Interaction between expression of angiogenic factors and pathology of the microvasculature and cardiomyocytes in myocardial tissue of patients with diabetes mellitus

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Abstract

Sudden death with myocardial infarction has always been a challenging issue for the investigators and forensic pathologists. When a person suffering from angina or myocardial infarction is simultaneously suffering from diabetes mellitus issue becomes even more complex for the investigators as usual signs & symptoms of MI may not manifest so as to rouse a suspicion of MI. This study will help the pathologists to understand the microscopic changes of diabetic cardiomyopathy better in a case having MI with diabetes. This study was done in 47 cases to know the pathology of microvasculature and cardiomyocytes in myocardial tissue of diabetic patients and expression of angiogenic factors.

Keywords: Sudden death, diabetes mellitus, myocardial infarction.

Introduction

The major cause of both morbidity and mortality in diabetic patients is cardiovascular disease. Diabetic cardiomyopathy is proposed to affect up to 50% of patients with Type 2 diabetes[1-4]. Light and electron microscopic ultra structural changes of the cardiomyocyte have been quantified in a range of animal models of diabetes including the dog[5], monkey[6], rabbit[7], mouse[8] and rat[9-12]. However, widely varying results have been reported with regard to the onset, type and severity of cardiomyocyte pathology[10] . Studies in humans are limited[13].

Pathogenesis of cardiomyocyte involvement in diabetes mellitus involves vascular endothelial cell dysfunction and cardiomyocyte necrosis[14]. Whilst most structural studies of the myocardial vasculature have focused on coronary arteries and intramural arterioles, few studies have assessed myocardial capillary structure in either clinical or experimental diabetes showing significant ultrastructural changes in cardiomyocytes with or without concomitant vascular changes in the diabetic heart[11,13,15]. These changes include thickening of endothelial cytoplasm, endothelial cell hypertrophy, cytoplasmic bridging and irregular thickening of capillary basement membrane[8, 11-13, 15-17].

Vascular endothelial growth factor (VEGF) has been shown to contribute to the development of collateral vessels[18] and is expressed in increased quantities in cardiomyocytes and arteriolar smooth muscle cells following MI in non-diabetic patients[19]. However, the expression of VEGF protein and mRNA, as well as its receptors, VEGFR1 and VEGFR2, are significantly decreased in the myocardium of both diabetic and insulin-resistant non-diabetic rats by 40-70%[20]. In diabetic patients, the normal molecular processes which regulate angiogenesis may be impaired, although there are no direct studies to substantiate this to date.

Emerging evidence suggests that HIF-1α and HIF-2α have important and independent effects on pathological angiogenesis. Cells respond to hypoxic stress through multiple mechanisms, including the stabilization of hypoxia-inducible factors (HIFs), which directly regulate the expression of more than 150 target gene[21].

Although functional changes in the diabetic heart are well established in the literature, the structural basis for these changes is not well defined. Most of the literature on ultra structural pathology of the micro vessels and cardiomyocytes is confined to animal models of diabetes, with studies in man being small and qualitative. It is therefore of considerable mechanistic and translational importance to define morphological and immunohistochemical changes in human diabetic myocardium. It was therefore decided to carry out a study with the objective to examine the interaction between expression of angiogenic factors and pathology of the microvasculature and cardiomyocytes in myocardial tissue of patients with diabetes mellitus.

Materials and methods

Biopsies from right atrial appendix were obtained of the forty seven patients underwent various cardiac procedures between June 2003 and January 2004at the cardiothoracic surgical department, Manchester Royal Infirmary. Before surgery, all patients underwent cardiac catheterization and coronary angiography. This study was approved by the local Ethics Committee, and all patients gave their written informed consent before participation. Patients were classified into three clinical groups:

1. Diabetic patients undergoing CABG alone (DIHD) (n=18).
2. Diabetic patients undergoing cardiac valve replacement with normal coronary angiography (D-IHD) (n=7).
3. Non-diabetic patients undergoing cardiac valve replacement with normal coronary angiography (control group) (n=22)

Formalin fixed paraffin embedded tissue were sectioned and stained with H&E for routine examination and primary and secondary antibody for immunohistochemical examination after antigen retrieval. Blocking was done using 1% hydrogen peroxide solution. All the tissues were stained by a panel of primary antibodies including vWF(M)+antiCD31a (monoclonal), vWF(P)+antiCD31 (polyclonal), antiVEGFb (polyclonal), antiVEGFR2b (monoclonal) and antiHIF-1αc(monoclonal). Biotin conjugated secondary antibody was applied at the recommended dilution in appropriate non-immune serum in TBS. Streptavidin-HRP and Biotin were diluted in TBS (pH 7.6) for 30 min. Microscopic assessment of the intensity of HIF-1α, VEGF and VEGFR2 staining on blood vessels and cardiomyocytes was semi quantified using a scale from 1 to 10 according to the intensity of staining.

Statistical analysis was performed using SPSS for Windows 14.0. All data are expressed the mean and standard error of mean (SEM). Clinical data were compared between groups by Kruskal-Wallis test or Mann-Whitney test, as appropriate. Linear regression analyses were performed to determine correlation between various pathological changes and angiogenic factor expression. Statistical significance was accepted for $P< 0.05$ (two-tailed).

Results

HIF-1α expression

Intensity and percentage of HIF-1α expression

There were significant differences in the intensity and percentage of HIF-1α expression in cardiomyocytes between the three groups (P= 0.001, P= 0.01). Thus, intensity of HIF-1αwas

Table1: Intensity and percentage of HIF-1α expression in controls and diabetic patients with and without ischemia

Parameters	Controls	D-IHD	DIHD	P-value
Intensity of HIF-1α staining	1.5 ± 0.3	2.8 ± 0.4	3.5 ± 0.4	0.001
Percentage of HIF-1α staining	30.7 ± 5.1	34.1 ± 5.7	48.2 ± 3.6	0.01

significantly increased in diabetic patients with and without ischemia compared to controls (P= 0.000, P= 0.03). In addition, the percentage of HIF-1α was increased in DIHD patients compared to D-IHD patients (P= 0.05) and controls (P= 0.007) (Table 1)

Interco-relation between HIF-1α expression and distal myocardial capillary pathology

There was a significant positive correlations between HIF-1α intensity with number of endothelial nuclei (P= 0.02). However, HIF-1α intensity correlated negatively with lumen area (P= 0.04). In addition, HIF-1α percentage showed a positive correlation with endothelial area (P=0.03), basement membrane

Table 2: Correlation between HIF-1α expression and distal myocardial capillary pathology

	Lumen area	Endothelial area	Pericyte area	BM area	Vessel area	Endothelial cellprofile	No of endo.cell nuclei	No of pericyte nuclei
HIF-1α intensity	R = 0.31 P = 0.04	R = 0.12 P = 0.32	R = 0.05 P = 0.86	R = 0.15 P = 0.24	R = 0.08 P = 0.7	R = 0.21 P = 0.2	R = 0.35 P = 0.02	R = 0.02 P = -0.62
HIF-1α percentage	R = -0.09 P = 0.45	R = 0.34 P = 0.03	R = 0.08 P = 0.7	R = 0.4 P = 0.01	R = 0.2 P = 0.18	R = 0.19 P = 0.35	R = 0.3 P = 0.02	R = 0.01 P = 0.87

P= P-value, R= Correlation Coefficient.

area (P= 0.01) and number of endothelial nuclei (P= 0.02) (Table3).

VEGF expression

VEGF expression in different blood vessel regions and cardiomyocytes

There was a significant difference in the expression of VEGF on vascular endothelium between the three groups (P= 0.002). Thus, it was significantly reduced in D-IHD patients compared to controls (P= 0.000). In addition, there was a non-significant trend towards a reduction in VEGF expression on the endothelium of DIHD patients compared to controls (P=0.06). No significant difference was found in VEGF expression on endothelium between DIHD and D-IHD patients (P= 0.11). There was no significant difference in VEGF expression on cardiomyocytes, basement membrane and pericytes between groups (P= 0.18, P= 0.09, P= 0.95) (Table4).

Inter-correlation between VEGF expression and distal myocardial capillary pathology in diabetic patients

There was a significant positive correlation between VEGF expression on endothelium and endothelial cell profile no./capillary (P= 0.01). In addition, VEGF expression on cardiomyocytes showed positive

Table 3: VEGF expression on cardiomyocytes, endothelium, basement membrane and pericytes in controls and diabetic patients with and without ischemia

VEGF Expression	Controls	D-IHD	DIHD	P Value
Cardiomyocyte	2.85 ± 0.21	2.66 ± 0.15	2.18 ± 0.31	0.18
Endothelium	2.62 ± 0.24	1.32 ± 0.13	1.62 ± 0.23	0.002
Basement Membrane	0.84 ± 0.09	0.79 ± 0.35	0.92 ± 0.11	0.09
Pericyte	0.12 ± 0.03	0.12 ± 0.06	0.08 ± 0.02	0.95

Correlation with endothelial cell profile no./capillary (P= 0.04) (Table4).

Table 4: Correlations between VEGF expression and myocardial capillary pathology

VEGF Expression	Lumen area	Endo area	Pericyte area	BM area	Vessel area	Endo cell profile	No of endo. cell nuclei	No of pericyte nuclei
Cardiomyocyte	R = 0.09 P = 0.8	R = 0.13 P = 0.32	R = 0.19 P = 0.4	R = 0.09 P = 0.7	R = 0.002 P = 0.9	R = 0.31 P = 0.04	R = 0.12 P = 0.5	R = 0.2 P = 0.12
Endothelial cell	R = -0.23 P = 009	R = 0.2 P = 0.18	R = 0.08 P = 0.63	R = 0.04 P = 0.65	R = 0.14 P = 0.4	R = 0.4 P = 0.01	R = 0.05 P = 0.74	R = 0.12 P = 0.3
Basement Membrane	R = 0.2 P = 0.1	R = 0.08 P = 0.6	R = -0.13 P = 0.12	R = 0.12 P = 0.8	R = -0.19 P = 0.2	R = 0.1 P = 0.3	R = 0.03 P = 0.8	R = 0.16 P = 0.3
Pericyte	R = 0.09 P = 0.86	R = 0.14 P = 0.3	R = 0.07 P = 0.9	R = 0.24 P = 0.1	R = 0.21 P = 0.17	R = 0.05 P = 0.9	R = 0.02 P = 0.8	R = 0.002 P = 0.96

P= P-value, R= Correlation Coefficient

Discussion

Epidemiological and clinical trial data confirm the higher incidence and prevalence of heart failure in diabetes. Although functional changes in the heart of diabetic patients are well established in the literature, the structural basis for these changes is not well defined. In the present study the expression of key angiogenic factors (HIF-1_α, VEGF, and VEGFR2) has been quantified in relation to both pathology and key clinical variables. We have shown a significant reduction in capillary density in diabetic patients with ischemia. This finding is in accordance with the finding of other researchers. It is well known that in diabetic myocardium there is an inadequate response to ischemia which results in poor collateralization[18,22, 23]. The impaired angiogenic response in diabetes has been explained by various mechanisms. It could be as a result of the presence of vascular dysfunction, characterized by endothelial and vascular smooth muscle impairment[24-26].

Reduced expression of VEGF and its receptors, along with reduction of other angiogenic factors and increase in expression of anti angiogenic proteins have been demonstrated in animals[20,27]. In the present study, HIF-1_αexpression was detected in cardiomyocytes and the expression of HIF-1_α was increased in diabetic patients compared to controls, but was maximally expressed in DIHD patients. Our observations are in contrast to other studies conducted in experimental animals[28]. Expression of HIF-1_α is an important process allowing the myocardium to adapt to a reduction in blood flow and oxygenation. Experimental studies demonstrate that it might be a marker of persisting ischemia which indicates the presence of viable myocardium reacting to revascularization for a long period after an MI[29, 30]. This explains our findings that expression of HIF-1_αon cardiomyocytes of diabetic patients is increased and may therefore indicate ongoing ischemia, which is the main activator for myocardial HIF-1_α production. The increase of HIF-1_α in D-DIHD patients suggests that although these patients had normal coronary arteries on angiography they still had micro vascular abnormalities characterized by low capillary density, reduced capillary luminal size, and thickening of basement membrane which could lead to ischemia and subsequent activation of HIF-1_α. Another explanation for increased HIF-1_α expression in D-IHD patients is it could be related to oxidative stress caused by ROS as hyperglycemia causes an increase of ROS and a state of pseudohypoxia which might activate HIF-1_αexpression[28, 31].

We show a significant negative correlation between the percentage of HIF-1_α expression and arteriole, capillary and total vessel density suggesting that HIF-1_α is upregulated by tissue hypoxia and ischemia. Thus, when vascular density increases via angiogenesis myocardial ischemia and hypoxia may reduce which subsequently down regulates HIF-1_α expression. In the current study, HIF-1_α expression correlates negatively with capillary luminal area whereas it correlated positively with basement membrane area and number of endothelial nuclei. These findings again support our hypothesis that coronary microangiopathy characterized by reduced luminal area, endothelial hypertrophy and thickening of basement membrane are associated with myocardial ischemia which stimulates HIF-1_αexpression.

The best characterized regulators of angiogenesis are VEGF/VEGF receptor system[32]. VEGF expression is upregulated in response to hypoxia[33, 34] and during physiological (wound healing) or pathological (tumor growth) needed for angiogenesis[35]. In the current study we define the expression VEGF and its receptor $VEGFR_2$ in atrial appendages of diabetic patients with and without ischemia. We show a reduction in VEGF expression on the vascular endothelium of diabetic patients compared to controls. Whereas, $VEGFR_2$ expression showed a trend towards an increase in diabetic patients compared to controls. This finding is in contrast to the finding of reduction in VEGF and VEGFR1 expression by 40-70%[20], absence of VEGFR1 and VEGFR2[36], and lowered VEGF, VEGFR1 and VEGFR2 in diabetic animals[37]. It is well known that in diabetic myocardium there is inadequate collateral formation as a result of an impaired angiogenic response. Thus the reduction in vascular density that we observe in the current study could be related to an impaired angiogenic response mediated by reduced VEGF in diabetic patient compared to controls. Recent experimental studies of diabetic myocardium have shown that low levels of VEGF precede all other features of diabetic cardiomyopathy and are followed by several changes which include increased apoptosis of endothelial cells, reduced numbers of circulating endothelial progenitor cells, reduced capillary density, and impaired myocardial perfusion. These changes lead to apoptosis and necrosis of

cardiomyocytes along with fibrosis and progressive diastolic and then systolic dysfunction [38]. Thus, downregulation of VEGF expression may initiate or aggravate diabetic cardiomyopathy. We show an increased expression of $VEGFR_2$ on vascular endothelium in diabetic patients compared to controls. This upregulation in $VEGFR_2$ expression in diabetic patients could be a compensatory mechanism for the reduction in VEGF expression. However, the compensation was not enough to mediate the growth action of VEGF.

References

1. Kengne AP, Amoah AG, Mbanya JC. Cardiovascular complications of diabetes mellitus in sub-Saharan Africa. *Circulation.* 2005;112(23):3592-3601.
2. Rajan SK, Gokhale SM. Cardiovascular function in patients with insulin-dependent diabetes mellitus: a study using noninvasive methods. *Ann N Y Acad Sci.* 2002;958:425-430.
3. Schannwell CM, Zimmermann T, Schneppenheim M, Plehn G, Marx R, Strauer BE. Left ventricular hypertrophy and diastolic dysfunction in healthy pregnant women. *Cardiology.* 2002;97(2):73-78.
4. Moir S, Hanekom L, Fang ZY, Haluska B, Wong C, Burgess M, Marwick TH. Relationship between myocardial perfusion and dysfunction in diabetic cardiomyopathy: a study of quantitative contrast echocardiography and strain rate imaging. *Heart.* 2006;92(10):1414-1419.
5. Regan TJ, Wu CF, Yeh CK, Oldewurtel HA, Haider B. Myocardial composition and function in diabetes. The effects of chronic insulin use. *Circ Res.* 1981;49(6):1268-1277.
6. Haider B, Yeh CK, Thomas G, Oldewurtel HA, Lyons MM, Regan TJ. Influence of diabetes on the myocardium and coronary arteries of rhesus monkey fed an atherogenic diet. *Circ Res.* 1981;49(6):1278-1288.
7. Bhimji S, Godin DV, McNeill JH. Myocardial ultrastructural changes in alloxan-induced diabetes in rabbits. *Acta Anat (Basel).* 1986;125(3):195-200.
8. Giacomelli F, Wiener J. Primary myocardial disease in the diabetic mouse. An ultrastructural study. *Lab Invest.* 1979;40(4):460-473.
9. Tarach JS. Histomorphological and ultrastructural studies of a rat myocardium in experimental conditions. *Z Mikrosk Anat Forsch.* 1976;90(6):1145-1157.
10. Jackson CV, McGrath GM, Tahiliani AG, Vadlamudi RV, McNeill JH. A functional and ultrastructural analysis of experimental diabetic rat myocardium. Manifestation of a cardiomyopathy. *Diabetes.* 1985;34(9):876-883.
11. Hsiao YC, Suzuki K, Abe H, Toyota T. Ultrastructural alterations in cardiac muscle of diabetic BB Wistar rats. *Virchows Arch A Pathol Anat Histopathol.* 1987;411(1):45-52.
12. Thompson EW. Structural manifestations of diabetic cardiomyopathy in the rat and its reversal by insulin treatment. *Am J Anat.* 1988;182(3):270-282.
13. Fischer VW, Barner HB, Larose LS. Pathomorphologic aspects of muscular tissue in diabetes mellitus. *Hum Pathol.* 1984;15(12):1127-1136.
14. Farhangkhoee H, Khan ZA, Kaur H, Xin X, Chen S, Chakrabarti S. Vascular endothelial dysfunction in diabetic cardiomyopathy: pathogenesis and potential treatment targets. *Pharmacol Ther.* 2006;111(2):384-399.
15. Saito K, Nishi S, Kashima T, Tanaka H. Histologic and ultrastructural studies on the myocardium in spontaneously diabetic KK mice: a new animal model of cardiomyopathy. *Am J Cardiol.* 1984;53(2):320-323.
16. Okruhlicova L, Tribulova N, Weismann P, Sotnikova R. Ultrastructure and histochemistry of rat myocardial capillary endothelial cells in response to diabetes and hypertension. *Cell Res.* 2005;15(7):532-538.
17. Silver MD, Huckell VF, Lorber M. Basement membranes of small cardiac vessels in patients with diabetes and myxoedema: preliminary observations. *Pathology.* 1977;9(3):213-220.
18. Abaci A, Oguzhan A, Kahraman S, Eryol NK, Unal S, Arinc H, Ergin A. Effect of diabetes mellitus on formation of coronary collateral vessels. *Circulation.* 1999;99(17):2239-2242.
19. Shinohara K, Shinohara T, Mochizuki N, Mochizuki Y, Sawa H, Kohya T, Fujita M, Fujioka Y, Kitabatake A, Nagashima K. Expression of vascular endothelial growth factor in human myocardial infarction. *Heart Vessels.* 1996;11(3):113-122.
20. Chou E, Suzuma I, Way KJ, Opland D, Clermont AC, Naruse K, Suzuma K, Bowling NL, Vlahos CJ, Aiello LP, King GL. Decreased cardiac expression of vascular endothelial growth factor and its receptors in insulin-resistant and diabetic States: a possible explanation for impaired collateral formation in cardiac tissue. *Circulation.* 2002;105(3):373-379.
21. Skuli N, Majmundar AJ, Krock BL, Mesquita RC, Mathew LK, Quinn ZL, Runge A, Liu L, Kim MN, Liang J, Schenkel S, Yodh AG, Keith B, Simon MC. Endothelial HIF-2alpha regulates murine pathological angiogenesis and revascularization processes. *J Clin Invest.*122(4):1427-1443.
22. Cohen M, Rentrop KP. Limitation of myocardial ischemia by collateral circulation during sudden controlled coronary artery occlusion in human

subjects: a prospective study. *Circulation.* 1986;74(3):469-476.

23. Coggins MP, Sklenar J, Le DE, Wei K, Lindner JR, Kaul S. Noninvasive prediction of ultimate infarct size at the time of acute coronary occlusion based on the extent and magnitude of collateral-derived myocardial blood flow. *Circulation.* 2001;104(20):2471-2477.

24. Rosen P, Ballhausen T, Stockklauser K. Impairment of endothelium dependent relaxation in the diabetic rat heart: mechanisms and implications. *Diabetes Res Clin Pract.* 1996;31 Suppl:S143-155.

25. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes.* 1999;48(9):1856-1862.

26. Momose M, Abletshauser C, Neverve J, Nekolla SG, Schnell O, Standl E, Schwaiger M, Bengel FM. Dysregulation of coronary microvascular reactivity in asymptomatic patients with type 2 diabetes mellitus. *Eur J Nucl Med Mol Imaging.* 2002;29(12):1675-1679.

27. Boodhwani M, Sodha NR, Mieno S, Xu SH, Feng J, Ramlawi B, Clements RT, Sellke FW. Functional, cellular, and molecular characterization of the angiogenic response to chronic myocardial ischemia in diabetes. *Circulation.* 2007;116(11 Suppl):I31-37.

28. Marfella R, D'Amico M, Di Filippo C, Piegari E, Nappo F, Esposito K, Berrino L, Rossi F, Giugliano D. Myocardial infarction in diabetic rats: role of hyperglycaemia on infarct size and early expression of hypoxia-inducible factor 1. *Diabetologia.* 2002;45(8):1172-1181.

29. Kido M, Du L, Sullivan CC, Li X, Deutsch R, Jamieson SW, Thistlethwaite PA. Hypoxia-inducible factor 1-alpha reduces infarction and attenuates progression of cardiac dysfunction after myocardial infarction in the mouse. *J Am Coll Cardiol.* 2005;46(11):2116-2124.

30. Parisi Q, Biondi-Zoccai GG, Abbate A, Santini D, Vasaturo F, Scarpa S, Bussani R, Leone AM, Petrolini A, Silvestri F, Biasucci LM, Baldi A. Hypoxia inducible factor-1 expression mediates myocardial response to ischemia late after acute myocardial infarction. *Int J Cardiol.* 2005;99(2):337-339.

31. Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, Nyengaard JR, van den Enden M, Kilo C, Tilton RG. Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes.* 1993;42(6):801-813.

32. Harry LE, Paleolog EM. From the cradle to the clinic: VEGF in developmental, physiological, and pathological angiogenesis. *Birth Defects Res C Embryo Today.* 2003;69(4):363-374.

33. Levy AP, Levy NS, Wegner S, Goldberg MA. Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. *J Biol Chem.* 1995;270(22):13333-13340.

34. Levy AP, Levy NS, Goldberg MA. Post-transcriptional regulation of vascular endothelial growth factor by hypoxia. *J Biol Chem.* 1996;271(5):2746-2753.

35. Li J, Hampton T, Morgan JP, Simons M. Stretch-induced VEGF expression in the heart. *J Clin Invest.* 1997;100(1):18-24.

36. Sasso FC, Carbonara O, Persico E, D'Ambrosio R, Coppola L, Nasti R, Campana B, Moschella S, Torella R, Cozzolino D. Increased vascular endothelial growth factor mRNA expression in the heart of streptozotocin-induced diabetic rats. *Metabolism.* 2003;52(6):675-678.

37. 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(5):827-834.

38. Yoon YS, Uchida S, Masuo O, Cejna M, Park JS, Gwon HC, Kirchmair R, Bahlman F, Walter D, Curry C, Hanley A, Isner JM, Losordo DW. Progressive attenuation of myocardial vascular endothelial growth factor expression is a seminal event in diabetic cardiomyopathy: restoration of microvascular homeostasis and recovery of cardiac function in diabetic cardiomyopathy after replenishment of local vascular endothelial growth factor. *Circulation.* 2005;111(16):2073-2085.