG, insulin, HbA_1 *c*, HOMA-IR, and HOMA-β between all studied groups as shown in Table 1. Meanwhile, the renal function tests for studied groups revealed highly significant differences ($p<0.01$) in serum urea, creatinine, GFR, and albumin/creatinine ratio of urine between all studied groups as shown in Table 1.

Table 1 Anthropometric and biochemical parameters of the studied groups.

Parameter	Group I	Group II	Group III	Group IV	*P*
Age (years)	54.59±9.71	56.52±8.12	58.47±9.97	54.50±9.75	0.52
BMI (Kg/m^2)	30.57±3.76	32.19±5.45	31.60±3.44	30.88±4.53	0.80
FBG (mg/dL)	90.17±9.67	162.52±71.21	194.26±48.01	211.53±79.2	0.001
FPI (U/mL)	12.65±2.77	9.65±2.44	11.98±3.43	15.76±6.01	0.02
HbA_1*c* (%)	5.54±0.21	8.44±1.97	8.81±1.42	9.56±1.88	0.001
HOMA-IR	50.88±18.01	70.55±26.10	112.64±27.63	178.59±77.99	0.001
HOMA-β (%)	2.55±0.87	1.38±0.32	1.49±0.496	1.54±0.62	0.001
B. urea (mg/dL)	23.85±5.37	25.35±6.56	28.32±9.33	40.940±5.04	0.001
S. creatinine (mg/dL)	0.57±0.10	0.68±0.19	0.83±0.24	1.52±0.37	0.001
GFR (mL/min/1.73 m2)	117.39±25.7	107.41±31.35	84.71±26.41	45.16±13.43	0.001
A/C ratio (mg/mmol)	1.38±0.41	1.95±1.08	10.28±5.21	32.3±8.87	0.001
β2M (μg/mL)	2.35±0.56	2.86±0.95	5.06±1.97	3.6±1.59	0.001

Group I (control), Group II (DM patients with with normoalbuminuria), Group III (DM patients with with microalbuminuria), Group IV (DM patients with with macroalbuminuria).
A/C ratio, *albumin:creatinine ratio;* ***BMI,*** *bodymass index;* ***FBG,*** *fasting blood sugar or glucose;* ***GFR,*** *glomerular filtrationrate;* ***FPI,*** *fasting plasma insulin;* ***β2M,*** *beta-2 microglobulin.*

As shown in Table 2, the Pearson correlation analysis of β2M results showed highly significant positive correlation ($p<0.01$) between β2M and HOMA-β, urea, and (A/C ratio) in normoalbuminuria groupMmcroalbuminuria group showed highly significant ($p< 0.01$) positive correlation with HOMA-IR, urea and creatinine, meanwhile indicate a highly significant ($p< 0.01$) negative correlation with GFR (Table 2). The results of β2M of macroalbuminuria group showed a highly significant ($p< 0.01$) positive correlation with urea and creatinine, while a highly significant ($p< 0.01$) negative correlation with GFR was observed (Table 2).

Receiver operating characteristics (ROC) curve was used to evaluate the usefullness of β2M in optimal diagnosis of diabetic nephropathy. In normoalbuminuria group, as in Table 3, the area under curve (AUC) was 0.7, sensitivity 65.2%, and specificity 63.6%. In addition, the AUC in microalbuminuria group was 0.98, sensitivity 90.5%, and specificity 90.9%. Meanwhile, in macroalbuminuria group, the AUC was 0.74, sensitivity 68.2%, and specificity 68.2%.

DISCUSSION

Table 2 **Pearson correlation analysis between β2M and other studied variables.**

	Group II		Group III		Group IV	
Variable	**r**	***p***	**r**	***p***	**r**	***p***
Age (years)	0.29	0.16	0.30	0.18	0.19	0.38
BMI (Kg/m^2)	-0.13	0.54	-0.02	0.90	-0.13	0.55
FBG (mg/dL)	-0.24	0.26	-0.00	0.97	0.04	0.86
FPI (U/mL)	0.15	0.47	-0.21	0.35	-0.09	0.68
HbA1*c* (%)	-0.40	0.08	0.12	0.60	0.10	0.65
HOMA-IR	-0.11	0.63	0.71	0.001	0.05	0.82
HOMA-β (%)	0.46	0.03	0.23	0.31	0.10	0.64
B. urea (mg/dL)	0.22	0.001	0.24	0.001	0.35	0.001
S. creatinine (mg/dL)	0.00	0.58	0.44	0.001	0.27	0.001
GFR (mL/min/1.73 m^2)	-0.01	0.43	-0.48	0.001	-0.22	0.001
A/C ratio (mg/mmol)	0.56	0.001	-0.08	0.72	0.10	0.64

Group II (DM patients with with normoalbuminuria), Group III (DM patients with with microalbuminuria), Group IV (DM patients with with macroalbuminuria).
A/C ratio, *albumin:creatinine ratio;* ***BMI,*** *bodymass index;* ***FBG,*** *fasting blood sugar or glucose;* ***GFR,*** *glomerular filtrationrate;* ***FPI,*** *fasting plasma insulin;* ***β2M,*** *beta-2 microglobulin.*

Table 3 **Receiver operating characteristics (ROC) curve analysis of β2M.**

	Group II	Group III	Group IV
AUC	0.70	0.98	0.74
Cut-off level (μg/mL)	2.61	3.44	2.72
Sensitivity (%)	65.2	90.5	68.2
Specificity (%)	63.6	90.9	68.2

Group II (DM patients with with normoalbuminuria), Group III (DM patients with with microalbuminuria), Group IV (DM patients with with macroalbuminuria).

The β2M is located on the surface of lymphocytes and other nucleated cells. The free molecules are also detectable in plasma as products of circulating cells, particularly from lymphocytes. Since the kidneys are the primary site of clearance, the serum β2M is highly dependent on renal function. As a result, the β2M has been used as a nephropathies biomarker.[13]

In the current study, the result of β2M level in sera of nephropathy patients indicated significant increase compared to that of control group. This was in agreement with Herrero-Morin *et al* study, in 2007,[14] which reported that β2M is wholly filtered at the glomerulus and then almost completely reabsorbed in the proximal tubule. In healthy conditions its production is constant, thus making it suitable as a replacement for GFR.[12]

Meanwhile, a study by Sedighi O and his colleagues[15] found that plasma β2M levels were higher in patients with CKD, and that this level increased as GFR decreased. Since β2M is removed by glomerular filtration and tubular catabolism in the kidneys, its plasma level is closely linked to GFR.[7]

Colombo *et al* (2019) have shown that β2M can be used as a biomarker of renal function decline from an eGFR of 30-75 ml.min^{-1} [1.73 m]$^{-2}$ in type 2 diabetes.[16] The β2M freely

passes through the glomerular filtration membrane, where it is absorbed and degraded almost entirely by the proximal tubules, without being secreted and returned to the blood.[16] Although only small amount of β2M might be filtered by the glomeruli, the majority are catabolized in the tubules, however; all these notes make it a good marker for kidney disease.[17]

In Indian patients with type II diabetes mellitus, the study was demonstrated higher association between β2M and increased risk of incident end-stage renal disease (ESRD), and thus β2M is associated with mortality in Indian patient with type II diabetes mellitus.[18]

Kim *et al*[19] and Cheung *et al*[20] studies found that the patients with high levels of β2M had higher prevalence of diabetic nephropathy and retinopathy than those with low β2M levels. In general, the effect of β2M is slightly higher on diabetes mortality than all other causes of mortality because its high levels were found to be associated with multiple diabetic complications.[19,20] In the present study, the β2M level was significantly correlated with all markers of kidney function (serum urea and creatinine). Patients with significantly higher β2M levels were more frequently seen in the microalbuminuria group compared to those with macroalbuminuria, which might reflect the renal treatment effects on β2M levels. Also, according to ROC curve analysis which indicted that β2M levels of type II diabetes mellitus with normoalbuminuria, greater than or equal to2.61(μg/mL) with 63.60% specificity and 65.20% sensitivity and the (AUC=0.70, p=0.02) that mean β2M is fair marker for predicting nephropathy in type 2 diabetics. While, β2M of type II diabetics in microalbuminuria group greater than or equal to 3.44 (μg/mL) with 90.90% specificity and 90.50%sensitivity and the (AUC=0.98, p=0.001 that serum mean β2M is prefect marker for predicting nephropathy in type 2 diabetes mellits. Meanwhile, β2M levels of type II diabetes mellitus in macroalbuminuria group greater than or equal to 2.72 (μg/mL) with 68.20% specificity and 68.20%sensitivity and the (AUC=0.74, p=0.00 6) that mean β2M is fair for predicting nephropathy in type II diabetes mellitus, and to confirm that our results more studies are needs.

CONCLUSIONS

Signs of renal damage appeared to be parallel to diabetic nephropathy and assessed by reduced glomerular filtration rate, and it is possible to use β2M as a predictive marker rather than prognostic marker for diabetic nephropathy.

ABBREVIATIONS

β2M, Beta-2-Microglobulin; BMI, Body Mass Index; FBS, Fasting Blood Sugar; GFR, Glomerular Filtration Rate HbA_1c, Hemoglobin A_1 c; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; INS, Insulin: ROC, Receiver Operator Characteristics.

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DECLARATIONS

Authors' contributions

SAA conceived the presented idea and wrote the manuscript. ZAH carried out the experiment and verified the analytical methods with support from SAA. INS developed the theory and performed the computations. All authors discussed the results, reviewed and approved the final manuscript.

Conflict of interest

The authors have no conflict of interest.

Ethical approvals

This study was approved by the Scientific Committee of Chemistry and Biochemistry Department, College of Medicine at University of Mustansiriyah, and by the scientific Committee of the National Diabetes Center (Ref. No.: 4786/9.11.2019). The objectives and methodology were explained to all participants and verbal consent had been taken.

Data availability

The data that support the findings of this study is available from the corresponding author, upon reasonable request.

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