

Beta-2 microglobulin as a predictor of peripheral arterial disease in diabetes: the effect of estimated glomerular filtration

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Abstract

Background: Peripheral arterial disease (PAD) is common in patients with type 2 diabetes mellitus. Its definitive diagnosis requires ultrasound or angiography. Beta-2 microglobulin (β_2 microglobulin) has been proposed as a diagnostic marker for PAD. The objective of the study was to evaluate the diagnostic value of β_2 microglobulin for PAD in patients with diabetes and varying renal function.

Design: This was a cross-sectional study.

Setting: An academic centre (University of Pretoria and Steve Biko Academic Hospital Diabetes Clinic).

Subjects: One hundred and eight convenience-sampled patients.

Outcome measures: Patients completed a questionnaire and had toe and arm blood pressure (toe-arm index), as well as serum β_2 microglobulin and creatinine, measured.

Results: Beta-2 microglobulin did not differ (p -value = 0.34) between those subjects with PAD ($n = 43$) and those without PAD ($n = 65$). In a linear regression model, the interaction term between estimated glomerular filtration rate categories and the inverse of β_2 microglobulin was highly significant (p -value = 0.001).

Conclusion: Although the sample size was small, β_2 microglobulin did not distinguish between subjects with and without PAD. Renal function and its effects on the association between β_2 microglobulin and PAD need further study.

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Introduction

Epidemiological evidence confirms an association between diabetes and increased prevalence of peripheral arterial disease (PAD). Individuals with diabetes have a two- to fourfold increase in the rates of PAD.^{1,2} Generally, they also have femoral bruits and absent pedal pulses,³ and rates of abnormal ankle-brachial indices ranging from 11.9-16%.^{4,5} The duration and severity of diabetes correlates with the incidence and extent of PAD.⁶

Diabetes changes the nature of PAD. Generally, patients with diabetes have more infrapopliteal arterial occlusive disease and vascular calcification than nondiabetic cohorts.⁶ The Hoorn study⁷ examined the rates of PAD in groups ranging from patients with normal glucose tolerance, to those with diabetes requiring multiple medications. The 7% prevalence of abnormal ankle-brachial indices in individuals with normal glucose tolerance increased to 20.9% in those requiring multiple hypoglycaemic medications.

Usually, patients with diabetes develop the symptomatic forms of PAD, intermittent claudication and amputation.⁸ In the Framingham cohort,⁹ the presence of diabetes increased the risk of claudication 3.5-fold in men and 8.6-fold in women. Worse, diabetes causes most nontraumatic lower extremity amputations in the USA.¹⁰ The relative risk of lower extremity amputation in patients with diabetes was 12.7 [95% confidence interval (CI): 10.9-14.9] compared with that of nondiabetic patients in the Medicare population, and as high as 23.5 (95% CI: 19.3-29.1) for persons with diabetes aged 65-74 years.¹⁰

The protein beta-2 microglobulin (β_2 microglobulin) is an 11.7-kDa nonglycosylated polypeptide comprising 99 amino acids. It is one of the major histocompatibility complex class molecules on the cell surface of all nucleated cells. It interacts with and stabilises the tertiary structure of the major histocompatibility complex class I alpha-chain.¹¹ This protein is present on almost all cells of the body. (Red blood cells are a notable exception.)¹²

In a study using proteomic profiling, β_2 microglobulin was identified as a biomarker for PAD.¹³ After identification, β_2 microglobulin was assessed in a validation study in a population at risk of acquiring PAD. Serum β_2 microglobulin was higher in patients with PAD undergoing coronary angiography ($n = 237$). Also, the combination of β_2 microglobulin and high-sensitivity C-reactive protein (CRP) levels correlated with PAD diagnosis, independent of other vascular risk factors and glomerular filtration rate (GFR) by stepwise regression analysis. This was consistent with the earlier observation in the smaller confirmation study.¹³

Increasing age and diagnosis of diabetes mellitus were the other independent correlates of PAD diagnosis. The odds ratio for the diagnosis of PAD for elevated β_2 microglobulin was 7.2 (95% CI: 1.6-31.3, p -value = 0.009). The odds ratio was 1.3 (95% CI: 1-1.7, p -value = 0.026) for high-sensitivity CRP.¹³ Because of its probable role in immunity and inflammation, the association of β_2 microglobulin with PAD or with alterations in vascular structure,¹⁴ could relate to vascular inflammation.¹⁵

The abovementioned study demonstrated the ability of β_2 microglobulin and CRP to predict PAD, independent of diabetes and GFR. However, no details were given on the prevalence of diabetes or the range of GFR. The results were also reported as standardised coefficients and not as measures of diagnostic accuracy.

It has been known for many decades that β_2 microglobulin increases with diminished renal function.¹⁶ The normal synthesis rate of β_2 microglobulin is 2-4 mg/kg/day and the normal plasma level is 1-4 mg/l, which varies inversely with GFR. Usually, it is filtered through the renal glomeruli. Part of it is absorbed and catalysed by the renal tubular cells, while the nonabsorbed part is excreted in the urine.¹⁷ We investigated the diagnostic accuracy of β_2 microglobulin, given a variation in renal function.

Method

Setting

The setting of this cross-sectional study was the Diabetes Clinic at the Steve Biko Academic Hospital in Pretoria. This is a tertiary diabetes clinic in a public (state) hospital. The University of Pretoria Ethics Committee approved the study (231/2008).

Subject selection

Included was a convenience sample of subjects who were 50 years of age and older, with at least one first toe.

Research procedures

Consecutive patients who met the inclusion criteria were asked to participate. After agreeing and giving their signed informed consent, patients provided a urine sample and a venous blood sample. The blood

samples were centrifuged. The serum and urine samples were snap frozen and stored at -70°C .

Patients completed a demographic and clinical questionnaire, which included the variables from the Rose questionnaire.¹⁸

In our vascular laboratory, after five minutes of supine rest, the brachial blood pressure was taken by a single investigator using a Baumanometer with a large cuff, if the mid-arm circumference was larger than 35 cm, and a stethoscope. This was carried out twice. If there was a difference in the systolic blood pressure (SBP) of more than 6 mmHg, a third and decisive measurement was taken. The average of the two closest SBP measurements was used to calculate the toe brachial index (TBI). Blood pressure (in both the toes and the arm) was taken with a strict two-minute interval.

Lastly, toe blood pressure was measured using a photoplethysmograph (DE Hokanson, MD6RP device). Before a measurement was taken, the foot temperature was measured using an infrared skin thermometer (Microlife™ Fr1DZ1). The temperature of the foot had to be at least 25°C , otherwise the feet were warmed up for two minutes and then the temperature was measured again. A photo sensor was attached to the distal part of the pulp space. A miniature pneumatic blood pressure cuff was placed at the base of the toe, encircling the distal phalanx. After this, the toe blood pressure was taken. This was always carried out by the same two researchers, one operating the Baumanometer, and the other, the photoplethysmograph. Only SBP was measured. A mean systolic toe pressure was calculated by averaging the values obtained from the left and right toes. In the event of a difference of more than 6 mmHg, a third measurement was taken. In the absence of either the right or left hallux, two values were obtained in the remaining hallux and averaged, taking the two values that were closest to each other. To calculate the TBI, the mean of two brachial SBP readings was used. The toe SBP was divided by the systolic brachial blood pressure. The TBI was calculated per toe.

A TBI of ≤ 0.75 in either foot was used to define PAD.^{19,20}

To evaluate the TBI as a continuous measurement, the TBI of the left and right foot was averaged. (A single measurement was used in the case of any absent foot.)

Laboratory measurements

β_2 microglobulin was assayed by nephelometry with Siemens N Latex B_2 Microglobulin/OQWU15 reagent using Siemens BN Prospec Nephelometer (Siemens Healthcare Diagnostics, Newark, USA), at the Department of Immunology, Tshwane Academic Division, National Health Laboratory Service. Serum and urine creatinine (non-isotype dilution mass spectrometry traceable) was measured at the National Health Laboratory Service Laboratory using the Jaffe method on the Synchron LX system.

Data analysis

The aim of the study was to determine whether β_2 microglobulin could distinguish between patients with and without PAD, and to investigate the association between β_2 microglobulin and the TBI, taking into account the renal function of the patients.

Groups were compared with t-tests/Mann-Whitney tests and chi-square tests. The association between the TBI and renal function [estimated GFR (eGFR)] was investigated with linear regression. We transformed β_2 microglobulin to the inverse of β_2 microglobulin (best approximation to normal). To determine the effect of renal function, we dichotomised the eGFR at 60 ml/minute/m². The regression analysis used the TBI of the left and right foot of the same patient if available, but corrected for clustering within the same patient. Stata® 1221 was used for all analyses. A p-value < 0.05 was regarded as statistically significant.

Results

One hundred and seventeen patients were seen, of whom 108 had complete data for analysis. Of these, 47% were men, 42% were black, 12% were current smokers, 30% were ex-smokers (stopped > 1 year ago), 93% had hypertension, 17% had a history of myocardial infarction and 81% were statin users.

In Table I, patients with and without PAD are compared. The only variable that distinguishes the two groups is smoking (p-value = 0.08). Beta-2 microglobulin was higher in the PAD group, but also had a higher standard deviation. Possibly, this partly explains why the difference was not significant. The sample size was not large enough to stratify on estimated glomerular function. The Spearman rank correlation coefficient between β_2 microglobulin and serum creatinine was 0.41 (p-value < 0.0001).

The National Kidney Disease Education Program²² recommends that laboratories report eGFR values of 60 ml/minute/1.73 m² (1 ml/second/1.73 m²) or greater simply as \geq 60 ml/minute/1.73 m². Thus, patients were categorised into those below 60 ml/minute/1.73 m², and those above.

In Figure 1, there is a negative linear relationship between inverse β_2 microglobulin and TBI. As β_2 microglobulin increases, TBI decreases, and vice versa, or inverse β_2 microglobulin. However, this relationship is not true for the subjects with an eGFR \geq 60 ml/minute/1.73 m².

In Table II, the regression coefficients for eGFR category (\geq 60 vs. < 60), inverse β_2 microglobulin, and the interaction term (eGFR category x inverse β_2 microglobulin) are given. The interaction term is highly statistically significant, as can be deduced by the regression lines that are not parallel in Figure 1. Sex and age did not relate to PAD or β_2 microglobulin, and so were not included in the models (data not shown).

Table I: A comparison between patients with and those without peripheral arterial disease

Characteristics	No PAD (n = 65)	PAD (n = 43)	p-value
Sex			
Male	29 (44.6%)	22 (51.2%)	0.64
Female	36 (55.4%)	21 (48.8%)	
Diabetes mellitus type			
Type 1	9 (14.8%)	4 (9.8%)	
Type 2	52 (85.2%)	37 (90.2%)	
Co-morbidities			
Amputation	3 (5.0%)	4 (9.8%)	0.44
Hypertension	54 (88.5%)	39 (97.5%)	0.14
Revascularisation	9 (15.0%)	7 (17.1%)	0.99
Myocardial infarction	10 (16.7%)	7 (17.1%)	0.83
Intermittent claudication	8 (12.3%)	9 (20.9%)	0.23
Smokers			
No	41 (67.2%)	18 (45.0%)	0.08
Yes	5 (8.2%)	7 (17.5%)	
Ex	15 (24.6%)	15 (37.5%)	
Race			0.41
White	26 (40.0%)	22 (51.2%)	
Black	27 (41.5%)	18 (41.9%)	
Coloured	5 (7.7%)	1 (2.3%)	
Indian	7 (10.8%)	2 (4.7%)	
Other			
Cholesterol (mmol/l)	4.65 (1.29)	4.67 (1.30)	0.96
Triglycerides (mmol/l)*	1.50, 1.10, 2.57	1.80, 1.32, 2.18	0.74
HDL (mmol/l)	1.02 (0.31)	1.01 (0.32)	0.93
LDL (mmol/l)	2.79 (1.18)	2.68 (0.92)	0.62
Creatinine (μ mol/l)*	91, 77, 102	90.5, 80.3, 112.3	0.50
TBI right	0.95 (0.11)	0.70 (0.17)	< 0.001
TBI left	0.93 (0.12)	0.64 (0.13)	< 0.001
β_2 microglobulin (mg/l)*	2.0, 1.6, 2.5	2, 1.7, 2.8	0.35
eGFR (ml/minute/1.73 m ²)	65.20 (21.67)	65.73 (21.06)	0.90

* median and interquartile range

eGFR: estimated glomerular filtration rate, HDL: high-density lipoprotein, LDL: low-density lipoprotein, PAD: peripheral arterial disease, TBI: toe brachial index

Discussion

In this relatively small sample of 108 patients with diabetes, β_2 microglobulin did not significantly distinguish between those patients with and those without PAD.

Patients with PAD had a greater variation in β_2 microglobulin levels, as depicted by the standard deviation, than those without PAD.

If the results are evaluated graphically using a regression interaction term, the relationship between β_2 microglobulin and TBI appears to be modified by the level of eGFR (the association of β_2 microglobulin with TBI is different at different levels of eGFR).

The results do not support the findings of Wilson et al.¹³ However, our findings were contrary to what we expected. We expected that as the eGFR decreased, higher β_2 microglobulin levels would not correlate with lower TBI levels. We observed the relationship being reversed when eGFR was ≥ 60 ml/minute per 1.73 m^2 .

Unfortunately, at the time that the study was carried out, our laboratory had not yet standardised the creatinine measurements (traceable to isotope dilution mass spectrometry). This would have allowed us to calculate eGFR according to the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) method, which gives better estimates, especially when the GFR is > 60 ml/minute per 1.73 m^2 .²³

Table I and Figure 1 show that the variation in β_2 microglobulin was greater in both the groups with PAD and the group with eGFR ≥ 60 ml/minute per 1.73 m^2 . This could explain why the same relationship was not observed across the categories of PAD and eGFR.

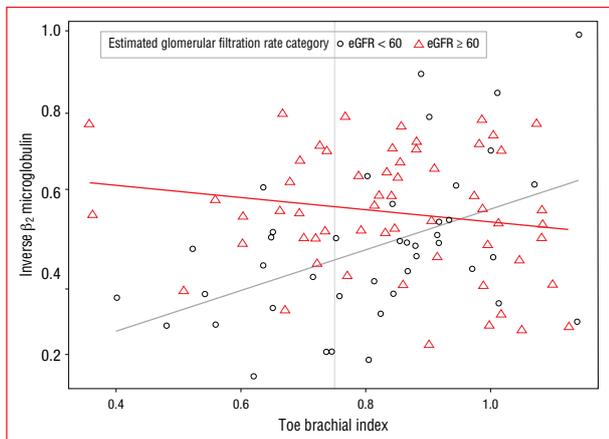
Another possible explanation for the discrepancy could be that the bouts of ischaemia that were postulated to cause the release of β_2 microglobulin are dependent on the stage of chronic kidney disease, as the stage of the disease possibly correlates with the degree and manifestations of atherosclerosis.

Our study was limited because of its cross-sectional nature and its small sample size. However, in clinical practice, a marker should be able to clearly distinguish

between diseased from nondiseased persons in 100 patients, otherwise its clinical usefulness is doubtful.

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eGFR: estimated glomerular filtration rate, TBI: Toe brachial index

Figure 1: The relationship between toe brachial index and inverse β_2 microglobulin by estimated glomerular filtration rate category

Table II: Toe brachial index regression coefficients adjusted for clustering of individuals

	Change in TBI (coefficient)	Standard error	95% CI (lower limit)	95% CI (upper limit)	p-value
eGFR category	0.33	0.11	0.12	0.55	0.003
Inverse β_2 microglobulin	0.41	0.11	0.20	0.62	< 0.001
Interaction term	-0.62	0.19	-0.99	-0.25	0.001
Constant	0.62	0.06	0.50	0.75	< 0.001

CI: confidence interval, eGFR: estimated glomerular filtration rate, TBI: toe brachial index