Angiogenic factors in adult with type 2 diabetes mellitus with renal complication

Laila Hamed Damanhouri and Kaltoom-AL-Sakkaf

Immunology Department, Medical Laboratory Technology Department, Faculty of Applied Medical science, King Abdulaziz University, P.O. Box 10415, Jeddah 21433, Saudi Arabia
Lailahhd71@hotmail.com

Abstract: Objective: This study was to investigate the role of vascular endothelial growth factor in renal complications in T2DM patients. **Methods:** 100 patients with T2DM (50 patients with diabetic nephropathy , 50 without complication) And fifty healthy subjects matched with patient's age . Plasma levels of VEGF, angiopoietin-2, adrenomedullin were measured by enzyme-linked immunosorbent assay. **Results:** VEGF and angiopoietin-2 plasma levels were significantly higher in diabetic patients versus healthy control (P =0.0001). Adrenomedullin plasma level was significantly higher only in T2DM with renal complications (P =0.0001). In T2DM patient without renal complications, significant positive correlations were found between blood glucose with VEGF (r= 0.322, P =0.022) and angiopentin-2 (r= 0.441, P =0.001). In T2DM patient with renal complications, significant positive correlations were found between angiopoitin-2 with serum urea (r= 0.529, P =0.0001) and serum creatinine (r= 0.754, P =0.0001) and between adrenomedullin with serum urea (r =0.649, P =0.0001), serum creatinine (r =0.807, P =0.0001); HbA1c (r= 0.407, P =0.003) and Ang-2 (r= 0.678, P =0.0001). **Conclusion:** our study revealed elevations of VEGF, Ang-2, and adrenomedullin in T2DM patients with renal complications. suggest the implication of these vascular markers in pathogenesis of renal complications in T2DM.
[Laila Hamed Damanhouri and Kaltoom-AL-Sakkaf. **Angiogenic factors in adult with type 2 diabetes mellitus with renal complication.** *J Am Sci* 2014;10(12):79-87]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 8

Key words: vascular endothelial growth factor, angiopoietin-2, adrenomedullin, diabetes mellitus, renal complication.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic condition and considered as a major cause of mortality and morbidity for its micro-vascular (such as retinopathy, nephropathy and neuropathy) and macro-vascular (coronary heart disease, peripheral vascular disease and stroke) complications [(1).] Diabetic nephropathy, a major microvascular complication of diabetes mellitus, affects approximately one-third of all diabetic patients [(2)] and is a leading cause of end-stage renal disease in Western countries [(3)] . Prevention and treatment of chronic renal insufficiency would require the application of therapies that specifically interfere with the pathogenesis of diabetic nephropathy. However, the molecular mechanisms involved in diabetic nephropathy are largely unknown.

The balance between pro- and anti-angiogenic factors regulates angiogenesis. Of the former, vascular endothelial growth factor (VEGF or VEGF-A) and the angiopoietin family of growth factors act exclusively on the endothelium. VEGF increases vascular permeability and is mitogenic for endothelial cells, acting early and at most points of the angiogenic cascade [(4)]. In contrast, angiopoietin-1 (Ang-1), by promoting endothelial cell survival, stabilizes endothelial interactions with supporting cells, limits vascular permeability (with little effect on endothelial proliferation) and may have a role in vascular maturation [(5)] . Angiopoietin-2 (Ang-2), on the other hand, may destabilise the vasculature [(6)] . In the absence of VEGF, this results in vessel regression but facilitates endothelial cell migration and proliferation in concert with VEGF [(7)] Hence, selective up-regulation of VEGF and Ang-2 may lead to aberrant proliferation of leaky, friable vessels. High levels of Ang-2 have been observed in diseases characterized by increased rate of vascular proliferation and endothelial damages/ injuries such as tumor progression, hypertension, coronary artery diseases, chronic kidney diseases, and proliferative diabetic retinopathy [(8,9,10)] .

Apart from HbA1c, which is a well-known risk factor for micro- and macrovascular complications, data suggest that growth factors, including VEGF, may have an important function in the modification of tissue damage and its acceleration. VEGF belongs to a family of multipotent cytokines that include VEGF-B, -C, -D, -E and placenta growth factor. The receptors for VEGF-A are VEGFR-1, VEGFR-2 and VEGFR-3 along with high-affinity transmembrane tyrosine kinase receptors. In the kidney, VEGF production by the podocytes plays an important role in endothelial cell survival, regeneration and repair within the glomeruli, and it helps maintain the integrity of the glomerular filtration barrier with VEGFR-2 [(11)] It was shown that VEGF induces vascular endothelial cell proliferation and migration

and increases the permeability of renal glomerular and retinal capillaries [12,13] It has been reported that the over-expression of Ang-2 in podocyte lead to proteinuria and apoptosis in glomerular endothelia [14].

Adrenomedullin, a potent vasodilator, has been reported to be widely distributed in various organs and tissues including the cardiovascular system (myocardium, vascular endothelium, and vascular smooth muscle), lung, kidney, and endocrine system. Adrenomedullin plays a role in glucose metabolism and insulin balance. Adrenomedullin inhibits insulin release after an oral glucose load. Therefore, it can be expected that adrenomedullin contributes to diabetes and even leads to the development of diabetic complications [15]. Adrenomedullin has vasodilator and antiproliferative actions in vascular smooth muscle and glomerular mesangium [16]., as a paracrine or autocrine mechanism [17]. Diabetes mellitus has chronic complications which are closely related to microangiopathy. These observations led us to speculate that adrenomedullin may play a role in diabetics.

The current cross sectional study was designed to determine circulating levels of angiogenic factors (vascular endothelial growth factor, angiopoietin-2, adrenomedullin) in patients with T2DM with established renal complications. Additionally, to investigate whether these angiogenic parameters were associated with glycemic control and duration of the disease. These may contribute to the development of new therapeutic approaches for prevention and treatment of diabetic vascular complications.

2. Subjects and methods

Participants

1. A total of 100 consecutive type 2 diabetic patients, mean+/- SD age 48.58 ± 8.41 years and diabetes duration 6.67 ± 2.85 years, treated at the diabetes outpatient clinic of the University Clinics of King Abel Aziz University, Hospital, Jeddah, Saudi Arabia from 1 January 2013 to 1 May 2014 were studied. The patients were subdivide into 50 patients (25 males and 25 females) with T2DM without complications (mean age 49.14 ± 9.32, range 23–82 years) and 50 patients (all were males) with T2DM with established diabetic nephropathy (mean age 48.02 ± 7.45, range 29-60 years). The diagnosis of T2DM was established before the beginning of this study, and it was made "as discussed by the diagnostic criteria of the American Diabetes Association [18]. Type 2 diabetic subjects with normal renal function (the diabetic group) were strictly defined as early morning spot urinary albumin-to-creatinine ratio (ACR) ≤3.3 mg per mmol/l (i.e., 30 mg/g) and consistently normal serum creatinine. The diabetic nephropathy group (n = 50) was defined according to the presence of proteinuria ≥1.0 g/day (equivalent to spot urinary ACR ≥113 mg per mmol/l [i.e., 1,000 mg/g]) or persistently elevated serum creatinine with a mean Modified Diet in Renal Disease (MDRD) formula–estimated glomerular filtration rate [19]. of □43 ml/min per 1.73 m^2 [20]. Fifty apparently healthy subjects (28 males and 22 females) matched with patient's age (mean age 46.38 ± 4.85, range 36.00–55.00 years) was recruited from hospital staff, friends, spouses and relatives of the patients and considered as healthy controls. All diabetic patients were treated either with humanized insulin therapy, oral hypoglycemic or both. Individuals were excluded from the diabetic nephropathy group when renal diseases attributable to other causes were suspected. These exclusion criteria included the presence of hematuria, renal insufficiency of unexplained origin, urinary tract infection, and history of rapidly progressive renal failure, glomerulonephritis, and polycystic kidney disease, diabetic complications except diabetic nephropathy. To avoid misclassification, we decided to only include subjects with well-establish nephropathy in the diabetic nephropathy group because recent data suggested that early forms of diabetic nephropathy might remit spontaneously [21]. The control subjects do not have any health problems, no family history of diabetes and are not receiving any medications or dietary supplements.

All participants were subjected to careful history taking laying stress on onset, duration, thorough clinical examination with special emphasis on signs of diabetic complications. Retinopathy was assessed by fundus examination that was by an ophthalmologist after maximum papillary dilatation using indirect ophthalmoscope to identify diabetic retinopathic changes, neuropathy (assessed using clinical history and physical examinations), or nephropathy as previously described.

Written informed consent was obtained from participants. This study was approved by The Ethics Committee of the Medical University of King Abdulaziz and the investigation was carried out in accordance with the principles of the Declaration of Helsinki as revised in 1996.

Laboratory procedures

Patients with T2DM had blood collected for hematology and biochemistry measurements at the time of their routine clinic visit. Three ml of fasting venous blood samples obtained from an antecubital vein before treatment were collected on EDTA tube, centrifuged at 3.000 g for 15 minutes and plasma samples were stored at -70°C till assay. The blood glucose level was measured by an automated enzymatic method. Circulating levels of hemoglobin

A1c (HbA1c), a clinical indicator of blood glucose control, was measured by Hitachi 911 autoanalyzer (Hitachi Co. Ltd., Tokyo, Japan). HbA1c determination is based on turbidimetric inhibition immunoassay for hemolyzed whole blood from Roche/Hitachi 911, Tokyo, Japan. Normal values of HbA1c [22]. ranged from 4.0 to 6.0%. HbA1c values were recorded for the previous 12-month period from the participants' clinic record and then averaged. Poor metabolic control was considered when HbA1c reached >8.0% [23] . Microalbuminuria was assayed using SERAPAK immuno-microalbumin Kit (Bayer Corporation, Benedict Ave, Tarry town, NY, USA). Plasma levels of VEGF, angiopoietin-2, adrenomedullin were measured by the ELISA method [Quantikine High Sensitivity Human by R&D System, Minneapolis, Minn., USA (VEGF); Weka Med Supplies Corp, The Ave, NY, USA (angiopoietin-2, adrenomedullin)] according to manufacturer protocols. Minimum detectable concentrations were determined by the manufacturer as 5.0 pg/ml (VEGF); 3 ng/L (angiopoietin-2) and 2 ng/L (adrenomedullin). Other routine biochemical tests were performed using standard methodology in clinical pathology laboratory.

Statistical analysis

Statistical Science for Social Package (SPSS Version 20, SPSS Inc., Chicago, IL, USA) was used for data analysis. Data were presented as mean (SD), minimum – maximum or number (%) as appropriate. Comparisons of multiple groups were performed using analysis of variance (ANOVA) for parametric variables. Pearson's correlation test was used for correlating parametric variables. For all tests, a probability (P) <0.05 was considered significant.

3. Results

The male were more than females in different studied groups (P =0.0001). The treatment in in T2DM patients was oral hypoglycemic and oral hypoglycemic & insulin (62.00% and 38.00%) and in T2DM with renal complication was only insulin (100.00%) with significance difference between groups (P =0.0001). Albuminuria was found only in patients with T2DM with renal complication (Table 1).

RBCs count and hemoglobin levels were significantly lower in T2DM without complications and in T2DM with renal complication versus healthy control (P =0.0001 for all) and in T2DM with renal complication versus T2DM without renal complication (P =0.0001 for all). Serum urea and creatinine were significantly higher in T2DM with renal complication compared to healthy control and T2DM without renal complication (P = 0.0001 for all). HbA1c and blood glucose were significantly higher in T2DM without complications and in T2DM with renal complication versus healthy control (P =0.0001 for all). VEGF and angiopoietin-2 plasma levels were significantly higher in T2DM without complications and in T2DM with renal complication versus healthy control (P =0.0001 for all). Adrenomedullin plasma level was significantly higher in T2DM with renal complications than T2DM without complications and healthy control (P =0.0001 for both) (Table 2).

Table: (1): Demographic characteristics of patients and controls.

Parameters	**Control** (n=50)	**T2DM without kidney diseases** (n=50)	**T2DM with kidney diseases** (n=50)	**Significance**
Age (years)	46.38±4.85 (36-55)	49.14±9.32 (23-82) 1P =0.065	48.02±7.45 (29-60) 1P =0.272, 2P=0.453	-
Gender male female	 28 (56.0%) 22 (44.0%)	 25 (50.0%) 25 (50.0%)	 50 (100.0%) -	**0.0001**
Duration of disease (years)	-	5.24±2.34 (2.00-10.00)	8.10±1.95 (5.00-14.00) **2P =0.0001**	**-**
Treatment no Oral hypoglycemic Insulin Oral hypoglycemic & insulin	 50 (100.0%)	 - 31 (62.0%) - 19 (38.0%)	 - - 50 (100.0%) -	**0.0001**
Albuminuria	-	-	50 (100.0%)	**0.0001**

Type 2 diabetes mellitus, T2DM; Data are expressed as mean+/- SD (minimum-maximum) or number (percentage). P: significance between different groups; 1P: significance versus control; 2P: significance type 2 diabetes mellitus. Significance between non-parametric parameters Chi-square test; parametric test using One way ANOVA using LSD test.

Table (2): Measured metabolic and angiogenic parameters of patients and control.

Parameters	**Control** (n=50)	**Type 2 diabetes mellitus without kidney diseases** (n=50)	**Type 2 diabetes mellitus with kidney diseases** (n=50)
Red blood cells	4.76±0.37 (4.01-5.40)	4.39±0.54 (2.91-5.20) **1P =0.0001**	3.29±0.50 (2.31-4.47) **1P =0.0001, 2P=0.0001**
Hemoglobin (gram/dl)	14.20±0.92 (13.00-16.20)	12.23±1.52 (9.00-14.60) **1P =0.0001**	9.48±1.22 (6.40-12.80) **1P =0.0001, 2P=0.0001**
Serum urea (mmol/l)	4.58±1.41 (2.00-8.80)	6.13±5.88 (1.90-32.90) 1P =0.306	20.88±11.31 (5.60-51.60) **1P =0.0001, 2P=0.0001**
Serum creatinine (umol/L)	67.86±13.48 (36.00-95.00)	65.40±34.35 (15.00-257.00) 1P =0.958	730.66±397.74 (131.00-1884.00) **1P =0.0001, 2P=0.0001**
HbA1c (%)	4.87±0.66 (3.90-6.00)	8.81 (1.52 (6.00-13.00) **1P =0.0001**	9.27±2.26 (7.00-16.00) **1P =0.0001**, 2P=0.156
Glucose (mmol/l)	5.44±0.48 (4.90-6.10)	9.92±2.42 (4.70-16.00) **1P =0.0001**	10.48±2.42 (7.70-17.00) **1P =0.0001**, 2P=0.159
VEGF (pg/ml)	84.976±9.19 (57.74-97.81)	154.386±31.09 (108.42-221.46) **1P =0.0001**	203.686±35.85 (112.90-289.46) **1P =0.0001, 2P=0.0001**
Angiopoietin-2 (ng/ml)	3.70±1.24 (1.23-7.16)	5.42±1.79 (2.44-8.51) **1P =0.0001**	12.46±5.30 (4.54-22.51) **1P =0.0001, 2P=0.0001**
Adrenomedullin (ng/ml)	1.36±0.91 (0.14-4.66)	1.93±1.36 (0.10-5.23) 1P =0.134	5.85±2.83 (1.09-10.00) **1P =0.0001, 2P=0.0001**

Data are expressed as mean+/- SD (minimum-maximum) or number (percentage)
P: significance between different groups; 1P: significance versus control; 2P: significance type 2 diabetes mellitus. Significance between non-parametric parameters Chi-square test; Parametric test using One way ANOVA using LSD test.

In T2DM patient without renal complications, significant positive correlations were found between RBCs count with hemoglobin (r= 0.756, *P* =0.0001); between serum urea with creatinine (r= 0.647, *P* =0.0001) and between blood glucose with HbA1c (r=0.450, *P* =0.001), VEGF (r= 0.322, *P* =0.022) and angiopentin-2 (r= 0.441, *P* =0.001). Meanwhile, significant negative correlations were found between RBCS count and hemoglobin with urea (r= -0.621, *P* =0.0001; r= -0.526, *P* =0.0001) and with creatinine (r= -0.441, *P* =0.001; r= -0.436, *P* =0.001) (Table 3).

Table (3): Correlation (r, P) between measured parameters in diabetic patients without renal complications.

Parameters	**Duration**	**Red blood cells**	**Hemoglobin**	**Urea**	**Creatinine**	**Glucose**	**HbA1C**	**VEGF**	**Angiopoietin-2**
Red blood cells (10^{12}/L)	0.145 (0.316)								
Hemoglobin (gram/dl)	0.122 (0.397)	**0.756 (0.0001)**							
Urea (mg/dl)	0.050 (0.738)	**-0.621(0.0001)**	**-0.526 (0.0001)**						
Creatinine (mg/dl)	0.154 (0.285)	**-0.442 (0.001)**	**-0.436 (0.002)**	**0.647 (0.0001)**					
Glucose (mg/dl)	-0.046 (0.749)	-0.041 (0.780)	-0.111 (0.441)	0.182 (0.222)	0.117 (0.416)				
HbA1C (%)	0.057(0.692)	0.014 (0.921)	-0.094 (0.515)	-0.112 (0.452)	-0.032 (0.827)	**0.450 (0.001)**			
VEGF (pg/ml)	-0.035 (0.812)	0.000 (0.998)	-0.219 (0.126)	0.013 (0.930)	0.139 (0.336)	**0.322 (0.022)**	0.180 (0.212)		
Angiopoietin-2 (ng/L)	-0.046 (0.751)	-0.152 (0.293)	-0.070 (0.631)	-0.067 (0.655)	0.063 (0.665)	**0.441 (0.001)**	-0.138 (0.339)	-0.049 (0.736)	
Adrenomedullin (ng/L)	0.030 (0.836)	0.283 (0.047)*	0.230 (0.109)	-0.074 (0.619)	-0.145 (0.315)	-0.167 (0.248)	-0.137 (0.342)	-0.116 (0.422)	-0.144 (0.317)

VEGF: vascular endothelial growth factor; HbA1c: glycosylated hemoglobin. Correlation using Person equation.

In T2DM patient with renal complications, significant positive correlations were found between RBCs count with hemoglobin (r= 0.731, *P* =0.0001); between serum creatinine with urea (r= 0.820, *P* =0.0001); between HbA1c with urea (r= 0.305, *P* =0.031), creatinine (r= 0.596, *P* =0.0001), blood glucose (r=0.890, *P* =0.0001); between angiopoitin-2 with serum urea (r= 0.529, *P* =0.0001) and serum creatinine (r= 0.754, *P* =0.0001) and between adrenomedullin with serum urea (r =0.649, *P* =0.0001), serum creatinine (r =0.807, *P* =0.0001); HbA1c (r= 0.407, *P* =0.003) and Ang-2 (r= 0.678, *P* =0.0001) (Table 4).

Table (4): Correlation (r, P) between measured parameters in diabetic patients with renal complications.

Parameters	Duration	Red blood cells	Hemoglobin	Urea	Creatinine	Glucose	HbA1C	VEGF	Angiopoietin-2
Red blood cells (10^{12}/L)	-0.159(0.269)								
Hemoglobin (gram/dl)	-0.062(0.670)	**0.731 (0.0001)**							
Urea (mg/dl)	-0.002 (0.989)	-0.039 (0.790)	0.000 (1.000)						
Creatinine (mg/dl)	-0.021 (0.886)	-0.056 (0.701)	-0.052 (0.721)	**0.820 (0.0001)**					
Glucose (mg/dl)	0.190 (0.187)	0.118 (0.415)	-0.086 (0.552)	0.192 (0.182)	-0.260 (0.069)				
HbA1C (%)	0.118 (0.416)	0.044 (0.764)	-0.063 (0.663)	**0.305 (0.031)**	**0.596 (0.0001)**	**0.890 (0.0001)**			
VEGF (pg/ml)	-0.089 (0.540)	-0.142 (0.324)	-0.034 (0.816)	0.019 (0.895)	0.097(0.502)	-0.030 (0.834)	0.034 (0.813)		
Angiopoietin-2 (ng/L)	-0.009 (0.950)	-0.100 (0.490)	-0.209 (0.146)	**0.529 (0.0001)**	**0.754 (0.0001)**	-0.058 (0.688)	-0.109 (0.453)	0.094 (0.517)	
Adrenomedullin (ng/L)	0.081 (0.574)	0.019 (0.893)	0.031 (0.830)	**0.649 (0.0001)**	**0.807 (0.0001)**	0.036 (0.801)	**0.407 (0.003)**	0.000 (1.000)	**0.678 (0.0001)**

VEGF: vascular endothelial growth factor; HbA1c: glycosylated hemoglobin. Correlation using Person equation.

4. Discussion:

VEGF, a survival and angiogenic factor with strong microvascular permeabilizing properties, may increase the permeability of the glomerular filtration barrier to circulating proteins [24]. In this study, VEGF plasma level was significantly higher in T2DM with and without renal complications compared with healthy control. Others had demonstrated elevated plasma VEGF in patients with diabetes ([25]. Increases in expression of both VEGF and its receptor have been reported in the kidney in rodents with type 1 or type 2 diabetes [26, 27].

The VEGF dysfunction has been found in diabetic conditions of retinopathy [28, 29].

Several rodent type 1 and 2 diabetes models have shown that the abnormal angiogenesis and immature vessels induced by glomerular hypertension and low nitric oxide (NO) bioavailability along with a high VEGF-A expression (uncoupling of VEGF-A with NO) cause glomerular hypertrophy, albuminuria, the expression of profibrotic growth factor and inflammatory cell infiltration in the kidneys. Blocking the increased VEGF-A with anti-pan-VEGF antibodies, which block all of the VEGFRs at the level of the tyrosine kinase, improves the diabetes-related early renal dysfunction, and especially the hyperfiltration [30]. A study with mostly type 2 patients concluded that the VEGF expression, at the transcript and protein levels, was decreased in diabetic nephropathy, and even in the early stages, and that this VEGF deficiency was associated with worsening proteinuria, glomerular capillary and peritubular capillary rarefaction and ischaemic interstitial fibrosis [31]

These findings suggest that when the VEGF level is too low it can be just as damaging as when the VEGF level is too high. Thus, the diverse biological effects of VEGF in diabetic nephropathy are due in part to 'uncoupling of VEGF-A with NO' [32].

Previous reports have shown that increased plasma levels of VEGF may be associated with proteinuria in patients with diabetes. Increased VEGF in the glomeruli may result in proteinuria through two possible different yet closely overlapping or related mechanisms. First, glomerular VEGF derived from podocytes is involved in the maintenance of the glomerular endothelium (including maintaining fenestration) and/or selective permeability to macromolecules. High levels of VEGF derived from podocytes can strongly bind to capillary endothelial cells through specific VEGF receptors, which may result in increased permeability or glomerular hyperfiltration by altering capillary fenestration or

basement membrane components or indirectly through the induction of nitric oxide and prostacyclin. Second it has been reported that VEGF stimulates increased synthesis of collagenase by endothelial cells, which result in the proteolytic disruption of the basement membrane may participate in the enhancement of proteinuria [33].

Angiopoietins are another family of growth factors in the development of diabetic nephropathy. Angiopoietin 1, 2 are ligands for Tie-2 receptor tyrosine kinase and angiopoietin 2 is a natural antagonist of angiopoietin-1 [34].

Changes in the expression of angiopoietins in the form of reduced ratio of angiopoietin 1 to angiopoietin 2 beside VEGF-A can be involved in the development of diabetic nephropathy [35].

In this study angiopoietin-2 plasma levels were significantly higher in T2DM with and without renal complications versus healthy control. The kidney endothelium itself has been identified as a rich source of Ang-2, so that chronic organ impairment might directly result in increased Ang-2 release from the kidney [36]. Alterations in the expression of the angiopoietins have been implicated in the progression of diabetic nephropathy. Clinical studies showed that circulating Ang-2 but not Ang-1 are elevated in type 2 diabetic patients [37,38] . Although the exact mechanism linking T2DM with increased levels of Ang-2 is still not clear, results from previous experimental studies revealed an increased Ang-2 expression in endothelial cells of pancreas, kidney, heart, brain, and retina exposed to the effects of hyperglycemia. This was accompanied by vascular damage, endothelial apoptosis, and decreased vascular density in these tissues [39] .

In this study, T2DM patient without renal complications, significant positive correlations were found between blood glucose with angiopentin-2. In T2DM patient with renal complications, significant positive correlations were found between angiopoitin-2 with serum urea and serum creatinine and adrenomedullin [40]. reported that levels of Ang-2 are negatively associated with the glomerular filtration rate [37]. reported significant associations between Ang-2 and VEGF levels in patients with diabetes mellitus. In mice model studies, hyperglycemia was associated with impaired wound healing due to the increased Ang-2 and decreased Tie-2 expression [41].

Electron microscopic studies in angiopoietin-2 overexpressing mice demonstrate glomerular endothelial apoptosis, but there is no evidence of glomerular capillary collapse or foot process effacement. These findings are consistent with the role of angiopoietin-2 in destabilizing endothelial cell integrity [14]. biological effects of angiopoietin-2 are depend on ambient levels of VEGF-A, such that vessel regression occurs if VEGF-A is lacking, whereas vessel destabilization followed by angiogenesis occurs if the local milieu is rich in VEGF-A [36].

It could be postulated that the increased levels of angiopoietin-2 alongside a VEGF-A rich milieu in glomeruli will lead to the destabilization of blood vessels and hence excessive angiogenesis as what happens during the initial phases of diabetes [42].

The raised blood glucose could be one of the primary determinants of circulating Ang-2 levels. Increased blood glucose in diabetes can exert toxic effects on the endothelium through a number of mechanisms. The accelerated formation and accumulation of glycation products associated with raised blood glucose may up-regulate both Ang-2 transcription and production [25]. Therefore, Anuradha et al. finding of selective elevation of Ang-2 and its relationship with HbA1c supports these in vitro observations. A cause–effect relationship between excess Ang-2 and albuminuria has been proposed in animals with inducible podocyte-specific overexpression of Ang-2 [14]

Selective up-regulation of VEGF and Ang-2 may promote vascular permeability, destabilization and sprouting [6,7, 36] finding of raised VEGF and Ang-2, but not Ang-1 levels suggests an adverse angiogenic milieu in patients with diabetes, favoring the aberrant proliferation of leaky, friable vessels that may be prone to rupture.

One mechanism identified in the pathogenesis of diabetic nephropathy was hemodynamic-mediated vascular injury[43]. Sustained increase in glomerular capillary pressure driven by increase in plasma flow had been observed, especially in early stages of nephropathy. The elevation in glomerular capillary pressure might be damaging to glomerular endothelial, epithelial, and mesangial cells, thereby initiating and contributing to the progression of nephropathy. In this study, adrenomedullin plasma level was significantly higher in T2DM with renal complications compare to its levels in T2DM without complications and healthy control, meanwhile, adrenomedullin plasma level was not elevated in T2DM without renal complication. [44]. Although hyperglycemia has been reported to be increase vascular adrenomedullin expression [45] . it may act only in the local vasculature and may not influence the circulating levels of adrenomedullin. In contrary, it has been previously reported that the plasma levels of adrenomedullin are increased in patients with T2DM [46, 47] Similarly, whether or not the presence of diabetic microangiopathy resulted in elevation in adrenomedullin levels was also controversial [48].

In this study, in T2DM patient with renal complications, significant positive correlations were found between adrenomedullin with serum urea, serum creatinine, HbA1c and ang-2. Vascular bed damage in diabetes may be originated by chronically stimulated high glucose and advanced glycosylated end product, which might be mediated through cytokines, including TGFβ and oxidized stress like oxidized low density lipoprotein. Such mechanical and humoral factors may be involved in the enhanced production of adrenomedullin. The present study may therefore indicate that the elevated adrenomedullin in plasma is derived from vascular beds, dependent on the development of microangiopathy, and plays a certain role in protection against microvascular disturbance in diabetic patients.

Limitations of the study

It has to be stressed that this present study is limited by its cross-sectional design. Although the mechanisms mentioned can describe the associations of VEGF, Ang-2 as well as adrenomedullin with type 2 diabetes mellitus nephropathy, the causal relationship is still unclear. Longitudinal studies on type 2 diabetic subjects could provide a better basis to elucidate the exact impact of angiogenesis in the pathogenesis of diabetes and its other micro- and macro-vascular complications.

Conclusion

In conclusion, our study revealed that plasma levels of VEGF, Ang-2 and adrenomedullin were elevated among subjects with T2DM with renal complications, which was further accentuated when nephropathy set in and their relation to metabolic and renal abnormalities. Therefore, these mediators might play a role in the pathogenesis of diabetic vasculopathy in renal endothelial damage/dysfunction.

Acknowledgments

This project was funded by Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah. The authors, therefore, acknowledge with thanks DSR technical and financial support.

Competing interests

The authors declare that they have no competing interests.

Corresponding Author:
Laila H. Damanhouri, Ph D, MSc, DCP, M.B.B.Ch.
E mail : Lailahhd71@hotmail.com
P.O. Box 10415, Jeddah 21433, Saudi Arabia

References

1. .Golden SH (2011). Emerging therapeutic approaches for the management of diabetes mellitus and macrovascular complications. Am J Cardiol. 108(3):59B–67B.
2. Stengel B, Billon S, Van Dijk PC. *et al.* (2003) . Trends in the incidence of renal replacement therapy for end-stage renal disease in Europe, 1990-1999. Nephrol Dial Transplant. 18 (9): 1824-1833.
3. Schieppati A, Remuzzi, G (2005). Chronic renal diseases as a public health problem: epidemiology, social, and economic implications. Kidney Int Suppl. 98: S7–S10
4. Ferrara N (2001). Role of vascular endothelial growth factor in regulation of physiological angiogenesis. Am J Physiol Cell Physiol. 280 (6): C1358–C1366
5. Pizurki L, L Zhou Z, Glynos K, *et al*, (2003). Angiopoietin-1 inhibits endothelial permeability, neutrophil adherence and IL-8 production. Br J Pharmacol.. 139(2): 329–336
6. Maisonpierre PC, Suri C, Jones PF, *et al* (1997) . Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. Science. 277(5322):55-60.
7. Lobov IB, Brooks PC, Lang RA (2002). Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival in vivo. Proc Natl Acad Sci USA. 99 (17):11205-11210.
8. Watanabe D, Suzuma K, Suzuma I, *et al* (2005). (Ohashi H, Ojima T, Kurimoto M, Murakami T, Kimura T, Takagi H. Vitreous levels of angiopoietin 2 and vascular endothelial growth factor in patients with proliferative diabetic retinopathy. Am. J. Ophthalmol. 139(3): 476–481.
9. Chung YC, Hou YC, Chang CN, Hseu TH (2006). Expression and prognostic significance of angiopoietin in colorectal carcinoma. J. Surg. Oncol. 94(7): 631–638.
10. Anuradha S, Mohan V, Gokulakrishnan K, *et al.* (2010). Angiopoietin-2 levels in glucose intolerance, hypertension, and metabolic syndrome in Asian Indians (Chennai Urban Rural Epidemiology Study-74). Metab. Clin. Exp. 59(6): 774–779.
11. Eremina V, Baelde HJ, Quaggin SE (2007). Role of the VEGF–a signaling pathway in the glomerulus: evidence for crosstalk between components of the glomerular filtration barrier. Nephron Physiol. 106(2): p32–p37

12. Aiello LP, Wong JS (2000). Role of vascular endothelial growth factor in diabetic vascular complications. Kidney Int Suppl. 58:113–119
13. Kvanta A (2006). Ocular angiogenesis: the role of growth factors. Acta Ophthalmol Scand. 84(3):282–288.
14. Davis B, Dei Cas A, Long DA, *et al.* (2007). Podocyte-specific expression of angiopoietin-2 causes proteinuria and apoptosis of glomerular endothelia. J Am Soc Nephrol. 18 (8):2320–2329.
15. Bunton DC, Petrie MC, Hillier C, Johnston F, *et al.* (2004). The clinical relevance of adrenomedullin: a promising profile? Pharmacol Ther. 103(3):179–201.
16. Kusaka I, Ishikawa S, Fujita N, *et al.* (1996). Inhibition by adrenomedullin of arginine vasopressin-activated mitogen-activated protein kinase in rat glomerular mesangial cells via CAMP production. Hypertens Res. 19(2):113-119.
17. Kato J, Kitamura K, Kangawa K, *et al.* (1995). Receptors for adrenomedullin in human vascular endothelial cells. Eur J Pharmacol. 289(2): 383-385.
18. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. (1997). Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 20 (7): 1183–1197.
19. Stevens LA, Coresh J, Greene T, *et al.* (2006). Assessing kidney function: measured and estimated glomerular filtration rate. N Engl J Med. 354 (23):2473–2483.
20. Widlansky ME, Gokce N, Keaney JF Jr, *et al.* (2003) . The clinical implications of endothelial dysfunction. J Am Coll Cardiol. 42 (7):1149–1160.
21. Araki SI, Handa M, Sugimoto T, *et al.* (2005). Factors associated with frequent remission of microalbuminuria in patients with type 2 diabetes. Diabetes. 54(10):2983–2987.
22. Heap J, Murray M, Miller S, *et al* (2004). Alterations in bone characteristics associated with glycemic control in adolescents with type 1 diabetes mellitus. J Pediatr. 144(1):56-62.
23. Iwanicka Z, Lewandowicz-Uszyńska A, Głab E, et.al (2006). Relationship between nitrogen oxide and the degree of metabolic control of diabetes mellitus type 1 in children and adolescents. Wiad Lek. 59(1-2):27-31.
24. Neufeld G, Cohen T, Gengrinovitch S, *et al* (1999). Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 13(1): 9–22
25. Blann AD, Belgore FM, McCollum CN, Silverman S, *et al* (2002). Vascular endothelial growth factor and its receptor, Flt-1, in the plasma of patients with coronary or peripheral atherosclerosis, or Type II diabetes. Clin Sci. 102(2): 187–194.
26. Cooper ME, Vranes D, Youssef S, Stacker SA, *et al.* (1999). Increased renal expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 in experimental diabetes. Diabetes. 48(11):2229 –2239.
27. Tsuchida K, Makita Z, Yamagishi S, *et al.* (1999). Suppression of transforming growth factor and vascular endothelial growth factor in diabetic nephropathy in rats by a novel advanced glycation end product inhibitor, OPB-9195. Diabetologia. 42(5):579 –588.
28. Praidou A, Androudi S, Brazitikos P, *et al.* (2010). Angiogenic growth factors and their inhibitors in diabetic retinopathy. Curr Diabetes Rev. 6(5):304–312.
29. Lenz T, Haak T, Malek J, *et al.* (2003). Vascular endothelial growth factor in diabetic nephropathy. Kidney Blood Press Res; 26(5-6):338–343
30. Nakagawa T, Kosugi T, Haneda M, *et al.* (2009). Abnormal angiogenesis in diabetic nephropathy. Diabetes. 58(7): 1471–1478
31. Lindenmeyer MT, Kretzler M, Boucherot A, *et al.* (2007). Interstitial vascular rarefaction and reduced VEGF-A expression in human diabetic nephropathy. J Am Soc Nephrol. 18(6): 1765–1776.
32. Nakagawa T (2007). Uncoupling of the VEGF-endothelial nitric oxide axis in diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease. Am J Physiol Renal Physiol. 292(6): F1665–F1672.
33. Kim NH, Oh JH, Seo JA, *et al.* (2005). Vascular endothelial growth factor (VEGF) and soluble VEGF receptor FLT-1 in diabetic nephropathy. Kidney Int. 67(1):167–177.
34. Yuan HT, Khankin EV, Karumanchi SA, *et al.* (2009) . Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in the endothelium. Mol Cell Biol. 29(12): 2011–2022.
35. Nakagawa T, Sato W, Glushakova O, *et al.* (2007). Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. J Am Soc Nephrol.. 18(2):539–550.
36. Kumpers P, Hellpap J, David S, *et al.* (2009). Circulating angiopoietin-2 is a marker and potential mediator of endothelial cell detachment in ANCA-associated vasculitis with

renal involvement. Nephrol Dial Transplant. 24(6):1845–1850.
37. Lim HS, Lip GY, Blann AD (2005). Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF, glycaemic control, endothelial damage/dysfunction and atherosclerosis. Atherosclerosis. 180 (1):113–118.
38. Lieb W , Zachariah JP, Xanthakis V, *et al.* (2010). Clinical and genetic correlates of circulating angiopoietin-2 and soluble Tie-2 in the community. Circ. Cardiovasc. Genet. 3(3): 300–306.
39. Cui X, Chopp M, Zacharek A, *et al.* (2011). Angiopoietin/Tie2 pathway mediates type 2 diabetes induced vascular damage after cerebral stroke. Neurobiol. Dis. 43(1): 285–292.
40. David S, Kümpers P, Lukasz A, *et al.* (2010). Circulating angiopoietin-2 levels increase with progress of chronic kidney disease. Nephrol. Dial. Transplant. 25(8): 2571–2576.
41. Qiao L, Lu SL, Dong JY, *et al.* (2011). Abnormal regulation of neo-vascularisation in deep partial thickness scalds in rats with diabetes mellitus. Burns.37(6): 1015–1022.
42. De Caestecker M (2007). Angiopoietin-2 and Glomerular Proteinuria. J Am Soc Nephrol. 18(8): 2217–2218.
43. O'Bryan GT, Hostetter TH (1997) The renal hemodynamic basis of diabetic nephropathy. Semin Nephrol. 17(2):93–100, 1997
44. Kinoshita H, Kato K, Kuroki M, *et al.* (2000). Plasma adrenomedullin levels in patients with diabetes. Diabetes Care. 23(2): 253–254.
45. Hayashi M, Shimosawa T, Fujita T (1999). Hyperglycemia increases vascular adrenomedullin expression. Biochemical and Biophysical Research Communications. 258(2): 453–456.
46. Hayashi M, Shimosawa T, Isaka M, *et al.* (1997). Plasma adrenomedullin in diabetes. Lancet. 350 (9089): 1449– 1450.
47. Nakamura T, Honda K, Ishikawa S, *et al.* (1998). Plasma adrenomedullin levels in patients with non-insulin dependent diabetes mellitus: close relationships with diabetic complications. Endocr J. 45(2):241–246.
48. Sharma K, Ziyadeh FN (1995). Hyperglycemia and diabetic kidney disease. The case for transforming growth factor-beta as a key mediator. Diabetes. 44(10): 1139-1146.

12/5/2014