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Original article

The association between endostatin and kidney disease and mortality in patients with type 2 diabetes

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Abstract

Aim. – Circulating endostatin, a biologically active derivate of collagen XVIII, is considered to be a marker of kidney disease and a risk factor for its related mortality. However, less is known of the role of endostatin in diabetes and the development of diabetic nephropathy. For this reason, our study investigated the associations between circulating endostatin and the prevalence and progression of kidney disease, and its mortality risk in patients with type 2 diabetes (T2D).

Methods. – This was a cohort study of 607 patients with T2D (mean age: 61 years, 44% women). Estimated glomerular filtration rate (eGFR), calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation, was used to assess the patients' kidney function decline and mortality.

Results. – Of the total study cohort, 20 patients declined by ≥20% in eGFR over 4 years, and 44 died during the follow-up (mean duration: 6.7 years). At baseline, participants with diabetic nephropathy (defined as eGFR < 60 mL/min/1.73 m^2) and/or microalbuminuria [defined as a urinary albumin-to-creatinine ratio (ACR) > 3 g/mol] had higher median levels of endostatin than those without nephropathy (62.7 μg/L *vs* 57.4 μg/L, respectively; $P = 0.031$). In longitudinal analyses adjusted for age, gender, baseline eGFR and ACR, higher endostatin levels were associated with a higher risk of decline (≥20% in eGFR, OR per 1 SD increase: 1.73, 95% CI: 1.13–2.65) and a higher risk of mortality (HR per 1 SD increase: 1.57, 95% CI: 1.19–2.07).

Conclusion. – In patients with T2D, circulating endostatin levels can predict the progression of kidney disease and mortality independently of established kidney disease markers. The clinical usefulness of endostatin as a risk marker in such patients merits further studies.

Keywords: Albumin-to-creatinine ratio; Angiogenesis; Chronic kidney disease; Community; Extracellular matrix remodeling; NIDDM

1. Introduction

Type 2 diabetes (T2D) is becoming increasingly more common and appears to be tailing the obesity epidemic worldwide [1,2]. As a consequence, the incidence of diabetic nephropathy and, in turn, cardiovascular disease (CVD) is also expected to increase dramatically.

Collagen XVIII is highly expressed in Bowman's capsule and the tubular basal membranes of the kidney [3,4]. Endostatin is a fragment of collagen XVIII formed during extracellular matrix (ECM) remodelling; it has anti-angiogenic effects that have been proposed to affect the development of both the micro- and macrovascular complications of diabetes [5]. Both endostatin and collagen XVIII have been shown to be modified in different types of renal disease, including glomerulonephritis

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and nephropathy in type 1 diabetes [6,7], and recent animal studies have pointed to a causal link between circulating levels of endostatin and physiological renal responses [8,9].

Circulating endostatin levels have been proposed as a promising marker for hypertensive end-target organ damage in the kidneys, vascular system and heart [10]. Moreover, endostatin levels were recently shown to parallel kidney function decline [11], and have been associated with an increased mortality risk in different settings [12,13]. However, data on the relevance of circulating endostatin in patients with T2D are scarce.

Based on previous experimental and clinical studies, it was hypothesized that endostatin is causally involved in the development of diabetic nephropathy and associated with adverse outcomes in T2D. For this reason, the present study evaluated the cross-sectional association between endostatin levels and kidney disease, and sought to determine whether endostatin levels are longitudinally associated with deterioration of kidney function and increased mortality in patients with T2D.

2. Methods

Data from the Cardiovascular Risk Factors in Patients with Diabetes: A Prospective Study in Primary Care (CARDIPP) were analyzed. The study was launched in 2005, and its baseline data collection was completed in November 2008. The general aim of CARDIPP was to investigate cardiovascular risk factors in middle-aged patients with T2D to facilitate early and individually adjusted risk interventions. Patients aged 55–65 years were consecutively recruited during their usual annual checkups at 22 primary healthcare centres in the counties of Östergötland and Jönköping in Sweden, irrespective of their previous blood pressure and CVD status. The centres varied in size and were located in different geographical areas, but all followed the national guidelines for diabetes care. Patients with other severe concomitant diseases, such as cognitive impairment and cancer, were not included.

A questionnaire was used to obtain information about lifestyle habits, including alcohol consumption and smoking status, while a standardized medical history provided data on diabetes duration, medication use and previous cardiovascular events. A single spot urine test and blood samples were taken at the healthcare centres in the morning, following at least 10 h of fasting, for laboratory analyses. Clinical data such as body weight, height and waist circumference were also recorded, and blood pressure was calculated as the average of three sitting measurements taken 1 min apart by specially trained nurses at each centre.

Altogether, CARDIPP enrolled 761 participants; 58 were missing serum samples in the biobank and an additional 71 had suffered a myocardial infarction or stroke prior to baseline data collection, and so were excluded from the present analyses, leaving a study sample of 632 patients with endostatin measurements. However, after further exclusion of those with missing baseline data for estimated glomerular filtration rate (eGFR) or albumin-to-creatinine ratio (ACR), 607 individuals remained for the present analyses. Repeat examinations of the participants were performed after approximately 4 years; at that examination, 372 participants had data for eGFR.

The present study was approved by the regional ethics review board based in Linköping, and all participants gave their written informed consent.

2.1. Laboratory analyses

Initially, the standard Swedish high-performance liquid chromatography (HPLC) Mono-S method was used to measure HbA_{1c}, although the data were later converted to the Diabetes Control and Complications Trial (DCCT) standards (%) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units (mmol/mol). Serum and urine creatinine were measured at a local clinical-chemistry laboratory (a SWEDAC-accredited medical laboratory in Östergötland), using an IDMS (Integrated Database Management System)-calibrated modified Jaffe method in an ADVIA 1800 analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). Coefficients of variation (CV) for the creatinine method were 4.3% at 90 μmol/L and 3.1% at 380 μmol/L. Urine albumin was analyzed with the same instrument as creatinine, using reagents from the manufacturer.

The patients' eGFR was based on creatinine according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [14], while urinary ACR was used as a measure of albuminuria. Kidney dysfunction was defined as an eGFR < 60 mL/min/1.73 m^2, and micro-/macroalbuminuria was defined as an ACR < 3 g/mol. The presence of diabetic nephropathy was defined as having kidney dysfunction and/or micro-/macroalbuminuria (MA) at baseline.

Blood and urine samples were frozen for later analyses; serum levels of endostatin were analyzed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit for endostatin (#DY1098, R&D Systems, Minneapolis, MN, USA). The microtitre plates were coated with a monoclonal anti-Endostatin antibody. After washing, the plates were blocked with a 1% bovine serum albumin (BSA) buffer (0.02 M NaH_2PO_4, 0.15 M NaCl, pH 7.2). After this step, the samples were added, after dilution to 1:40 in BSA buffer, together with calibrators. Eight calibrator points were analyzed as duplicates, while the samples were analyzed as singletons. After incubation and washing, a biotinylated anti-Endostatin antibody, diluted in BSA buffer, was added. After another incubation and washing cycle, a streptavidin–horseradish peroxidase (HRP) conjugate was added. After the plate was again incubated and washed, a substrate solution was added. Substrate development was stopped and its colour intensity measured. These assays had a total CV of approximately 7%. All laboratory tests were performed blinded with no knowledge of the patients' outcomes.

2.2. Definitions of outcomes

The patients' GFR decline was defined as the absolute decline in eGFR at 4 years after baseline compared with baseline values.