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Is Placental Growth Factor an angiogenic marker in type 2 diabetes mellitus?

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Abstract

Introduction: Type 2 diabetes mellitus (T2DM) known by vascular complications due to abnormal angiogenesis leading to micro and macro vascular complications. PlGF a close homolog of VEGF, shares receptors with VEGF, and stimulates angiogenesis. By up regulating PlGF and the signaling subtype of VEGFR-1, endothelial cells amplify their responsiveness to VEGF in many pathological conditions.

Objectives: To compare and estimate serum PlGF and VEGF levels in patients with T2DM.

Materials and Method: A case control study conducted with 30 type 2 diabetes mellitus and 30 age and sex matched healthy subjects as controls. All samples analyzed for FBS, HbA_{1C} in an automated analyzer (Beckmann coulter AU480) and serum VEGF, PlGF was estimated by Biotin double antibody sandwich ELISA. Data analysis was done by using relevant statistical tests.

Results: Mean PlGF and VEGF was significantly higher in cases as compared to control with p value of <0.0001 respectively. Serum PlGF showed positive correlation with VEGF, FBS and HbA_{1C} with 'r' value of 0.867 (p=<0.0001), 0.555 (p=0.0014), 0.5951 (p=0.0005) respectively.

Conclusion: Serum PlGF was significantly higher in cases than in controls and showed positive correlation with serum VEGF. PlGF stimulates angiogenesis, which is implicated in all the micro vascular complications of type 2 diabetes mellitus. Hence detecting PlGF early in the course of disease helps to prevent complications and in the management of complications of type 2 diabetes mellitus.

Keywords: Type 2 Diabetes Mellitus, Angiogenesis, PlGF-Placental Growth Factor, VEGF-Vascular Endothelial Growth Factor, VEGFR 1 and 2- Vascular Endothelial Growth Factor Receptor 1 and 2.

Introduction

Type 2 Diabetes mellitus (DM) is a group of disorders characterized by chronic hyperglycemia, which leads to many metabolic as well as vascular complications. Apart from hyperglycemia, abnormal angiogenesis may cause or contribute in the pathogenesis of vascular abnormalities seen in retina, kidneys, fetus, and impaired wound healing, increased risk of transplant rejections, and abnormal formation of coronary collaterals. This abnormal angiogenesis mainly occurs in the form of excessive or insufficient angiogenesis in various organ systems in the body.[(1)] Angiogenesis, a process of formation of new capillary network (micro vascular) as a result of hypoxia or other stimuli.[(2)] Angiogenesis a complex multisequential process which involves interaction between various angiogenic mediators such as Vascular endothelial growth factor (VEGF), Fibroblast growth factor (FGF2), Transforming growth factor-β and angiopoeitins and antiangiogenic mediators such as angiostatins, endostins, and thrombospondins, various growth factors, cytokines, endolthelium and extracellular matrix(ECM).[(1)] Process of angiogenesis is characterized by ECM degeneration, endothelial cell (EC) proliferation, EC survival, EC migration, EC morphology changes, and EC anastomoses.[(3)] VEGF a family of angiogenic factors which includes VEGF (A-D) and Placental Growth Factor (PlGF). VEGF family interacts with various tyrosine kinase receptors such as Fms related tyrosine kinase 1 (Flt 1), fetal liver kinase 1, and Flt 4. VEGF family acts as mitogens for vascular EC, and stimulate endothelial progenitor cells (EPCs) migration from bone marrow. VEGF-A (through VEGFR-1 and 2) family is associated with development, maintainance, and remodeling of vasculature.[(4)] Diabetes mellitus is characterized by reduced number of EPCs, the extent of which is directly proportional to HbA_{1C} levels.[(5)] The disease process leads to defective signaling in VEGF which results in abnormal Flk 1 activation affecting various processes of angiogenesis as mentioned above. All these leads to increased VEGF levels and abnormal angiogenesis. Placenta growth factor (PlGF) a member of VEGF family is which has emerged as an pro-angiogenic factor which binds specifically to VEGFR-1 receptor. Placental growth factor a member of VEGF family endoded by PLGF gene is secreted as a glycosylated homodimer.[(6)] Many studies of PlGF deficient mice have demonstrated that PlGF is involved in pathological angiogenesis including choroidal[(7)] and retinal neovascularization.[(8)] Such studies also demonstrated role of PlGF in extravasation of plasma and collateral vessel growth in ischaemia and wound healing and its role in inflammation.[(8)] And it has been also shown that PlGF stimulates angiogenesis and collateral growth in ischaemic heart and limb with an efficacy equal to VEGF. It has been demonstrated that PlGF and VEGF plays a synergistic role in pathologic angiogenesis. And PlGF thought to amplify VEGF

driven angiogenesis and activates vascular endothelial cells.[8] And further this can represent that PlGF acts as an additional and safer (given to specificity to VEGFR-1) target for the treatment of neovascular disorders.[9]

Hence present study was taken to assess and correlate serum PlGF and VEGF levels in patients with type 2 diabetes mellitus.

Materials and Method

Study was done on 30 diagnosed cases of type 2 diabetes mellitus out patients attending Victoria hospital attached to BMC & RI. Study also included 30 age and sex matched healthy individuals from general population as controls. Patients with liver dysfunction, essential hypertension, neoplastic diseases and on its treatment, also, Pregnant and lactating mothers were excluded from the study. After written informed consent, 5ml fasting venous blood sample was obtained and centrifuged, separated serum was used to estimate fasting blood sugar, HbA_{1C} by chemiluminescence method in an autoanalyser Beckmann coulter AU480 and serum PlGF and VEGF were estimated by Biotin double antibody sandwich Enzyme Linked Immuno Sorbent Assay (ELISA).

Study design: A case control study to estimate serum PlGF and VEGF levels in patients with type 2 DM. and to assess the correlation of serum PlGF with serum VEGF, FBS, and HbA_{1C} was undertaken.

Statistical methods: descriptive statistical analysis has been carried out in the present studies results on continuous measurement as present on Mean ± SD (Min-Max) and results on categorical measurements are present in Number (%). Significance is assessed at 5% level of significance. Pearson's correlation is used to assess significance of correlation.

Statistical softwares: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver. 2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

Statistical Analysis

Table 1: Age distribution among cases and controls

Age in yrs.	Cases	Controls	Total
30-40	6(20%)	2(7%)	8(13%)
41-50	6(20%)	14(47%)	20(67%)
51-60	13(43%)	11(36%)	24(40%)
61-70	5(17%)	3(10%)	8(13%)
Total	30	30	60
Mean ± SD	52.43±10.32	51.33±7.34	

P value: 0.6360 t score: 0.4758 NOT SIGNIFICANT

Table 2. Gender distribution between cases and controls

Gender	Cases	Controls	Total
Male	13(43%)	13 (43%)	26(43%)
Female	17(57%)	17(57%)	34(57%)
Total	30	30	60

Among 30 cases 13 were males (43%) and 17 were females (57%). Samples were gender matched.

Table 3: Comparison of mean FBS in the two groups studied

FBS (Mean ± SD)	Cases	Controls
	290.6±148.11	97.67±9.78
t score	7.119	
p value	0.0001*	

Mean FBS was significantly higher in cases as compared to control group.

Table 4: Comparison of mean PlGF and VEGF between the two groups studied

Subjects	Total no.	Mean ± SD	
		PlGF	VEGF
Cases	30	93.19 ± 56.47	96.22 ± 16.871
Controls	30	45.393 ± 16.12	56.83 ± 16.93
t score		4.4579	9.027
p value		0.0001**	0.0001**

Mean serum PlGF and Serum VEGF was significantly higher in cases as compared to controls with p value of 0.0001 respectively.

Table 5: Correlation between serum PlGF and serum VEGF, FBS, and HbA_{1C} in diabetic group

Parameters		FBS	HbA_{1C}	VEGF
Serum PlGF	r score	0.555	0.5951	0.867
	p value	0.0014**	0.0005**	<0.0001**

Serum PlGF showed positive correlation with FBS, HbA_{1C} and serum VEGF.

Scatter Plot - 1: Between serum PlGF and VEGF

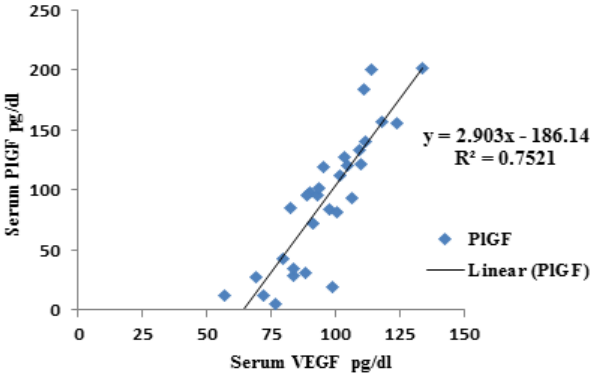

Fig. 1

Discussion

In the present study it was observed that serum PlGF and serum VEGF were significantly higher in cases (diabetic group) as compared to controls. When correlation analysis was done it was found that serum PlGF showed large positive correlation with FBS and HbA1C and very large correlation with serum VEGF levels. Angiogenesis a major pathological change associated with many complex diseases such as cancer, atherosclerosis, age related macular degeneration, arthritis and diabetes mellitus.[6] Study done by Peter Carmeliet et al[8] showed that PlGF and VEGF act

synergistically in inducing pathological angiogenesis suggesting endothelial cells upregulate PlGF expression and signaling subtype of VEGFR-1 thus amplifying their responsiveness to VEGF during angiogenic switch in pathological conditions. Same study also showed that deficiency of PlGF did not affect embryonic angionenesis but loss of PlGF impaired angiogenesis, plasma extravasation, collateral growth formation during ischemia, inflammation and wound healing. Study done by Sandro D Falco[6] showed that one possibility for PlGF in amplifying VEGF is that, as PlGF bind exclusively to VEGFR-1 and displaces VEGF-A from VEGFR-1 which can bind to VEGFR-2, resulting in abnormal angiogenesis. Study done by Yoshinori Mitamura[10] showed correlation of PlGF levels with VEGF levels in vitreous fluid suggesting a cooperative role PlGF in the progression of proliferative diabetic retinopathy. In the present study positive correlation between serum PlGF and VEGF suggests there is a role of PlGF in excessive angiogenesis in type 2 diabetes mellitus resulting microvascular complications of retinopathy and nephropathy. In this study it was also seen that serum PlGF levels were correlated positively with levels of glycated Hb (HbA_{1C}).

Conclusion

This case control study showed there is significant increase in serum PlGF and VEGF levels in type 2 diabetes mellitus patients indicating excessive abnormal angiogenesis leading to many vascular complications involving multiple organ systems mainly microvascular which includes retinopathy and nephropathy which are the cause of higher morbidity of the disease. Hence measuring serum PlGF levels may help in identifying extent of pathological angiogenesis. Additionally it may be of help in management of diabetic microvascular complications because of its specificity to VEGFR-1 receptors acting as safe target for anti angiogenic compounds. Further large scale prognostic studies are required to prove this.

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