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Association of biomarkers of inflammation and oxidative stress with the risk of chronic kidney disease in Type 2 diabetes mellitus in North Indian population ☆

Stuti Gupta, Jasvinder K. Gambhir *, OP Kalra, Amar Gautam, Kirtikar Shukla, Mohit Mehndiratta, Sunil Agarwal, Rimi Shukla

Departments of Biochemistry and Medicine, University College of Medical Sciences (University of Delhi) & GTB Hospital, Delhi, 110095 India

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ABSTRACT

Chronic kidney disease (CKD) is a major cause of morbidity and mortality worldwide. It results from diverse etiologies, diabetes being a frontrunner amongst them. Type 2 diabetes mellitus (DM) is being increasingly recognized as a proinflammatory state with increased oxidative stress which enormously increases the risk of micro and macro vascular diseases. This study was planned to explore the possible association between tumor necrosis factor-alpha (TNF-α), urinary monocyte chemoattractant protein-1 (uMCP-1), high-sensitivity C-reactive protein (hsCRP) and parameters of oxidative stress in patients with Type 2 diabetes mellitus (DM) and diabetic chronic kidney disease (DM–CKD). Fifty patients each were recruited in DM, DM–CKD and healthy control groups. Plasma TNF-α, hsCRP and uMCP-1 levels as inflammatory mediators were measured by ELISA, reduced glutathione (GSH), ferric reducing ability of plasma (FRAP) as parameters of antioxidant activity and malondialdehyde (MDA) as marker of oxidative stress, were measured spectrophotometrically. Plasma TNF-α, hsCRP and uMCP-1 were significantly higher in DM–CKD compared to DM and healthy controls. Lipid peroxidation, measured as MDA was significantly higher in patients with DM–CKD as compared to patients with DM and healthy controls. Further, antioxidant capacity of blood measured as FRAP and GSH was found to be significantly lower in patients with DM and DM–CKD as compared to healthy controls ($p < 0.001$). Plasma TNF-α and uMCP-1 showed a significant positive correlation with HbA_{1c} ($r = 0.441, 0.643$), hsCRP ($r = 0.400, 0.584$) and MDA ($r = 0.423, 0.759$) and significant negative correlation with GSH ($R = -0.370, -0.800$) and FRAP ($r = -0.344, -0.684$) Increased inflammatory markers viz. TNF-α, hsCRP and uMCP-1 and markers of oxidative stress i.e. increased MDA and decreased GSH and FRAP in DM–CKD suggest an important role of inflammation and oxidative stress in the pathogenesis of renal damage in diabetic patients.

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most challenging health concerns of the 21st century. T2DM is a proinflammatory state with increased oxidative stress, predisposing the patients to macro- and micro-vascular complications. Diabetic nephropathy (DN), a microvascular complication of long term uncontrolled DM is the single most frequent cause of end-stage renal disease (ESRD) (Ritz, Rychlik, Locatelli, & Halimi, 1999), accounting for approximately 40% of new cases of ESRD every year (Kramer & Molitch, 2005).

Although metabolic and hemodynamic alterations are considered the main cause of renal damage in diabetes, the accumulating evidence now indicates that immunologic and inflammatory mechanisms also play a significant role in its development and progression (Tuttle, 2005; Mora & Navarro, 2006). Diabetics have been shown to produce excessive inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) (Zinman, Hanley, Harris, Kwan, & Fantus, 1999), and few studies have also reported association between inflammatory biomarkers and DN (Navarro, Mora, Muros, & García, 2006; Yeo, Hwang, Park, Choi, Huh, & Kim, 2010; Lu, Randell, Han, Adeli, Krahn, & Meng, 2011; Taslipinar et al., 2011; Fernández-Real, Vendrell, García, Ricart, & Vallès, 2012). Most attention has been focused on the role of TNF-α, which is a pleiotropic proinflammatory cytokine primarily synthesized by monocytes, macrophages and T cells and plays an important role in pathogenesis of DN. Many studies in animal models have shown that intrinsic renal cells, including glomerular, mesangial, endothelial and tubular cells, are also able to produce this cytokine (Jevnikar et al., 1991; Nakamura et al., 1993; Sugimoto, Shikata, Wada, Horiuchi, & Makino, 1999; Dong, Swaminathan, Bachman, Croatt, Nath, & Griffin, 2007; Zhang,

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* Corresponding author. Tel.: +91 9811641277.
E-mail address: jassigambhir@yahoo.co.in (J.K. Gambhir).

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Ramesh, Norbury, & Reeves, 2007). TNF-α is not only cytotoxic to renal cells (Bertani et al., 1989), but also induces the production of reactive oxygen species (ROS) in diverse cells, including mesangial cells (Raedke, Meier, Topley, Fluge, Habermehl, & Resch, 1990). C-reactive protein (CRP), a member of pentatraxin family is the prototypic marker of inflammation which has been reported to be associated with T2DM and early stages of nephropathy, however its association with DN is not completely understood (Navarro, Mora, Macia, & Garcia, 2003; Navarro et al., 2006; Navarro-González & Mora-Fernandez, 2008). Recent evidence has highlighted the production of monocyte chemoattractant protein-1 (MCP-1) by diabetic kidneys as a major promoter of inflammation, renal injury and fibrosis in diabetic nephropathy (Tesch, 2008). Urinary levels of MCP-1 (uMCP-1) closely reflect kidney MCP-1 production and correlate significantly with levels of albuminuria, serum glycated albumin, urine *N*-acetylglucosaminidase (NAG) and kidney CD68+ macrophages in human and experimental diabetic nephropathy (Furuta et al., 1993; Gu, Tseng, & Rollins, 1999; Chow, Ozols, Nikolic-Paterson, Atkins, Tesch, et al., 2004).

Accumulating evidence, both experimental and clinical, has suggested that there is a close link between hyperglycemia, oxidative stress, inflammation and diabetic complications including nephropathy (Pan et al., 2010). ROS have been shown to cause renal damage via vasoconstriction, vascular smooth muscle cell growth and migration, endothelial dysfunction, modification of extracellular matrix proteins and increased renal tubular reabsorption. Oxidative stress may also be implicated in promoting a low grade systemic inflammation in patients with T2DM (Amalich et al., 2000). Activation of nuclear factor-kappa B (NF-κB) through oxidative stress induced by hyperglycemia increases the concentration of proinflammatory cytokines (Esposito et al., 2002). A study has shown that elevation of TNF-α in turn increases oxidative stress leading to renal injury in streptozotocin induced diabetes in rats (Kuhad & Chopra, 2009).

In spite of improvement in our knowledge on DN, from a pathophysiologic point of view, the intricate mechanisms in chronic hyperglycemia leading to the development of renal injury are complex and not yet fully understood. Previous reported studies have not included both inflammatory biomarkers and oxidant–antioxidant parameters in a single setting, in addition, no association between uMCP-1, a marker of renal injury in DN and other parameters has been shown in the aforementioned studies. Therefore, the present work aims to evaluate inflammatory cytokines (TNF-α), inflammatory chemokine (MCP-1), inflammatory marker (hsCRP) and oxidant–antioxidant status in order to investigate the relationship between inflammation and oxidative stress in patients with DN.

2. Methodology

2.1. Subjects

The present study included 150 subjects attending Medicine OPD/Nephrology Outpatient Clinic at the University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi. The subjects were divided into 3 groups (n = 50) viz; Group I: healthy controls (HC), Group II: patients with Type 2 diabetes mellitus without nephropathy (DM), Group III: patients with Type 2 diabetes mellitus with nephropathy (DM–CKD). Healthy subjects with systolic and diastolic blood pressure 120 mm Hg and 80 mm Hg respectively, and fasting and postprandial plasma glucose less than 100 ml/dL and 140 ml/dL were recruited as controls from volunteers and staff of UCMS and GTB Hospital. Diagnosis of T2DM was made according to revised American Diabetes Association criteria (American diabetic association, 2012). All diabetic subjects with retinopathy and dipstick positive proteinuria and microalbuminuria were clustered in group III. All patients in group III were in pre-dialysis stage. The sensitivity of the dipstick "Urine Test 11 MAU" from Piramal Diagnostic, India is 10–15 mg/dL. Further spot urine samples were analyzed for urinary albumin creatinine ratio which was 0.40 ± 0.33 in the DM–CKD group.

To avoid potential confounding factors, the patients having acute and chronic infections, fever, malignancy, other renal disorders, cirrhosis of liver and congestive heart failure were excluded. All the patients in group III had retinopathy however patients with macrovascular complications like stroke and CAD were excluded. Patients who were on inhibitors of renin–angiotensin aldosterone system, aspirin and vitamin D analogues were advised to stop these drugs for one week before inclusion in the study. The protocol of this study was approved by the Institutional Ethics Committee for Human Research and informed written consent was taken from all the participants.

2.2. Methods

Relevant clinical history and physical examination were recorded. Arterial blood pressure was measured using mercury sphygmomanometer with the patient in the sitting position after 5 min of rest. The glomerular filtration rate was estimated by 'Modification of Diet in Renal Disease Abbreviated Equation (MDRD)': [eGFR = 186 × (plasma Cr)$^{-1.154}$ × (age)$^{-0.203}$ × (0.742 if female) × (1.210 if African American) (Levey, Bosch, Lewis, Greene, Rogers, & Roth, 1999).

Fasting blood sample was withdrawn from ante-cubital vein under aseptic precautions and collected into vacutainers containing salts of fluoride & oxalate for plasma glucose and EDTA vials for various other parameters. For estimation of reduced glutathione 200 μL of whole blood was used and estimated immediately after collection of sample. For glycosylated hemoglobin 200 μL whole blood was kept as aliquot at 4°C–8°C and estimation was carried out within 1 week of collection. Rest of the EDTA blood sample was subjected to centrifugation at 3000 rpm for 10 min to separate the plasma. GST, MDA and FRAP were estimated from plasma immediately after collection. Remaining plasma was stored in aliquots at −80°C till further use for the estimation of TNF-α and hsCRP. Random mid-stream urine sample was collected for the estimation of the MCP-1 and albumin.

Reduced glutathione (GSH) was estimated by the method of Tietze (1969) and the antioxidant capacity of blood was measured as ferric reducing ability of plasma (FRAP) by the method of Benzie and Strain (1996). Plasma malondialdehyde (MDA) was measured according to the method of Satoh (1978). Glycosylated hemoglobin ($HbA1_C$) was measured by ion-exchange resin chromatography using commercially available kits (Fortress, UK). Plasma and urine samples were stored at −80°C until assayed by ELISA for the measurement of plasma TNF-α (Diaclone, France; sensitivity less than 8 pg/mL), hsCRP (Calbiotech, USA; sensitivity less than 0.005 mg/mL) and uMCP-1 (Weldon, California; sensitivity less than 7.8125 pg/mL). Routine biochemical parameters were assayed in automated analyzer using commercial kits. All these investigations were carried out once at the time of entry into the study.

2.3. Statistical analysis

SPSS-17 software was used for statistical analysis. The results were expressed as mean ± standard deviation (SD). Demographic data and routine biochemical parameters were compared among the subject groups using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test. Pearson's correlation analysis was used to determine correlation between different parameters. The mean difference was considered significant at $p < 0.05$.

3. Results

The baseline demographic characteristics and biochemical parameters of both healthy subjects and diabetic groups are shown in Table 1. There were no significant differences between diabetic patients and controls with respect to sex distribution and BMI. The