Comparison Between Biomarkers of Kidney Injury, Inflammation, and Oxidative Stress in Patients with Diabetic Nephropathy and Type 2 Diabetes Mellitus

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Keywords. diabetic nephropathy, type 2 diabetes mellitus, inflammation, oxidative stress

Introduction. This study was conducted to compare parameters of kidney injury, oxidative stress and inflammation in people with diabetic nephropathy (DN) and type 2 diabetes mellitus (T2DM).
Methods. In a cross-sectional study, 57 cases with DN and 57 cases with T2DM were included in the study. Fasting blood samples were obtained to determine parameters of kidney injury, oxidative stress and inflammation.
Results. The current study showed that patients with DN had higher tumor necrosis factor-α (TNF-α) (167.0 ± 40.1 vs. 151.4 ± 37.4 ng/L, $P < .05$) and matrix metalloproteinase-2 (MMP-2) concentrations (1625.2 ± 631.0 vs. 1391.5 ± 465.4 ng/mL, $P < .05$) compared with T2DM cases. Moreover, we observed a non-significant increase in MMP-9 levels among patients with DN compared with individuals with T2DM (4864.4 ± 1934.3 vs. 4239.2 ± 1853.9 ng/L, $P > .05$). Furthermore, advanced glycation end products (AGEs) levels in patients with DN were higher than that of patients with T2DM (8511.7 ± 1799.9 vs. 7660.7 ± 1711.9 AU, $P < .05$), but the difference in malondialdehyde value was not significant. Finally, we found that total protein levels in cases with DN were enhanced compared with individuals with T2DM (7.1 ± 0.5 vs. 6.9 ± 0.6 mg/dL, $P < .05$); however, other markers of kidney injury did not change.
Conclusions. In conclusion, the results of present study revealed that few markers of inflammation and oxidative stress including TNF-α, MMP-2, AGEs levels and total protein levels in patients with DN were significantly higher than that of patients with T2DN. Further studies are necessary to confirm these findings.

IJKD 2020;14:31-5
www.ijkd.org

Original Paper

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic condition with a variety of comorbidities, which affects different organs in the body and can cause latter disabilities, including amputation, renal failure, stroke and blindness.[1] Kidney disease due to diabetes mellitus which termed as diabetic nephropathy (DN) is one of the most common complications of T2DM and a strong predictor of end-stage renal disease in these patients.[2] It occurs in more than one-third of diabetic subjects regardless of the type of diabetes.[3] The pathogenesis of DN is complex and different factors including genetic background, epigenetic factors, inflammatory

response and oxidative stress are involved in the development of DN.[4,5] In addition, abnormal intracellular metabolism pathways induced by insulin resistance and hyperglycemia, dyslipidemia, glomerular hypertrophy, hyperfiltration and formation of advanced glycation end products (AGEs) have been identified to involve in the initiation and development of DN.[6]

In patients with DN, structural damages such as glomerular basement membrane thickening occur before functional changes and following the progression of disease, proteinuria increases, which is a major risk factor for reduction in kidney function and glomerular filtration rate (GFR).[7] Several investigators have compared clinical signs and metabolic changes between diabetes status and diabetic complications. Mu *et al*.[8] revealed that tumor necrosis factor-α (TNF-α) levels in patients with DN were higher than patients with T2DM. In addition, it was demonstrated that matrix metalloproteinases (MMP) levels are implicated in diabetic complications such as retinopathy, cardiomyopathy, peripheral neuropathy and nephropathy.[9]

The evidence suggested that MMP-2 and MMP-9 are linked to renal hypertrophy and abnormal extra cellular matrix deposition, which are hallmarks of DN.[10] However, the results of early studies are not conclusive, one study showed that urine MMP2/creatinine and MMP9/creatinine ratio were not different between patients with type 1 diabetes mellitus (T1DM) and normal subjects.[11] Therefore, we conducted this cross-sectional study to compare markers of oxidative stress, inflammation and kidney injury between patients with T2DM and DN.

MATERILAS AND METHODS

Participants

This is a cross-sectional study which was carried out among 57 cases with DN and 57 cases with T2DM, aged 40-85 years old referred to Shahid Beheshti Clinic in Kashan, Iran from July 2017-October 218. We defined DN as diabetic renal disease with proteinuria > 0.3 g/24 hours, with or without circulating levels of serum creatinine.[12]

Ethics Statements

This investigation was conducted in accordance with the Declaration of Helsinki and the informed consent form was taken from all patients. The ethics committee of Kashan University of Medical Sciences (KAUMS) approved the research.

Assessment of Anthropometric Measures

Weight and height of participants were determined in an overnight fasting status using a standard scale (Seca, Hamburg, Germany). BMI was calculated as weight in kg divided by height in meters squared.

Assessment of Outcomes

Renal function was determined by the Cockroft-Gault (CG) formula in mL/min [140 - age (years)] × [weight (kg)]/72 × (serum creatinine) ×0.85 if female].[13] Ten mL blood samples in a fasting status were taken from each patient at the KAUMS reference laboratory. Serum TNF-α, MMP-2 and MMP-9 was quantified by the use of ELISA kits (Crystal day, Shanghai, China) with inter- and intra-assay CVs below 7%. Enzymatic kits of Pars Azmun (Tehran, Iran) were used to determine serum creatinine (Jaffe method), blood urea nitrogen (BUN) and total protein concentrations with inter- and intra-assay CVs below 5%. The plasma MDA levels were assessed by the use of the thiobarbituric acid reactive substances (TBARs) spectrophotometric test[14] with inter- and intra-assay CVs of 1.3 and 2.2%, respectively. Serum AGEs were quantified by the fluorometeric methods.

Statistical Methods

To evaluate if the variables in the study were normally distributed or not, we applied the Kolmogrov-Smirnov test. To detect differences in anthropometric measures as well as in markers of kidney injury, oxidative stress and inflammation between the two groups, we applied independent *t*-test. All statistical analyses were done using Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Mean age, height, weight, and BMI of study participants were not statistically different between the two groups (Data not shown).

The current study showed that patients with DN had higher TNF-α (167.0 ± 40.1 vs. 151.4 ± 37.4 ng/L, $P < .05$) and MMP-2 concentrations (1625.2 ± 631.0 vs. 1391.5 ± 465.4 ng/mL, $P < .05$) than cases with T2DM (Table). Furthermore, we observed a non-

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	T2DM Group (n = 57)	DN Group (n = 57)	*P*[2]
TNF-α, ng/L	151.4 ± 37.4	167.0 ± 40.1	< .05
MMP-2, ng/mL	1391.5 ± 465.4	1625.2 ± 631.0	< .05
MMP-9, ng/L	4239.2 ± 1853.9	4864.4 ± 1934.3	> .05
MDA, μmol/L	3.6 ± 1.1	3.9 ± 0.8	> .05
AGEs, AU	7660.7 ± 1711.9	8511.7 ± 1799.9	< .05
AGEs, AU/g protein	1083.2 ± 248.3	1245.4 ± 259.3	< .05
Total protein, mg/dL	6.9 ± 0.6	7.1 ± 0.5	< .05
Urine protein, mg/dL	65.7 ± 28.4	69.4 ± 27.7	> .05
Urine creatinine, mg/dL	92.8 ± 47.9	92.3 ± 48.1	> .05
Creatinine, mg/dL	1.5 ± 0.5	1.6 ± 0.5	> .05
BUN, mg/dL	24.5 ± 13.2	24.0 ± 14.4	> .05
CG, mL/min	45.6 ± 16.2	45.7 ± 15.8	> .05
TFPG, mg/dL	122.1 ± 41.9	115.7 ± 39.5	> .05
Insulin, μIU/mL	19.9 ± 4.8	19.8 ± 4.6	> .05

[1]Data are means ± SD.
[2]Obtained from independent t test.
AGEs, advanced glycation end products; BUN, blood urea nitrogen; CG, Cockcroft-Gault formula to estimate of creatinine clearance; DN, diabetic nephropathy; FPG, fasting plasma glucose; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; MDA, malondialdehyde; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor-α

significant increased level of MMP-9 in patients with DN compared with individuals with T2DM (4864.4 ± 1934.3 vs. 4239.2 ± 1853.9 ng/L, $P > .05$). Additionally, AGEs levels in patients with DN were higher than that of patients with T2DM (8511.7 ± 1799.9 vs. 7660.7 ± 1711.9 AU, $P < .05$), but the difference in MDA values was not significant. Finally, we found that total protein levels in people with DN were enhanced compared with individuals with T2DM (7.1 ± 0.5 vs. 6.9 ± 0.6 mg/dL, $P < .05$); however, other markers of kidney injury did not change.

DISCUSSION

In the present cross-sectional study, we found that TNF-α, MMP-2, AGEs levels and total protein excretion in patients with DN were significantly higher than that of patients with T2DN; however, comparison of circulating levels of MDA and MMP-9, and other variables related to kidney function did not show any significant differences.

Inflammatory Markers

DN is associated with serious complication of diabetes such as increasing inflammatory response; progressive renal injury and oxidative damage.[4] This current study showed that patients with DN have higher TNF-α and MMP-2 concentrations than that of patients with T2DN. In addition, we observed a non-significant increase in MMP-9 levels in patients with DN compared with individuals with T2DM. In agreement with our findings, TNF-α levels in patients with DN were higher than that of patients with T2DM.[8,15] TNF-α is a pro-inflammatory cytokine, which primarily produced from monocytes and macrophages. Renal mesangial cell exposed to inflammatory markers may exert cytotoxic actions.[16] In addition, TNF-α induces an imbalance between the mediators of vasoconstriction and vasodilatation and elevates the endothelium permeability that is associated with the changes of GFR.[15,17] Furthermore, it is linked to an increase in sodium retention and renal hypertrophy, which are involved in the pathogenesis of DN.[18] Kim *et al*.[19] revealed that gene expression of two isoforms of MMP-2 was upregulated in both streptozotocin murine model of T1DM and renal biopsies of human DN. MMP-2 and MMP-9 expression were increased in both medullary regions and cortical of type 2 diabetic rats.[20] In patients with T1DM, MMPs levels were associated with a decrease in GFR, cardiovascular disease and all-cause mortality.[21] Overexpression of MMPs specifically MMP-2 may promote renal fibrosis through the degradation of MMP-2 and MMP-9.[22] In addition, increased MMP-9 activity is associated with leukocyte infiltration and albuminuria, which implicates a pathogenic role in the initiation and progression of DN.[23]

Biomarkers of Oxidative Stress

Our findings revealed that AGEs levels in patients with DN were significantly higher than that of patients with T2DM, but the difference between MDA values was not significant. Interestingly, it was shown that urinary AGEs concentrations were significantly elevated in diabetic patients with kidney disease than patients with non-diabetic chronic kidney disease.[24] However, one study showed that there was a significant increase in MDA concentrations in patients with DN compared with both normal subjects and patients with T2DM.[25] Oxidative stress plays an important role in the pathogenesis of micro- and macrovascular complications related to diabetes.[26] AGEs are able to influence the structure of extra cellular matrix protein by the formation of collagene cross-link.[9] In diabetic state, hyperglycemia induces generation of AGEs and reactive oxygen species, which in turn cause ultramicroscopic damage in kidney structure and produce functional defects such as microalbuminuria and reduced GFR.[27] In some tissues, AGEs formation can stimulate cytokine release and accelerate inflammatory response,[28,29] which in turn may enhance apoptosis in DN.[30] In addition, AGEs accumulation induces free radical production and decreases nitric oxide concentrations.[31] Previous studies revealed that AGEs concentrations mainly influenced by the quality of diabetes control.[32,33]

Parameters of Kidney Function

The results of present study demonstrated that total protein in patients with DN was elevated compared with individuals with T2DM; however, other markers of kidney injury did not change between two groups. In patients with T2DM, decreased GFR is attributed to ischemic stroke/transient ischemic attack, peripheral neuropathy and all cerebrovascular diseases.[34] In both diabetic and non-diabetic individuals, a lower GFR is associated with a higher risk of acute kidney injury.[35] Moreover, proteinuria is considered as a risk factor for progression of chronic kidney disease and death in both T2DM and DN groups.[36]

CONCLUSION

In conclusion, the results of present study revealed that few markers of inflammation and oxidative stress including TNF-α, MMP-2, AGEs levels and total protein levels in patients with DN were significantly higher than that of patients with T2DN. Further studies are necessary to confirm these findings.

ACKNOWLEDGEMENTS

The present study was supported by a grant from the Vice-chancellor for Research, KAUMS Iran.

CONFLICTS OF INTEREST

Nothing to declare .

AUTHOR CONTRIBUTIONS

EA and ZA contributed in conception, data collection and manuscript drafting. AS, EA, PG, NA, RS-C, RSh, and ZB contributed in conception, data collection and manuscript drafting.

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Received June 2019
Revised August 2019
Accepted October 2019