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 intrapopliteal arterial occlusive disease and vascular calcification than nondiabetic cohorts.\(^7\) The Hoorn study\(^7\) 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, \( \beta_2 \) microglobulin was identified as a biomarker for PAD.\(^\text{13}\) After identification, \( \beta_2 \) microglobulin was assessed in a validation study in a population at risk of acquiring PAD. Serum \( \beta_2 \) microglobulin was higher in patients with PAD undergoing coronary angiography (\( n = 237 \)). Also, the combination of \( \beta_2 \) 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.\(^\text{13}\)

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 \( \beta_2 \) microglobulin was 7.2 (95% CI: 1.6-31.3, p-value = 0.009). The odds ratio was 1.3 (95% CI: 1.1-1.7, p-value = 0.026) for high-sensitivity CRP.\(^\text{13}\) Because of its probable role in immunity and inflammation, the association of \( \beta_2 \) microglobulin with PAD or with alterations in vascular structure\(^\text{14}\) could relate to vascular inflammation.\(^\text{15}\)

The abovementioned study demonstrated the ability of \( \beta_2 \) 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 \( \beta_2 \) microglobulin increases with diminished renal function.\(^\text{16}\) The normal synthesis rate of \( \beta_2 \) 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.\(^\text{17}\) We investigated the diagnostic accuracy of \( \beta_2 \) 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.\(^\text{18}\)

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.\(^\text{19,20}\)

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**

\( \beta_2 \) microglobulin was assayed by nephelometry with Siemens N Latex \( \beta_2 \) 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 β₂ microglobulin could distinguish between patients with and without PAD, and to investigate the association between β₂ 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 the TBI and renal function. The Spearman rank correlation coefficient was calculated to account the renal function of the patients. The only variable that distinguishes the two groups is smoking (p-value = 0.08). Beta-2 microglobulin increases, TBI decreases, and vice versa, if the results are evaluated graphically using a regression interaction term, the relationship between β₂ microglobulin and TBI appears to be modified by the level of eGFR (the association of β₂ microglobulin with TBI is different at different levels of eGFR).

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             | 7 (12.3%)     | 17 (40.9%)  |         |
| **Race**        |               |             |         |
| White           | 26 (40.0%)    | 22 (51.2%)  | 0.41    |
| 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 (µmol/l)* | 91, 77, 102 | 90.5, 80.3, 112.3 | 0.30 |
| TBI right       | 0.95 (0.11)   | 0.70 (0.17) | < 0.001 |
| TBI left        | 0.93 (0.12)   | 0.64 (0.13) | < 0.001 |
| β₂ microglobulin (mg/l)* | 2.0, 1.6, 2.5 | 2.17, 2.7, 2.8 | 0.35 |
| eGFR (ml/minute/1.73 m²)* | 65.20 (21.67) | 65.73 (21.06) | 0.90 |

* median and interquartile range

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

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 β₂ microglobulin and serum creatinine was 0.41 (p-value < 0.0001).

The National Kidney Disease Education Program recommends that laboratories report eGFR values of 60 ml/minute/1.73 m² (1 ml/second/1.73 m²) or greater simply as ≥ 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 β₂ microglobulin and TBI. As β₂ microglobulin increases, TBI decreases, and vice versa, or inverse β₂ microglobulin. However, this relationship is not true for the subjects with an eGFR ≥ 60 ml/minute/1.73 m². In Table II, the regression coefficients for eGFR category (≥ 60 vs. < 60), inverse β₂ microglobulin, and the interaction term (eGFR category x inverse β₂ 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 β₂ microglobulin, and so were not included in the models (data not shown).

Discussion

In this relatively small sample of 108 patients with diabetes, β₂ microglobulin did not significantly distinguish between those patients with and those without PAD. Patients with PAD had a greater variation in β₂ 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 β₂ microglobulin and TBI appears to be modified by the level of eGFR (the association of β₂ microglobulin with TBI is different at different levels of eGFR).
The results do not support the findings of Wilson et al.1 However, our findings were contrary to what we expected. We expected that as the eGFR decreased, higher β2-microglobulin levels would not correlate with lower TBI levels. We observed the relationship being reversed when eGFR was ≥ 60 ml/minute per 1.73 m².

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 m².23

Table I and Figure 1 show that the variation in β2-microglobulin was greater in both the groups with PAD and the group with eGFR ≥ 60 ml/minute per 1.73 m². This could explain why the same relationship was not observed across the categories of PAD and eGFR.

Another possible explanation for the discrepancy is that the bouts of ischaemia that were postulated to cause the release of β2-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 disease from nondiseased persons in 100 patients, otherwise its clinical usefulness is doubtful.

References

1. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA. 2002;287(19):2570–2582.
2. Newman AB, Siscovick DS, Manolio TA, et al. Ankle-brachial index as a marker of atherosclerosis in the Cardiovascular Health Study. Circulation. 1993;88(3):837-845.
3. Abbott RD, Brand FN, Kannell WB. Epidemiology of some peripheral arterial findings in diabetic men and women. Am J Med. 1990;88(4):376-381.
4. Meijer KT, Hoeks AP, Rutgers D, et al. Peripheral arterial disease in the elderly: the Rotterdam Study. Arterioscler Thromb Vasc Biol. 1998;18(2):185-192.
5. Hlati WR, Hoag S, Hamman RF. Effect of diagnostic criteria on the prevalence of peripheral arterial disease. Circulation. 1995;91(5):1472-1479.
6. Jude EB, Oyibo SO, Chalmers N, Boulton AJ. Peripheral arterial disease in diabetic and nondiabetic patients. Diabetes Care. 2001;24(8):1433-1437.
7. Beks PJ, Mackaay AJ, De Neeling JN, et al. Peripheral arterial disease in relation to glycaemic level in an elderly Caucasian population: the Hoorn study. Diabetologia. 1995;38(1):86-96.
8. Uusitupa M, Niikami LT, Sihvonen O, et al. Five-year incidence of atherosclerotic vascular disease in relation to general risk factors, insulin level, and abnormalities in lipoprotein composition in non-insulin-dependent diabetic and nondiabetic subjects. Circulation. 1990;82(1):27-36.
9. Kannell WB, McGree DL. Update on some epidemiologic features of intermittent claudication: the Framingham Study. J Am Geriatr Soc. 1985;33(1):13-18.
10. Centers for Disease Control and Prevention. Diabetes-related amputations of lower extremities in the Medicare population: Minnesota, 1993-1995. MMWR Morb Mortal Wkly Rep. 1998;47(31):649-652.
11. Brasciale TJ. Antigen processing for presentation by MHC class I molecules. Curr Opin Immunol. 1992;4(1):59-62.
12. B2M gene. PubMed [homepage on the Internet]. c2012. Available from: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene&CmdShwResult=DetailView&TermDest ar=567
13. Wilson AM, Kimura E, Harada RK, et al. Beta-2-microglobulin as a biomarker in peripheral arterial disease: proteomic profiling and clinical studies. Circulation. 2007;116(12):1396-1403.
14. Saip J, Utug M, Yoshida E, et al. Relationship of beta2-microglobulin to arterial stiffness in Japanese subjects. Hypertens Res. 2005;28(4):505-511.
15. Tazukui I, Murray GD, Lee AJ, et al. C-reactive protein, interleukin-6, and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population: Edinburgh Artery Study. Circulation. 2005;112(7):976-983.
16. Viberli GC, Keen H, Mackintosh D. Beta-2-microglobulinaemia: a sensitive index of diminishing renal function in diabetes. Br Med J (Clin Res Ed). 1981;282(6258):95-98.
17. Al-Taee K, Al-Safl Al-Falahi YS, Al-Banna IA. The clinical significance of β2-microglobulin in end-stage renal disease. Saudi J Kidney Dis Transpl. 2003;14(4):492-496.
18. Rose GA, Blackburn H, Gillum RF, Prineas RJ. Cardiovascular survey methods. Geneva: World Health Organization; 1982.
19. Ramsey DE, Manke DA, Summer DS. Toe blood pressure: a valuable adjunct to ankle pressure measurement for assessing peripheral arterial disease. J Cardiovas Surg. 1983;24(1):43-48.
20. Williams DT, Harding KG, Price P. An evaluation of the efficacy of methods used in screening for lower-limb arterial disease in diabetes. Diabetes Care. 2005;28(9):2206-2210.
21. StataCorp. 2011. Stata Statistical Software: release 12. College Station, TX: StataCorp LP.
22. National Kidney Disease Education Program [homepage on the Internet]. c2011. Available from: www.nkdep.nih.gov/labprofessionals
23. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-612.

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

| eGFR category | Change in TBI (coefficient) | Standard error | 95% CI (lower limit) | 95% CI (upper limit) | p-value |
|---------------|-----------------------------|----------------|---------------------|---------------------|---------|
| eGFR < 60     | 0.33                        | 0.11           | 0.12                | 0.55                | 0.003   |
| eGFR ≥ 60     | 0.41                        | 0.11           | 0.20                | 0.62                | < 0.001 |
| Inverse β2-microglobulin | 0.62                        | 0.06           | 0.50                | 0.75                | < 0.001 |

E: estimated glomerular filtration rate, TBI: toe brachial index