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Soluble tumor necrosis factor receptor 1 is associated with diminished estimated glomerular filtration rate in colombian patients with type 2 diabetes

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ABSTRACT

Aims: The tumor necrosis factor α (TNF-α) family of inflammatory molecules plays a crucial role in the pathogenesis of type 2 diabetes mellitus (DM2) complications. TNF-α soluble receptors 1 (sTNFR1) and 2 (sTNFR2) have been associated with chronic kidney disease in DM2 patients. This cross-sectional study intended to determine serum concentrations of sTNFR1 and sTNFR2 in Colombian patients and correlated them with various clinical variables, especially kidney function.

Methods: 92 Colombian patients with DM2 were recruited. Anthropometric variables, glycemic control parameters, lipid profile and renal function were assessed for each patient. Levels of sTNFR1 and sTNFR2 were determined using ELISA. Patients were stratified in two groups according to reduced estimated glomerular filtration rate (eGFR) (<60 ml/min/1.73 m^2) and normal eGFR (≥60 ml/min/1.73 m^2).

Results: Significantly elevated levels of sTNFR1 and sTNFR2 were observed in the diminished versus normal eGFR group. Also, significant differences were noticed between both groups in haemoglobin A1c (HbA1c) values, percentage of hypertensive subjects treated with angiotensin receptor blocker (ARB) and subjects treated with metformin. No differences were observed regarding body mass index (BMI), albuminuria and lipid profile. Multivariable linear regression analysis revealed that sTNFR1 alone showed a significant association with low eGFR ($p = 0.009$). However, after adjusting for age, the association weakens. Moreover, sTNFR1 and sTNFR2 showed a linear negative correlation with eGFR ($r = -0.448$, $p < 0.001$ and $r = -0.376$, $p < 0.001$, respectively). A positive correlation was also seen between sTNFR1 and HbA1c, whereas a negative correlation between both sTNFRs and high-density lipoprotein (HDL) cholesterol was found.

Conclusion: Elevated levels of sTNFRs, especially sTNFR1, are associated with loss of kidney function in Hispanic patients with DM2. Future studies should focus on social and genetic determinants of inflammation and their association with CKD in this ethnicity.

1. Introduction

Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine predominantly secreted by immune cells. It exerts its effects by interacting with two specific membrane receptors: TNF receptor 1 (TNFR1, CD120a, p55) and TNF receptor 2 (TNFR2, CD120b, p75), each with specific functions in the cell (Wajant, Pfizenmaier, & Scheurich, 2003). Both membrane receptors and TNF-α itself are cleaved by the TNF-α converting enzyme (TACE), also known as disintegrin and metalloprotease protein – 17 (ADAM-17), and are released into circulation (Blobel, 2005; Bradley, 2008). TNF-α may be found in the serum in its free form or bound to the soluble TNF-α receptors (sTNFR1 and sTNFR2) (Blobel, 2005). The exact function of sTNFRs has not been completely elucidated; studies have suggested they operate as decoys for TNF-α, binding to it and impeding its interaction with membrane-bound receptors (Aderka, 1996; Van Zee et al., 1992). Both soluble TNF receptors have been postulated as anti-inflammatory molecules (Xanthoulea et al., 2004). Special attention has been raised regarding the role of sTNFR1 and sTNFR2 in diabetic nephropathy, since several studies show an important association between elevated levels of these molecules and decline in kidney function in DM2 patients (Izumi et al., 2013; Lin, Hu, Mantzoros, & Curhan, 2010; Lin, Hu, Rimm, Rifai, & Curhan, 2006).

Among the micro-vascular complications of DM2, diabetic nephropathy accounts for nearly 50% of end-stage renal disease (ESRD) cases

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worldwide (Collins, Foley, Gilbertson, & Chen, 2015). In the United States up to 42% of diabetic patients suffer from chronic kidney disease (CKD) (Bailey, Wang, Zhu, & Rupnow, 2014). Although precise data for CKD in Latin America are lacking, the incidence of ESRD patients needing renal replacement therapy (RRT) in this region has increased dramatically, and diabetes is the leading cause (Gonzalez-Bedat et al., 2015). The pathophysiology of CKD in DM2 patients is complex, involving hemodynamic pathways (e.g. renin-angiotensin-aldosterone system), oxidative stress mediators (advanced glycation end-products), and profibrotic cytokines (transforming growth factor β) (Arora & Singh, 2013). Chronic-low grade inflammation may play a crucial role in the pathogenesis of kidney damage, due to the action of several pro-inflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), and especially tumor necrosis factor α (TNF-α) (Navarro-González, Mora-Fernández, Muros de Fuentes, & García-Pérez, 2011; Wada & Makino, 2013).

The relation between sTNFRs and kidney disease has been studied predominantly in Caucasian and Asian DM2 patients. sTNFRs have been proposed as early biomarkers and predictors of diabetic nephropathy (Gohda & Tomino, 2013). However, to the best of our knowledge, this link has not been extensively investigated in Hispanic patients. Several of these previous studies exclude subjects due to several clinical conditions such as obesity, hypertension and hyperlipidemia because they are modifiers of chronic low-grade inflammation. The aim of this study was to determine concentrations of sTNFR1 and sTNFR2 in Colombian patients with type 2 diabetes mellitus and correlate them not only to renal function, but also to anthropometric variables (body mass index, waist circumference), lipid profile, and medication usage.

2. Subjects and methods

This study was conducted on the premises of the National University of Colombia, once approved by the institutional Ethics Committee in accordance with the Helsinki Declaration. The objectives and procedures of the study were explained to patients, and a signed informed consent form was obtained from all participants. Patients considered for this study were outpatients belonging to the "Program for the Prevention of Diabetes Complications" of the Lipids and Diabetes Laboratory, Faculty of Medicine, National University of Colombia. All of them were diagnosed with DM2 based on the American Diabetes Association (ADA) criteria (American Diabetes Association, 2015). Exclusion criteria included active autoimmune or neoplastic diseases, psychiatric disorders receiving medication, pregnancy, or age under 18. A total of 98 Colombian patients with type 2 diabetes mellitus were eligible and enrolled in the present study. In a period of three months, each of these patients received their usual follow-up medical exam, during which anthropometric variables, body mass index (BMI) (kg/m^2) and waist circumference (cm) were determined. Blood pressure (mmHg) was determined clinically with a mercury sphygmomanometer. Results from their routine metabolic and lipid profile were assessed: plasma glucose (mg/dl), triglycerides (mg/dl), total cholesterol (mg/dl), HDL cholesterol (mg/dl), and serum creatinine (mg/dl). LDL cholesterol was calculated using the Friedewald formula (Friedewald, Levy, & Fredrickson, 1972). Haemoglobin A1c (HbA1c) was reported in NGSP units (%) and in IFCC units (mmol/mol). All laboratory exams were determined from blood samples taken after an 8 hour fast, in a range of 1 to 3 days previous to medical consultation. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation as recommended by current guidelines (L. et al., 2013).

On the morning of the trial day (a range of 1 to 3 days after medical exam), after a 12 hour fast, blood samples for determining sTNFR1 and sTNFR2 were drawn from the antecubital vein of each patient. Also, a urine sample for the determination of urine albumin-to-creatinine ratio (UACR) was collected. None of the patients showed evidence of current acute illness, significant infectious disease or acute exacerbation of chronic disease. All subjects did not consume alcohol or perform heavy exercise at least one week prior to trial day. Medications received by each patient were assessed. Collected blood samples were centrifuged at 3500 rpm for 5 min. Separated serum was stored at −80 °C until the day of the assay. sTNFR1 and sTNFR2 concentrations were determined using the enzyme-linked immunosorbent assay (ELISA) method according to manufacturer`s instructions (R&D Systems, Minneapolis, USA).

2.1. Statistical analysis

Data are presented as means ± standard error of the mean (SEM). Statistical analyses were conducted using SPSS and R statistical software. Patients were divided into two groups for comparison: subjects with reduced GFR (<60 ml/min/1.73 m^2), and subjects with normal GFR (≥60 ml/min/1.73 m^2). A Student's T test was used to compare mean values of both groups. Posteriorly, a multivariable regression analysis was performed to determine the influence of significant variables on the eGFR. Also, a Pearson analysis was used to estimate correlations between values of sTNFRs and the other clinical variables. A p value less than 0.05 was considered significant.

3. Results

3.1. Reduced vs normal eGFR

Of the 98 eligible patients, 92 were included in the current study; the other 6 subjects experienced difficulties during blood extraction or could not collect the urine sample for UACR determination. Table 1 shows the demographic and clinical characteristics of this population. Fifty-two men and 40 women participated. Mean age at the time of the study was 69.4 ± 7.6 years. The mean eGFR was 69 ± 12.8 for the 92 patients; 24 subjects presented reduced eGFR (52 ± 5.1, range of 41 to 59 ml/min/1.73 m^2), while 68 subjects had normal filtration rate (75 ± 8.7, range of 60 to 96 ml/min/1.73 m^2). For men, the mean eGFR was 70 ± 11.7, for women 68 ± 14.2. When analyzing the impact of gender over eGFR, there were no statistical differences between men and women ($p = 0.55$).

When comparing the clinical characteristics of the two groups, significant differences were found in age ($p < 0.001$), HbA1c ($p = 0.042$), treatment with metformin ($p = 0.027$) and hypertension treated with angiotensin receptor blocker (ARB) ($p = 0.045$).

Significant differences were found between both groups regarding levels of sTNFRs. Patients with low eGFR presented increased levels of sTNFR1 compared to the normal eGFR group (1733.8 ± 508.6 pg/ml vs 1277.1 ± 434.8, $p < 0.001$). A similar result was observed for sTNFR2 (4028. 3 ± 1064.8 vs 3126.1 ± 936.7, $p < 0.001$).

Posteriorly, a multivariable regression analysis was performed to determine the influence of the significant variables over eGFR. Table 2 depicts the results of the model; whereas univariable analysis showed HbA1c, metformin use, hypertension treated with ARB, sTNFR1 and sTNFR2 were associated with reduced eGFR, multivariable regression analysis revealed only sTNFR1 showed a significant correlation with eGFR < 60 ml/min/1.73 m^2 ($p = 0.009$). However when the model is adjusted for age, the association between sTNFR1 and low eGFR weakens.

3.2. Correlation of sTNFRs with other variables

A linear correlation was performed to estimate the correlations of sTNFR1 and sTNFR2 with the other clinical variables. Table 3 shows a summary of the results. There was a significant inverse correlation between sTNFR1 levels and eGFR (r: −0.448, $p < 0.001$), and between sTNFR2 levels and eGFR ($r = -0.376$, $p < 0.001$). In terms of risk, for every increase of 100 pg/ml in sTNFR1 the probability of having eGFR < 60 ml/min/1.73 m^2 increases by 15% (OR = 1.152, $p = 0.034$). Also, a positive correlation was observed between sTNFR1