Published in final edited form as:

J Investig Med. 2010 August ; 58(6): 804–806. doi:10.231/JIM.0b013e3181e96203.

Both Vascular Endothelial Growth Factor and soluble Flt-1 are increased in Type 2 Diabetes but not in Impaired Fasting Glucose

Debashis Nandy, MD,

Division of Endocrinology and Metabolism, Mayo Clinic College of Medicine Rochester, MN 55905

Debabrata Mukhopadhyay, PhD*, and Ananda Basu, MD

Division of Endocrinology and Metabolism, Mayo Clinic College of Medicine Rochester, MN 55905

Abstract

Objective—Inadequate vascular remodeling is contributory to increased cardiovascular events in people with type 2 diabetes (DM) and impaired fasting glucose (IFG). Vascular Endothelial Growth Factor (VEGF) and its regulatory molecule soluble Flt-1 (sFlt-1) play important roles in atherogenesis.

Research Design—We measured fasting plasma concentrations of VEGF and sFlt-1 in 11 ND (age 46.1±2.1 yrs., BMI 26.1±0.9 kg/m², glucose 5.0±0.1 mM), 15 IFG (age 52.9±1.8 yrs., BMI 32.7±1.3 kg/m², glucose 6.4±0.1mM) and 8 DM (age 55.8±3.2 yrs., BMI 30.0±1.0 kg/m², glucose 9.3±0.5 mM) subjects.

Results—Plasma VEGF (42.1±4.0 vs. 24.2±0.9 vs. 29.4±3.8 pg/ml respectively) and sFlt-1 (119.4±4.9 vs. 58.9±3.2 vs. 56.7±1.2 pg/ml respectively) concentrations were higher (p<0.04) in DM than IFG and ND subjects. While VEGF concentrations were significantly lower (p<0.05) in IFG than ND subjects, sFlt-1 concentrations did not differ between the IFG and ND subjects.

Conclusions—Though plasma VEGF concentrations were higher (35%) in DM than ND subjects, VEGF action on vascular remodeling was likely attenuated by higher sFlt-1 concentrations in DM. In contrast, IFG subjects did not have major perturbations in either VEGF or sFlt-1 levels. Further studies defining the roles of these mediators in DM and IFG are necessary to extend these observations.

Keywords

VEGF; sFlt-1; diabetes

Introduction

Type 2 diabetes mellitus (DM) is a cardiac risk equivalent with a 2-4 fold greater prevalence of cardiovascular disease than nondiabetic individuals. Diabetic individuals without prior

basu.ananda@mayo.edu Tel: 507-255-6515 Fax: 507-255-4828 . *Department of Biochemistry and Molecular Biology Mayo Clinic College of Medicine Rochester, MN 55905. .

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

myocardial infarction have at least as high a risk of myocardial infarction as nondiabetic individuals with a prior history of myocardial infarction (1). Furthermore, inadequate vascular remodeling and insufficient collateral formation are critical responses to hypoxia that are likely contributory to increased cardiovascular events in DM and impaired fasting glucose (IFG). A large international contemporary database of established atherothrombotic disease showed a prevalence of DM of 44% and that of IFG of ~37% (2). This staggering statistics showing that almost 80% of those with macrovascular disease have either DM or IFG is a testimony to the burden of the disease process.

Vascular Endothelial Growth Factor (VEGF) is a heparin binding growth factor that is mostly secreted by vascular endothelial cells and is a crucial determinant of vascular remodeling and collateral formation by binding to its receptor VEGF-Receptor-1 (fms-like tyrosine kinase or Flt-1). This binding is normally inhibited by a naturally occurring soluble form of VEGFR-1 (sFlt-1). Elevated plasma levels of sFlt-1 have been linked to the development of toxemia of pregnancy likely resulting from impaired placental vascular development (3; 4).

To our knowledge, there have been no prior simultaneous assessments of plasma VEGF and sFlt-1 in those with IFG or DM. To elucidate this further, we performed simultaneous measurements of VEGF and sFlt-1 in cohorts of healthy nondiabetic subjects (ND), IFG and type 2 DM. We report that subjects with DM had plasma VEGF levels that were ~35% higher than ND and IFG subjects. In contrast, plasma sFlt-1 levels were 2 fold elevated in DM than ND and IFG subjects. This could have substantial implications in the pathogenesis of macrovascular disease and collateral formation in those with DM.

Materials and Methods

After approval from the Mayo IRB, blood samples were collected after an overnight fast in 11 ND, 15 IFG and 8 DM subjects. None of the subjects had any history of overt cardiovascular, hepatic, renal disease or hypertension. The ND subjects did not have a history of diabetes in first degree relatives. All oral antidiabetic agents were discontinued two weeks prior to sampling in DM subjects. None of the DM subjects were on insulin therapy. None of the IFG subjects were on antidiabetic medication. None of the subjects underwent vigorous physical activity for a week prior to sampling. Fasting plasma glucose and HbA1c were measured along with VEGF and sFlt-1 concentrations.

VEGF was measured by QuantiGlo® Chemiluminescent Human VEGF assay (R&D Systems, Minneapolis, MN). The amount of VEGF bound was measured by a microplate luminometer (5). The coefficient of variation for intra-assay was 1.8% at 78.8 pg/ml, 2.2% at 1629 pg/ml and 3.5% at 11,984 pg/ml and for inter-assay was 7.9% at 75.2 pg/ml, 4.1% at 1639 pg/ml and 6.4% at 11,705 pg/ml. This assay measured free VEGF₁₆₅ in plasma with minimal interference from sFlt-1.

Plasma soluble VEGF R1/Flt-1 was measured by Quantikine® Human Soluble VEGF R1/Flt-1 immunoassay (R&D Systems, Minneapolis, MN) that employed the quantitative sandwich enzyme immunoassay technique. Proportion of the amount of Flt-1 bound was measured by a microplate luminometer that reads the microplate at 450nm within 30 minutes and λ correction at 540 nm or 570 nm. The coefficient of variation for intra-assay was 2.6% at 96.6 pg/ml, 3.8% at 596 pg/ml and 3.2% at 1213 pg/ml and for inter-assay was 9.8% at 112 pg/ml, 7.0% at 645 pg/ml and 5.5% at 1279 pg/ml. All data is expressed as mean $\pm$ sem. ANOVA was used to determine differences between the three groups. A p-value of < 0.05 was determined as statistically significant and students unpaired t-test was performed to detect differences between individual groups.

Results

The subject characteristics are provided in Table 1. By design, fasting plasma glucose was higher ($p<0.0001$) in the DM and IFG subjects than ND individuals; fasting glucose was also higher ($p<0.01$) in the DM than IFG subjects and between IFG and ND subjects. HbA1c concentrations were also higher in the DM than IFG and ND subjects but were no different between IFG and ND subjects. Both age and body mass index differed ($p<0.04$) between groups with differences in both these parameters occurring between the ND and each of the two other groups. There were no differences in age or body mass index between the DM and IFG subjects.

Plasma VEGF concentrations (Fig 1 Upper Panel)

Plasma VEGF concentrations were higher ($p<0.001$) in the DM than IFG and ND subjects (42.1 ± 4.0 vs. 24.2 ± 0.9 vs. 29.4 ± 3.8 pg/ml respectively). Further analyses confirmed that DM subjects had higher VEGF levels than both IFG subjects ($p<0.001$) and ND subjects ($p<0.04$). There was also a slight but significant ($p<0.02$) difference in the VEGF levels between IFG and ND subjects.

Plasma sFlt-1 concentrations (Fig 1 lower Panel)

Plasma sFlt-1 concentrations were also higher ($p<0.0001$) in the DM than IFG and ND subjects (119.4 ± 4.9 vs. 58.9 ± 3.2 vs. 56.7 ± 1.2 pg/ml respectively). Further analyses confirmed that DM subjects had higher sFlt-1 levels than both IFG ($p<0.0001$) and ND ($p<0.0001$) subjects. In contrast to VEGF levels however, there were no differences ($p=0.6$) in sFlt-1 levels between IFG and ND subjects.

Correlation between plasma glucose, VEGF and sFlt-1 concentrations (Fig 2)

There were significant correlations ($p<0.01$) between fasting glucose concentrations and VEGF levels ($r^2=0.46$) (upper panel) and also between fasting glucose and sFlt-1 levels ($r^2=0.7$) (lower panel) in the entire cohort. However, within the IFG and ND groups, taken together or separately, there were no correlations between fasting glucose, VEGF or sFlt-1 concentrations. There were no correlations between HbA1c, VEGF and sFlt-1 concentrations in the cohorts studied.

Discussion

Our results show that DM subjects without overt macrovascular disease had higher plasma VEGF and sFlt-1 concentrations than individuals with IFG or ND. Furthermore, individuals with IFG had slightly but significantly lower plasma VEGF concentrations while their sFlt-1 concentrations did not differ when compared to ND individuals. Contrary to prior report (6), our data clearly reveal dramatic increases in sFlt-1 concentrations that is up to two-fold higher in DM than IFG and ND subjects. These initial observations suggest intriguing possibilities regarding vascular remodeling issues in both DM and IFG subjects.

The activity of VEGF mediated angiogenesis is limited by the extent of its binding to circulating soluble receptor Flt-1 (7). Our results suggest the possibility that in those with diabetes, although VEGF levels are increased when compared to IFG and ND subjects, the dramatic doubling of sFlt-1 concentrations likely limit the angiogenic potential of VEGF, that could lead to impaired vascular remodeling and collateral formation. That said, in the absence of measuring VEGF bioactivity in plasma as well as exploring the stoichiometric relationship between VEGF and sFlt-1, our line of reasoning remains speculative and needs to be further evaluated in future studies. In contrast, in IFG subjects, although the sFlt-1 levels were no different when compared to those with ND, the slight but significant fall in

VEGF levels could potentially be a factor in impaired angiogenesis and vascular remodeling in these individuals.

However, use of VEGF and/or sFlt-1 as biomarkers for cardiovascular disease in individuals with type 2 diabetes will need to be further explored and tested. Further population based studies will need to be performed to compare the predictive power of these novel biomarkers with conventional CV risk markers.

Elevated levels of VEGF have been found in the vitreous humor (8) and plasma (9) of those afflicted with diabetic retinopathy, in the urine of those with diabetic nephropathy (10) and in plasma of individuals with DM with or without vascular disease (6; 11). In contrast, while reduced expression of VEGF has been found in myocardium of subjects with DM with acute coronary syndrome (12), other studies have found higher myocardial VEGF mRNA and protein levels but lower Flt-1 mRNA and protein levels in diabetic subjects with chronic congestive cardiac failure than their nondiabetic counterparts (13). Few existing studies have examined plasma sFlt-1 levels in individuals with DM. While sFlt-1 have been shown to be increased in the urine in those with diabetic nephropathy (10), there were no changes in plasma sFlt-1 levels in those with diabetic retinopathy (9).

In the only other study that measured VEGF and sFlt-1 levels in subjects with DM (6), VEGF concentrations were increased in those with DM and vascular disease (but not in those without vascular disease) while there were no alterations in sFlt-1 levels when compared to nondiabetic subjects.

The positive correlations between fasting plasma glucose and both VEGF and sFlt-1 concentrations for the entire cohort was intriguing suggesting a mechanistic link between rising glucose concentrations and regulation of angiogenesis. While a relationship between glycemic control as measured by HbA1c and VEGF levels have been reported earlier (11; 14), there have been no prior information of correlations between glycemic status and sFlt-1 levels. Additionally, there have been studies that have shown reduction in plasma VEGF concentrations with improved glycemic control (11; 15; 16). Whether that translates into improved vascular outcomes is uncertain. Large clinical trials either has (17; 18) or has not affected (19) major cardiovascular outcomes with improved glycemic control in diabetes. However, it remains to be seen whether a combined approach of improvement in glycemic control linked with modulation of angiogenic factors could pave the way for improving the considerable cardiovascular morbidity in these high-risk individuals.

Acknowledgments

This work was carried out with the support of NIH grants HL072178 and HL070567 to D.M. and Mayo Foundation Clinical Research award to A.B. We also acknowledge support from the General Clinical Research Center of Mayo Clinic College of Medicine and our study volunteers.

References

1. Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med*. 1998; 339:229–234. [PubMed: 9673301]
2. Bhatt DL, Steg PG, Ohman EM, Hirsch AT, Ikeda Y, Mas JL, Goto S, Liao CS, Richard AJ, Rother J, Wilson PW. International prevalence, recognition, and treatment of cardiovascular risk factors in outpatients with atherothrombosis. *JAMA*. 2006; 295:180–189. [PubMed: 16403930]
3. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine

- kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*. 2003; 111:649–658. [PubMed: 12618519]
4. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*. 2004; 350:672–683. [PubMed: 14764923]
 5. Jelkmann W. Pitfalls in the measurement of circulating vascular endothelial growth factor. *Clin Chem*. 2001; 47:617–623. [PubMed: 11274009]
 6. Blann AD, Belgore FM, McCollum CN, Silverman S, Lip PL, Lip GY. 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 (Lond)*. 2002; 102:187–194. [PubMed: 11834138]
 7. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med*. 2003; 9:669–676. [PubMed: 12778165]
 8. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med*. 1994; 331:1480–1487. [PubMed: 7526212]
 9. Lip PL, Belgore F, Blann AD, Hope-Ross MW, Gibson JM, Lip GY. Plasma VEGF and soluble VEGF receptor FLT-1 in proliferative retinopathy: relationship to endothelial dysfunction and laser treatment. *Invest Ophthalmol Vis Sci*. 2000; 41:2115–2119. [PubMed: 10892852]
 10. Kim NH, Oh JH, Seo JA, Lee KW, Kim SG, Choi KM, Baik SH, Choi DS, Kang YS, Han SY, Han KH, Ji YH, Cha DR. Vascular endothelial growth factor (VEGF) and soluble VEGF receptor FLT-1 in diabetic nephropathy. *Kidney Int*. 2005; 67:167–177. [PubMed: 15610240]
 11. Lim HS, Blann AD, Chong AY, Freestone B, Lip GY. Plasma vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2 in diabetes: implications for cardiovascular risk and effects of multifactorial intervention. *Diabetes Care*. 2004; 27:2918–2924. [PubMed: 15562207]
 12. Marfella R, Esposito K, Nappo F, Siniscalchi M, Sasso FC, Portoghese M, DiMarino MP, Baldi A, Cuzzocrea S, DiFilippo C, Barboso G, Baldi F, Rossi F, D'Amico M, Giugliano D. Expression of angiogenic factors during acute coronary syndromes in human type 2 diabetes. *Diabetes*. 2004; 53:2383–2391. [PubMed: 15331549]
 13. Sasso FC, Torella D, Carbonara O, Ellison GM, Torella M, Scardone M, Marra C, Nasti R, Marfella R, Cozzolino D, Indolfi C, Cotrufo M, Torella R, Salvatore T. Increased vascular endothelial growth factor expression but impaired vascular endothelial growth factor receptor signaling in the myocardium of type 2 diabetic patients with chronic coronary heart disease. *J Am Coll Cardiol*. 2005; 46:827–834. [PubMed: 16139132]
 14. Lim HS, Lip GY, Blann AD. Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF, glycaemic control, endothelial damage/dysfunction and atherosclerosis. *Atherosclerosis*. 2005; 180:113–118. [PubMed: 15823283]
 15. Ashraf A, Mick G, Meleth S, Abdullatif H, Wang X, McCormick K. Effect of insulin on plasma vascular endothelial growth factor in children with new-onset diabetes. *J Clin Endocrinol Metab*. 2005; 90:4920–4923. [PubMed: 15914522]
 16. Kakizawa H, Itoh M, Itoh Y, Imamura S, Ishiwata Y, Matsumoto T, Yamamoto K, Kato T, Ono Y, Nagata M, Hayakawa N, Suzuki A, Goto Y, Oda N. The relationship between glycemic control and plasma vascular endothelial growth factor and endothelin-1 concentration in diabetic patients. *Metabolism*. 2004; 53:550–555. [PubMed: 15131756]
 17. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med*. 2005; 353:2643–2653. [PubMed: 16371630]
 18. Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med*. 2003; 348:383–393. [PubMed: 12556541]
 19. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*. 1998; 352:837–853. [PubMed: 9742976]

Figure 1.

Upper Panel A. shows plot of plasma VEGF concentrations in healthy nondiabetic subjects (open circles), subjects with impaired fasting glucose (open triangles) and subjects with type 2 diabetes (closed squares). The horizontal lines represent mean of each group.

Lower Panel B. shows plot of plasma sFlt-1 concentrations in healthy nondiabetic subjects (open circles), subjects with impaired fasting glucose (open triangles) and subjects with type 2 diabetes (closed squares). The horizontal lines represent mean of each group.

Figure 2. Shows correlations between fasting plasma glucose concentrations and VEGF levels (top panel) and sFlt-1 concentrations (bottom panel) in the entire cohort.

Table 1

Volunteer Characteristics: ND: Nondiabetic; IFG: Impaired fasting glucose; DM: Type 2 diabetes

	ND (n=11)	IFG (n=15)	DM (n=8)
Age	46.1±2.1	52.9±1.8 [#]	55.8±3.2 [*]
Sex	5♂6♀	9♂6♀	4♂4♀
BMI	26.1±1.0	32.7±1.3 [#]	30.0±1.0 [*]
Glucose	5.0±0.1	6.4±0.1 [#]	9.3±0.5 [*] @
HbA1c	5.8±0.2	6.0±0.2	7.9±0.5 [*] @

*
p< 0.05 DM vs. ND#
p< 0.05 IFG vs. ND@
p< 0.05 DM vs. IFG