

The Effects of Blocking Angiotensin Receptors on Early Stages of Diabetic Nephropathy

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ABSTRACT

Background: This study aimed to investigate the beneficial effects of angiotensin receptor blockers (ARBs) on markers of endothelial function in patients with early stage of diabetic nephropathy (DN).

Methods: This cross-sectional study was conducted on 32 participants with IDDM from January 2010 until May 2011 in Isfahan, Iran. The participants were candidate for receiving ARBs or angiotensin-converting enzyme inhibitors (ACEIs) to decrease microalbuminuria. The inclusion criteria were as follows: the age of onset of insulin-dependent diabetes mellitus (IDDM) less than 15 years; normal glomerular filtration rate (GFR); normal blood pressure; normal cardiovascular examination; negative urine culture, receiving no medications except insulin. Microalbuminuria was measured in two fasting urine samples with a sampling interval of at least 1–2 months by ELISA method. Patients with two abnormal results were included. Microalbumin to creatinin ratio equal to or more than 30 mg/gm was considered abnormal. The fasting blood samples to determine serum nitric oxide (NO) and vascular cell adhesion molecule (VCAM) were obtained at the time 0 (before starting the study), and after 2 months of receiving ARB medication. Valsartan tablet (Diovan, Novartis Company) with a dose of 1 mg/kg/day up to 80 mg/day in a single dose was administered.

Results: Urine microalbumin to creatinin ratio after valsartan consumption was lower than microalbumin level before the medication, $P < 0.05$. After valsartan consumption, serum VCAM-1 level reduced and NO level increased significantly, $P < 0.05$.

Conclusion: Angiotensin receptor blockers may reduce VCAM-1 and microalbuminuria and may increase NO levels in early stages of DN. Thus administration of ARBs might be considered even in early stages of DN.

Key words: Angiotensin receptor blocker, diabetic nephropathy, endothelial dysfunction, valsartan

INTRODUCTION

Diabetic nephropathy (DN) is a major cause of end-stage renal disease (ESRD) affecting nearby 20%–30% of diabetic patients worldwide.^[1-3] Therefore, preventing DN as a serious

microvascular complication of IDDM to reduce the risk of ESRD is a clinical priority.

Mechanisms by which kidney glomerular, interstitial, and vascular functions are injured consist of inflammation, oxidative stress, endothelial dysfunction, and accelerated fibrosis.^[4] Endothelium dysfunction that has been described in DM consists of impairment in many aspects of endothelial functions including anti-inflammatory, antiproliferative, and vasodilatation.^[1,5,6] In vessels, a balance between vasodilatation and vasoconstriction is achieved by normal endothelial function.^[4] Vascular inflammation is a result of combining damage in vasomotor response, augmenting cell proliferation, increasing platelet aggregation, and vascular permeability.^[2] Furthermore, endothelial dysfunction has been reported as the early sign of atherosclerosis and atherogenesis.^[4,7] The renin-angiotensin-aldosterone system (RAAS) has a main role in the progression of DN.^[8] Inhibition of the renin-angiotensin system (RAAS) may be effective in preventing DN through amending all above mentioned complications.^[4] Findings from several studies in animal models and subsequent clinical trials in DN showed that systematic use of RAAS blocking agents including angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) would reduce the risk of ESRD.^[1,5] Long-lasting prescription of ACEIs (Enalapril) and or ARBs (Olmesarten) has been demonstrated to ameliorate microalbuminuria in patients with insulin-dependent and independent diabetes mellitus (NIDDM and IDDM).^[9,10]

However, there is limited evidence about effects of blocking RAS system on endothelial dysfunction in young IDDM patients with microalbuminuria and early stages of DN, i.e., glomerular filtration rate (GFR) equals or more than 90 ml/min/1.73 m² and normal blood pressure.^[11]

This study aimed to investigate the beneficial effects of 2 months oral treatment of ARB on markers of endothelial function such as nitric oxide (NO) and vascular cell adhesion molecule (VCAM) in young IDDM patients with microalbuminuria and normal renal function.

METHODS

We randomly assigned 32 eligible IDDM patients with confirmed microalbuminuria in

this cross-sectional study from January 2010 until May 2011 in Isfahan, Iran. To select the participants, the medical file of 270 IDDM patients who had been referred to Isfahan Endocrine and Metabolic Research Centre was re-evaluated. Among them, 32 eligible patients who were candidate for receiving ARBs or ACEIs and met the inclusion criteria were recruited. The inclusion criteria were as follows: the age of onset of IDDM less than 15 years (children onset disease); GFR equals or more than 90 ml/min/1.73 m²; normal blood pressure at three consecutive blood pressure measurements (less than 95% for age and height and gender); normal cardiovascular examination (approved by a fixed cardiologist); negative urine culture, receiving no medications except insulin.

Microalbuminuria was measured in two fasting urine samples with a sampling interval of at least 1–2 months.^[12] The patients who had two abnormal results were recruited in the study. Microalbumin to creatinin ratio equaled to or more than 30 mg/gm was considered abnormal. Regarding abnormal microalbuminuria, the selected patients were candidate to receive ARBs or ACEIs as a part of approved management to treat microalbuminuria.

The fasting urine samples for measuring microalbumin and creatinin were used when the blood sugar was in the acceptable range (fasting blood sugar less than 140 mg/dl and trace or negative urine dipstick results for glucose). Microalbumin was measured by ELISA method on the fasting first morning urine sample. The blood samples to determine serum NO and VCAM were obtained at the time 0 (before starting the study) and after 2 months of receiving the medication. Valsartan tablet (Diovan, angiotensin receptor blocker from Novartis Company) with a dose of 1 mg/kg/day up to 80 mg/day in a single dose was administered. This medication was selected because of extended half-life and ease of administration.

The serum level of nitrite (stable NO metabolite) was measured using a colorimetric assay kit (Cayman, USA) based on Griess reaction, as previously described.^[13] For nitrite measurement, briefly, after pouring serum into wells, sulphanilamide solution was added to all experimental samples, and after incubation, *N*-1-naphthylethylenediamine dihydrochloride solution was added. Then, absorbance was measured by a microreader in 540° nm wavelength.

The serum levels of VCAM were quantified by ELISA kit (Bendermed, UK) according to the manufacturer's instruction.

In addition to the above-mentioned factors (endothelial markers), total cholesterol, triglyceride, HDL, LDL, and hemoglobin A1C were determined in the fasting state.

Written informed consent from the participants was obtained before enrolment in the study. The study was approved by institutional review boards and carried out in accordance with declaration of Helsinki guidelines.

Statistical analysis

The data are reported as the mean \pm SE. A statistical software package, SPSS (version 16), was used to perform statistical analysis. The data were tested for normality and homogeneity of variance. Paired student's *t*-test was used to assess the significance of any change within groups. Statistical significance was accepted at $P < 0.05$.

RESULTS

Thirty two patients with child-onset IDDM were recruited. The mean of age was 12.65 ± 0.38 years. Male to female ratio was $\frac{1}{2}$. Mean of height was 160.16 ± 10.24 cm. Mean of body weight was 60.46 ± 13.54 kg. Means of serum triglyceride and cholesterol levels were 98.25 ± 8.08 (SE) mg/dl and 155.90 ± 5.76 (SE) mg/dl, respectively. Mean serum levels of HDL and LDL were 49.03 ± 2.24 (SE) mg/dl and 89.46 ± 3.83 (SE) mg/dl, respectively. Mean of HBA1C before and after prescribing the medication were 7.84 ± 0.35 (SE) and 7.01 ± 0.78 (SE), respectively; $P > 0.05$. Urine microalbumin to creatinin ratio after valsartan consumption was lower than microalbumin level before the medication, $P = 0.0001$ [Table 1]. After valsartan consumption, serum VCAM-1 level reduced and NO level increased significantly [Table 2]. Microalbumin levels were positively correlated with VCAM (before valsartan); $r = 0.340$, $P = 0.04$.

DISCUSSION

In this study, the response of endothelia dysfunction and microalbuminuria to ARBs in normotensive young IDDM patients was evaluated. We demonstrated that an 8-week course of administrating valsartan (ARBs) in IDDM patients

Table 1: Characteristics of the participants

Characteristics	Mean	SE
Weight (kg)	60.46	2.70
Height (cm)	160.16	2.04
Triglyceride (mg/dl)	98.25	8.08
Total cholesterol (mg/dl)	155.90	5.76
High density lipoprotein (mg/dl)	49.03	2.24
Low density lipoprotein (mg/dl)	89.46	3.83
Hemoglobin A1C	7.84	0.35

Table 2: Markers of endothelial function before and after valsartan (Diovan) consumption

Parameter	Mean \pm SE		Significance
	Before Diovan	After Diovan	
Urine microalbumin/creatinin (mg/gm)	45.72 ± 3.36	15.12 ± 2.20	$P = 0.0001$
Serum VCAM-1	251.06 ± 3.27	211.53 ± 1.98	$P = 0.0001$
Serum NO	0.23 ± 0.003	0.29 ± 0.002	$P = 0.0001$

before attaining overt stages of DN was able to recover endothelial dysfunction by increasing NO and diminishing VCAM levels. In addition, the level of microalbuminuria was decreased after 8 weeks of receiving valsartan. Although at the end of the study a minority of patients still had abnormal amounts of microalbumin to creatinine ratio, the difference between the values before and after valsartan was significant. While numerous studies have assessed the effect of RAS inhibition on DN, most of them evaluated only microalbuminuria as an alternate to DN in patients with impaired renal function.^[14] The results of Collaborative Study Group's captopril trial have emphasized on the protective role of RAS inhibition in patients with DN and renal failure.^[15] Nonetheless, the benefit of RAS inhibition by ACEIs was not approved in patients with normal GFR by this group. The results of DIRECT program did not support the preventive effects of candesartan (ARBs) in reducing microalbuminuria in IDDM patients with a low vascular burden.^[16] Mauer *et al.* showed that early administration of RAS blocking agents did not reduce DN progression.^[17] However, the results of a cohort study by Bakris *et al.* showed that telmisartan was more effective than losartan in reducing microalbuminuria in type 2 diabetes.^[18]

In fact, in DN endothelium dysfunction occurs in both great and small arteries leading to cardiovascular and renal diseases.^[19] In the capillary and arteriolar endothelium, dysfunction leads to insulin resistance, impaired fibrinolysis, microalbuminuria, dyslipidemia, and even hypertension.^[20,21] Therefore, microalbuminuria was supposed to be a herald sign of early stages of DN.^[5,6] Furthermore, its presence has been introduced is an important predictor of endothelial dysfunction in DN regardless of the degree of renal impairment.^[22] Whereas microalbuminuria has been known as one of the most important markers of initiation of end organ damage, reducing NO bioactivity and increasing oxygen free radicals are the early indicators of endothelial dysfunction.^[4,23] The balance between vasodilators and vasoconstrictors largely is maintained by NO. In addition to being a key player for the vasodilator effects, NO has been known as second messenger for actions of many growth factors, hormones, and coagulation factors besides to its inhibitory effects on adhering VCAM-1 and ICAM-1 to the arterial wall.^[24-27] Higher production of prostanoid vasoconstrictors and increased oxidative degradation of NO have been reported as early mechanisms of diabetes-induced endothelial dysfunction.^[28,29] Furthermore, asymmetric dimethylarginine (ADMA) accumulation in diabetes may impair endothelial vasodilator dysfunction.^[30] The results of Calver *et al.*'s study revealed that patients with IDDM had a lower forearm blood flow response to locally infused L-NMMA (an inhibitor of endogenous NO synthesis) and SNP (an exogenous donor of NO).^[31] In our patients, serum NO levels rose dramatically after consuming valsartan. Irrespective of short-course therapy, increasing NO levels were significant.

In addition to microalbuminuria, cell adhesion molecules (such as VCAM-1 and ICAM-1) that mediate adhesion and relocation of leukocyte into the arterial wall, have been reported as markers of endothelial dysfunction.^[32,33] These markers are widely affected by angiotensin II (AG II) through inducing cytokine release.^[34,35] Vascular cell adhesion molecule-1 has been known as independent predictors of atherosclerosis in diabetes. Romuk *et al.* demonstrated increased levels of VCAM-1 on type 2 but not in type 1 diabetes.^[7] The lowering effect of ACEI but not ARBs on VCAM-1 level in

non-diabetic hypertensive patients was described by Jilma *et al.*^[36]

A double-blind placebo-controlled study on a small sample size of hypercholesterolemic normotensive volunteers, revealed diminishing c-VCAM-1 levels after consuming either ARBs or ACEIs.^[37] Gasic and colleagues demonstrated the lowering effect of ACEIs (fosinopril) on VCAM-1 levels in type 2 diabetes (NIDDM).^[38] The unique characteristic of ARBs in blocking AT1 receptor promotes using these types of drugs to reduce proteinuria, microalbuminuria, and renal dysfunction in type 2 diabetes (NIDDM).^[39-41] While angiotensin II increases VCAM-1 levels, NO down regulates its amounts at cellular level.^[42] Irrespective of Romuk *et al.*'s study, we revealed lowering effects of ARBs on VCAM levels in IDDM patients. Although microalbumin level was correlated with VCAM before administering valsartan, this correlation was not achieved after finishing the short-course evaluation.

In conclusion, we demonstrated that the increment of NO and decrement of VCAM-1 occurred after an 8-week course of consuming valsartan in IDDM patients. Recruiting normotensive diabetic patients with normal GFR allowed us to show the effect of ARBs in modulating endothelial function besides to decreasing microalbuminuria in the absence of hypertension and kidney failure.

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