The relationship between plasma vascular endothelial growth factor and plasma insulin like Growth factor-i levels on diabetic nephropathy in Patients with type 2 diabetes

Mohga Shafik Abdalla[1], *Hayat Mohammad Sharada* [1], *Sameh Adel shakor* [2], *Ashraf Ismael Imen* [3], *and Neveen Sayed ElTokhey*[1]
[1]*Chemistry Department, Faculty of Science, Helwan University*
[2] Internal Medicine Department, *National Institute of Diabetes and Endocrinology* (NIDE), [3]Clinical pathology *Department, National Institute of Diabetes and Endocrinology* (NIDE)

ABSTRACT

Plasma Vascular endothelial growth factors (p VEGFs) as well as plasma insulin like growth factors- I (pIGFs- I) has been implicated in the pathogenesis of diabetes mellitus. This study was performed to determine whether alternations of p VEGFs and pIGFs are related to diabetic nephropathy in type 2 diabetic patients. ***Patients & Methods:*** *We examined the association of pVEGFs and pIGFs concentrations with fasting glucose levels, glycosylated hemoglobin (HbA1c %), urinary measured renal parameters i.e. creatinine clearance and albuminuria in 75 patients with type 2 diabetes and 25 healthy controls. Study subjects were divided into four groups using urinary albumin-to-creatinine ratio (ACR).* ***Results:*** *We confirmed that (i) both pVEGFs and pIGFs showed remarkable increase in all diabetic groups with worsen A/Cr ratio, as compared with controls. (ii) p VEGFs and pIGFs were increased in diabetic patients as long as glycemic control was not achieved. (iii) Vascular endothelial growth factor in plasma as well as plasma insulin like growth factors elevations were also revealed statistically. (iv) Direct positive correlation between pVEGFs and pIGFs-I with glycemic control index, albuminuria were noticed.* ***Conclusion:*** *The release of both p VEGFs as well as pIGFs was increased during the earlier stage of diabetic nephropathy and were significantly correlated with urinary albumin excretion. This suggested that pVEGFs could be used as an early sensitive marker for the diagnosis before the stage of microalbuminuria. and for predicting disease progression to start therapy very early.*

Key words*: type2 diabetes mellitus-diabetic nephropathy-plasma vascular endothelial growth factors-plasma insulin like growth factors.*

INTRODUCTION

Diabetes is a class of metabolic disorders characterized by hyperglycemia; the two most prevalent types are distinguished by lack of insulin (type 1) or insufficient insulin (type 2) [(1)].

Dallo & Weller [(2)]**,** estimated that 20 - 30 % of people with type 2

diabetes will develop evidence of overt nephropathy; an advanced stage disease marked by proteinuria that leads to over 20 % of type 2 diabetic progressed to end stage renal disease (ESRD) within 20 years.

A higher proportion of individuals with type 2 diabetes are found to have micro albuminuria and overt nephropathy shortly after the diagnosis of their diabetes, because diabetes is actually present for many years before the diagnosis is made and also because the presence of albuminuria may be less specific for the presence of diabetic nephropathy[3].

Among the many potential pathogenic mechanisms which are responsible for the development of diabetic kidney disease, growth factors have been suggested to be important players [4]. PVEGFs production is known to be stimulated by high glucose levels , advanced glycated end products (AGEs) , p IGFs , angiotensin II and hypoxia [5]. p VEGFs have been proposed to play a role in the development of diabetic renal changes in type 2 diabetes [6].

pVEGFs as well as p IGF-I may enhance the permeability of the glomeruli to macromolecules, leading to albuminuria and nephropathy[7] .

Aim of the work:

The objective of the present study was detecting the relationship between plasma vascular endothelial growth factor and plasma insulin like growth factor with nephropathy in type 2 diabetic patients.

PATIENTS & METHODS

Seventy-five patients with type 2 diabetes attending outpatients clinic of the National Institute of Diabetes and Endocrinology (NIDE) and 25 controls were involved in this study.

All patients were matched in age, sex and socioeconomic classes. They had no known disease history.

All subjects included in this study were free from viral infections; this was confirmed by serologic testing "i.e. HIV, HCV and HBV" which were done as an important adjunct to any clinical evaluation in the diagnosis of acute or severe chronic diseases i.e. liver diseases, because of the possibility of hepatitis associated glomerulonephritis. Also, pregnant women were excluded.

Groups Classification:

Individuals included in this study were classified to:

1. *Control group:* 25 individuals , who were matched in age (40–65 years), sex (1: 1).
2. *Diabetic group:* 75 diabetic patients who were matched controls in age and sex, classified into 3 subgroups (Group I, II and III) on the concept of pathogenesis of diabetic nephropathy[8].

Study subjects were divided on the basis of urinary albumin- to-creatinine ratio as markers of diabetic nephropathy to: 25 *normoalbuminuric* diabetic patients (Group I: A/Cr < 30), 25 *microalbuminuric* diabetic patients (Group II: A/Cr: 30 - 299) and 25 *overt proteinuric* diabetic patients (Group III: A/Cr ≥ 300).

Methods: Venous blood samples were collected from all subjects after over night fasting without therapy. Clotted and anti-coagulant EDTA samples were centrifuged while, whole blood samples were collected using EDTA as anticoagulant without any centrifugation.

Morning midstream urine samples were collected in sterile containers.

Serum, plasma and urine samples were aliquot and stored at - 80 ^{0}C until required for testing.

Laboratory parameters estimations were done for all subjects.

1. Routine laboratory investigations: - Fasting plasma glucose, serum creatinine, urinary creatinine and creatinine clearance using Beckman autoanalyzer. - Microalbuminuria using ELISA was assayed according to method of ***Mogensen***[9]. – Glycated hemoglobin using HPLC was done according to method of ***Little et al.***[10].
2. Plasma vascular endothelial growth factor according to method of ***Honkanen et al.***[11] and plasma insulin like growth factor-I according to method of ***Zapf et al.***[12] were measured by enzyme linked immunoassay.

Data were presented as the mean ± S.D. using Student's unpaired t-test for comparisons of data and used chi-square tests for comparisons of proportions coefficients were used for determining correlations between growth factors levels and different laboratory parameters with the use of statistical package of social science.

RESULTS

In table (1), the diagnosed diabetic cases showed significant increase ($p < 0.001$) in pVEGFs levels as compared with control and in between diabetic groups themselves.

pIGFs were significantly increased ($P< 0.01$) in all diabetic groups as regard to control group. While there was no significant change ($p> 0.05$) in pIGFs in between different diabetic groups.

Table (1): pVEGFs and p IGFs-I levels in all groups under study.

Study groups	Growth Factors	
	p. VEGF(ng/ml)	p. IGFs-I (ng/ml)
Control		
Mean ± S.D.	**21.24 ± 6.40**	**54.74 ± 16.50**
G I: A/Cr < 30	***	***
Mean ± S.D.	**83.17 ± 18.15**	**94.55 ± 20.63**
% Change *from control*	**291.57%**	**72.73 %**
G II: A/Cr: 30-299	# ***	***
Mean ± S.D.	**121.62 ±24.32**	**94.08 ± 18.82**
% Change *from control*	**472.60 %**	**71.87 %**
G III: A/Cr ≥ 300	¥ # ***	***
Mean ± S.D.	**180.17 ± 36.03**	**96.92 ± 19.38**
% Change *from control*	**748.26 %**	**77.06 %**

*Data are expressed as mean of **25** patients' ± S.D.*
******** Values are **very highly significant; P< 0.001** as compared to **control group**.*
*# Values are **very highly significant; P< 0.001** as compared to **G I**.*
*¥ Values are **very highly significant; P< 0.001** as compared to **G II**.*

Direct positive correlation was stated between pIGFs-I and pVEGFs in albuminuric diabetic groups. Both pVEGFs and pIGFs-I were shown direct positive correlation with glycated hemoglobin in albuminuria diabetic groups.

Table (2): **Serum fasting glucose level and glycosylated hemoglobin for all groups under study.**

Study groups	Sugar Profile	
	Fasting glucose(mg/dl)	HbA1c (%)
Control		
Mean ± S.D.	88.91 ± 26.81	4.83 ± 1.46
G I: A/Cr < 30	***	***
Mean ± S.D.	230.52 ± 50.30	8.78 ± 1.92
% Change *from control*	159.27 %	81.78 %
G II: A/Cr: 30-299	***	***
Mean ± S.D.	234.32 ± 46.86	9.72 ± 1.94
% Change *from control*	163.55 %	101.24 %
G III: A/Cr ≥ 300	***	¥ ***
Mean ± S.D.	276.52 ± 55.30	11.02 ± 2.20
% Change *from control*	211.01%	128.16 %

Data are expressed as mean of **25** patients' ± S.D.
******* Values are ***very highly significant; P< 0.001*** as compared to **control group.**
¥ Values are ***highly significant; P< 0.01*** as compared to **G I.**

Direct positive correlation was represented between microalbuminuria, pVEGFs and pIGFs-I. There was no significant change ($p > 0.05$) in *serum creatinine* between diabetics and control as well as in between different diabetic groups, while; a highly significant decrease ($p < 0.001$) in the measurement of *urinary creatinine* and *creatinine clearance* were regarded in G II and III as compared to control group. As well, significant decrease ($p < 0.001$) was shown in urinary creatinine and creatinine clearance levels in G II and III in comparison with G I.

Micro albuminuria measurement in G II and G III revealed highly significant increase ($p < 0.001$) with respect to both control and G I.

Direct positive correlation was founded between microalbuminuria and glycosylated hemoglobin percentage.

Table (3): Renal functions in all groups under study.

Study groups	Renal Functions			
	S.Creatinine (mg/dl)	**Micro-albuminuria (mg/dl)**	**U. Creatinine (g/L)**	**Creat. Clearance (ml/min.)**
Control **Mean ± S.D.**	**0.81 ± 0.24**	**27.88 ± 8.41**	**121.82 ±36.73**	**132.43±39.93**
G I: A/Cr < 30 **Mean ± S.D.** % Change *from control*	**0.77 ± 0.17** **- 4.94 %**	**22.30 ± 4.87** **- 20.01 %**	**124.71± 27.21** **2.37 %**	**109.76±21.95** **- 17.12 %**
G I: A/Cr : 30-299 **Mean ± S.D.** % Change *from control*	**0.74 ± 0.15** **- 8.64 %**	**# ***** **116.36 ± 23.27** **317.36%**	**# ***** **82.24± 16.45** **- 34.06 %**	**106.26±21.25** **- 19.76 %**
G III: A/Cr ≥ 300 **Mean ± S.D.** % Change *from control*	**0.84 ± 0.17** **3.70 %**	**# ***** **399.24 ± 79.85** **1331.99 %**	**# ***** **75.12± 15.02** **38.34 %**	**¥ *** **94.64±18.93** **- 28.54 %**

Data are expressed as mean of **25** patients' ± S.D.

******* Values are ***very highly significant; P< 0.001*** and ***significant***; **** P< 0.05*** as compared to **control group.**

Values are ***very highly significant; P< 0.001*** and ***significant***; ***¥ P< 0.05 as*** compared to G **I.**

DISCUSSION

Diabetes is a world wide public health problem with expectations that type 2 patients comprise approximately 85 - 90 % of all cases of diabetes[13].

The risk of vascular diseases either micro or macro vascular are increased two to four folds at least in type 2 patients compared with non diabetic subjects [14].

Of new diagnosed patients with type 2 diabetes, 8% have already nephropathy ; since many patients with type 2 diabetes are diagnosed in a later stage of the disease, changes are higher in their kidneys that seems to be already damaged [15].

The discovered vasoconstrictors and angiogenesis regulators, such as vascular endothelial growth factors (VEGFs) and insulin like growth factors (IGFs) have been intensely studied for possible pathogenic roles in diabetic vascular complications [16].

The early diagnosis of diabetes and its devastating complications by elevating growth factors measurement i.e. IGFs in retinopathy and VEGFs in nephropathy, can be taken to protect and prevent diabetic complications [17] .

This study focus on metabolic severity of nephropathy evaluations according to the degree of proteins in urine with duration of diabetes; where diabetic nephropathy is micro vascular complication of longstanding diabetes; the earliest accessible manifestation of this development is the presence of albumin in urine[18].

Our results revealed that all urinary measured parameters in all these groups i.e.: albumin, creatinine and creatinine clearance were highly affected by duration of disease as well as degree of hyperglycemia while serum creatinine was slightly affected.

Duration of diabetes, albumin excretion rate (AER) are emerged as powerful risk factors for progression to macroalbuminuria as best available marker for tracing the risk of developing diabetic nephropathy [19].

The increase in albumin excretion in urine between different diabetic groups from normo- to micro- up to overt proteinuria in contrast with decrease excretion rate of creatinine in urine as well as creatinine clearance and slight change in serum creatinine was explained on the basis of renal injury that glomerular filtration rate decreases as microalbuminuria occurs; at this point; loss of more nephrons occur , each remaining nephrons works harder , glomerulus therefore receives more blood at a higher pressure and fitters more fluid with low molecular weight proteins into tubules lastly lead to further nephrons loss; overt proteinuria and ending with progressive renal failure[20].

There was statistically positive direct correlation found in this study between microalbuminuria and glycosylated hemoglobin percentage. There is a significant positive correlation between urinary albumin excretion rate and glycosylated hemoglobin (HbA1c %) in patients

with macroalbuminuria that with worsen diabetic control , increase probability of nephropathy , increases nephron hyper filtration, results in increase albumin excretion rate and decrease creatinine clearance ; these results clarified that with increase occurrence of albumin in urine which defined as microalbuminuria ; consider as a sing of diabetic nephropathy.

These findings were confirmed by ***Takebayashi et al.*** [(21)] who showed a significant positive correlation between urinary albumin excretion rate and HbA1c % in patients with macroalbuminuria and added explanation that with worsen diabetic control, increase probability of nephropathy , increases nephron hyper filtration , result in increase albumin excretion rate and decrease creatinine clearance; these results clarified that with increase occurrence of albumin in urine which defined as microalbuminuria; consider as a sing of diabetic nephropathy.

This study showed; remarkable increase in diabetic concentrations of plasma vascular endothelial growth factors as compared with control group as well as significant noticeable increase in between different diabetic groups under study with increase pVEGFs concentration. This was confirmed by statistically correlations created in this thesis between control and diabetics groups under study as well as in between diabetic groups themselves for pVEGFs.

In ***2005, Flyvbjerg et al.*** [(22)] concluded that VEGF, IGF-I and cytokines are implicated in the pathogenesis of diabetic kidney diseases ; so , our study focus on studying pIGFs beside plasma vascular endothelial growth factor . The results obtained showed elevation in plasma insulin like growth factor - I (IGF-I) concentrations in all diabetic groups under study as compared with control group, while non significant noticeable increase in between different diabetic groups under study.

Direct positive correlation was found in this study between microalbuminuria existence and elevation in measured growth factors levels: pVEGFs and pIGFs.

VEGFs overrelease may enhance the permeability of the glomeruli to macro molecules leading to albuminuria [(23)]. Also there was strong positive correlation between metabolic and hemodynamic factors i.e. pVEGFs and pIGFs indicators of vascular complications concur in the development of diabetic nephropathy and albuminuria as an indicator for renal hyperpermeability[(24)].

In ***2005, Medical World Communication*** [(25)]**,** reported that pVEGF levels have been found to correlate with microalbuminuria, therefore ; the increase of the systemic VEGF levels have been suggested as pathogenic factor in diabetic nephropathy.

The explanation was that VEGF is a key pathogenic factor of diabetic nephropathy where over expression of VEGF increases hyper permeability and increase hyperfiltration of proteins in urine[(7)]. In addition, ***Assem et al.*** [(26)]**,** reported that the measurement of VEGF as angiogenic factors was important to

predict cases at high risk development of incipient diabetic nephropathy (persistent microalbuminuria) with a sensitivity of 60 – 100 % and a specificity of 86.67 -93.33 %.

Now, understanding the role of pVEGF on elevating the possibility of presence of diabetic kidney diseases (nephropathy) lead to why did plasma vascular endothelial growth factor concentration elevate as well as insulin like growth factor-I? This question was answered from our results statistics proved that there was direct positive correlation between pVEGFs and pIGFs with hyperglycemia as well as glycated hemoglobin as well.

This means hyperglycemia and diabetic complications index (HbA1c) were the inducible factors to different measured growth factors either pIGFs or p VEGFs. This was confirmed by ***Ashraf et al.*** [(27)], who mentioned that the presence of hyperglycemia is associated with elevation of growth factors i.e. pVEGFs and pIGFs-I and found highly significant correlation between glycated hemoglobin, pVEGFs and pIGFs-I as well. Also, ***Amino et al.*** [(28)] reported that VEGF production is known to be stimulated by high glucose levels, advanced glycosylated end products , pIGFs-I , Angiotensin II and hypoxia.

Zhang et al. [(29)] as well as ***Araf et al.*** [(30)] reported that hyperglycemia activates polyol pathway in tissues as well as hexosamine pathway which activates protein kinase-C by diacyl glycerol which plays great roles in vascular disease so, the longstanding hyperglycemia which activate synthesis of advanced glycated end products (AGEs) formation interrelated with polyol formation and oxidative stress which are responsible for activating release of mediators of cell to cell communication, including growth factors such as: TGFß, IGF- I and VEGF.

In conclusion, our study evaluate the role of pVEGFs and pIGFs-I in type 2 Egyptian diabetic patients and their relation to diabetic nephropathy as well as raises the possibility that both plasma vascular endothelial growth factors and insulin like growth factor as well could detect very early before other markers and be a sensitive marker of diabetic nephropathy. Also, this is very important to find and define the more powerful predictors for diabetic nephropathy. These tests indicates that a person had slight kidney damage, and still treatment options that may help prevent further damage are available and preserve remaining renal functions.

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