

ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETES MELLITUS AND ITS RELATION WITH GLYCAEMIC CONTROL- RESULTS OF AN OBSERVATIONAL STUDY

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ABSTRACT

BACKGROUND

The aim of the study is to examine endothelial dysfunction in type 2 diabetes with inadequate glycaemic control and to study improvement in endothelial function after glycaemic control.

MATERIALS AND METHODS

An observational study was performed in type 2 diabetic patients (35-60 years) with inadequate glycaemic control. Endothelial function was evaluated by non-invasive method. Flow associated dilation (%FAD) change in lumen diameter of brachial artery after glyceryl trinitrate spray (%GTN) were calculated. After achieving glycaemic control for >3 months, HbA1c and endothelial function test were compared with baseline.

RESULTS

Out of 73 patients (mean age 50.79±4.96 years) enrolled, 46.5% were male and 41.09% were overweight. The mean duration of diabetes was 4.84 ± 3.2 years. Significant difference was noted in HbA1c, fasting blood glucose and postprandial blood glucose after treatment (p<0.05 for all). Mean %FAD before glycaemic control was 7.34 ± 2.16 whereas mean % GTN was 14.60 ± 2.96; 89% patients had % FAD <10%. Significant improvement was observed in %FAD after glycaemic control (p<0.05) but not in %GTN (p=0.47). Percentage FAD significantly increased after glycaemic control in patients with HbA1c <7% (p<0.05). No significant difference was seen in % GTN in patients with HbA1c less than or more than 7% (p>0.05). %FAD did not improve in patients with baseline HbA1c >7% (p>0.05). After anti-diabetic therapy, reduction in HbA1c (p <0.05) and %improvement in FAD (p< 0.01) was better in patients with baseline HbA1c <7% compared to those with >7%. HbA1c and body mass index had significantly negative correlation with %FAD before as well as after glycaemic control.

CONCLUSION

Uncontrolled type 2 diabetes mellitus is associated with impaired endothelial function. Negative correlation is observed between endothelial dysfunction and glycaemic status of type 2 diabetes patients. Glycaemic control results in improvement of endothelial dysfunction.

KEYWORDS

Endothelial Dysfunction, Glycaemic Control, Type 2 Diabetes Mellitus.

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BACKGROUND

Diabetes is a growing concern across the world and more so in India because of high prevalence and associated complications. About 65.1 million people between 20-79 years in India have diabetes resulting in prevalence of

8.56%. Microvascular and macrovascular complications of diabetes are very well documented.¹ Cardiovascular disease is one of the major complications of diabetes mellitus.² Diabetes is associated with two to four times more risk of cardiovascular diseases. Vascular endothelium plays dual role in maintaining cardiovascular health. It offers protection by acting as a physical barrier between vessel wall and its lumen and also by release of several mediators. Endothelial dysfunction results in loss of physiological properties of the endothelium rendering person vulnerable to the diabetes related complications.¹ Endothelial dysfunction is known to play a very important role in the pathogenesis of diabetes related complications. Hyperglycaemia is the main cause for endothelial dysfunction in diabetes mellitus.³ It is also postulated that oxidative stress may precede the development of endothelial dysfunction.⁴ Similarly, increased

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levels of lipids, hs-CRP, intimal medial thickening, reduced nitrite levels, could also contribute to the risk of endothelial dysfunction in these patients.⁵ Improved strategies for prevention and treatment of cardiovascular disease in diabetes patients are needed. In this regard, endothelial function can be a useful prognostic marker.^{3,6} The data about the impact of glycaemic control on endothelial dysfunction in Indian type 2 diabetes patient are limited.

Objective

The objective of the current study was to examine endothelial dysfunction in cases of type 2 diabetes with inadequate glycaemic control and to compare change in endothelial function before and after adequate glycaemic control.

MATERIALS AND METHODS

This observational study was performed in adult type 2 diabetic patients between 35 to 60 years of age with inadequate glycaemic control i.e. glycosylated haemoglobin (HbA1c) more than 7% and duration not more than 15 years attending outpatient clinic or admitted in wards from December 2007 to July 2009. Patients with coronary vascular disease, hypertension, peripheral vascular disease, smokers, those with renal failure (Defined as serum creatinine >2 mg %), sickle cell disease, evidence of any acute infectious, inflammatory or severe concurrent illness and body mass index (BMI) ≥40 kg/m² were excluded. Similarly, patients with history of cerebrovascular episodes were also not included in the present study.

After complete clinical examination, all patients underwent investigations including fasting blood glucose, serum creatinine, serum electrolytes and fasting lipid profile. A resting twelve lead electrocardiogram was recorded in every patient. Enrolled patients were evaluated for endothelial function by non-invasive method and regularly followed up in diabetic outpatient clinic. Their treatment pattern, compliance to treatment and blood glucose levels were reviewed regularly for ensuring stabilisation of controlled blood glucose levels. Treatment was modified for control of blood glucose levels by adding or changing oral hypoglycaemic agents. Dosages of insulin were modified for cases who were on already on insulin (Regular or NPH) for achieving glycaemic control. These drugs were used for achieving control of blood glucose levels and trying to avoid hypoglycaemic episodes. After achieving glycaemic control for more than three months, HbA1c and endothelial function test were repeated and compared with baseline readings. Assessment of endothelial function was done by Doppler study of brachial artery as described by Celermajer et al (1992)⁷ and found useful in Indian patients.⁸ In this study, diameter of brachial artery was assessed by vascular Doppler probe of 7.5 to 10 MHz of Esaote Megas GP 2-D Echo and Doppler machine. The measurement of the brachial artery diameter was scanned in supine position, right brachial artery 2-5 cm above the elbow. The scanned area was marked to measure the same segment of brachial artery repeatedly and changes in the diameter will be noted. The readings were taken for four times.

Reading	Time of reading
First	The patient was allowed to rest for 10 minutes and first reading taken
Second	Reactive hyperaemia: The cuff was inflated by pneumatic tourniquet for four minutes till 300 mmHg of pressure over forearm and reading was taken within one minute of cuff deflation
Third	After 15 minutes of rest
Fourth	After glyceryl trinitrate (GTN) spray: Reading was taken after 3-4 minutes of consumption of sublingual single dose of 0.4 mg GTN

Table 1. Recordings of Brachial Artery Diameter

Flow associated dilation (%FAD) was calculated as change in lumen diameter (LD) of brachial artery (BA) after reactive hyperaemia from reading at rest. Change in lumen diameter of brachial artery (BA) after glyceryl trinitrate (GTN) spray (%GTN) from reading at rest was also calculated.

Endothelial dysfunction was defined as %FAD less than 10%⁹ whereas glycaemic control was defined as fasting blood glucose ≤120 mg/dL and postprandial blood glucose ≤ 180 mg%.

Statistical Analysis

Continuous variables are presented as mean and standard deviation whereas categorical variables are given as percentages. Significance of difference between means of two independent groups was assessed by using unpaired 't' test whereas paired 't' test was applied for comparing the differences in means of the same groups. Pearson's correlation coefficient (r) was calculated for studying the relationship between two continuous variables, the significance of which was tested with a 't' test. P value of <0.05 was considered as statistically significant.

RESULTS

A total of 73 inadequately controlled type 2 diabetes patients were enrolled in the study. Thirty four patients (46.5%) were male and thirty nine (53.4%) were female. The mean±SD age of patients in this study was 50.79±4.96 years. A total of forty-seven (64.38%) patients were in age group of 45 to 55 years. The number (%) of patients between 35 to 45 years and 55 to 60 years were 14 (19.17%) and 12 (16.43%) respectively.

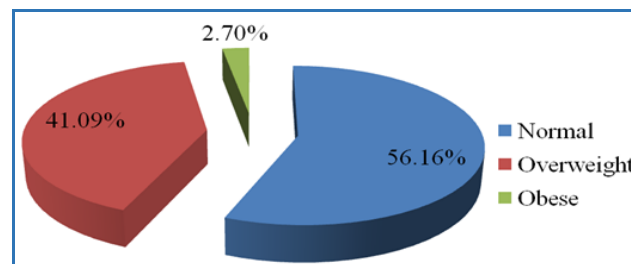


Figure 1. Distribution of Cases According to BMI

Normal: 18.5-24.99 kg/m²; Overweight: 25-29.99 kg/m²; Obese: 30-39.99 kg/m².

A total of 56.16% patients had normal body mass index (BMI) while 41.09% were overweight (Figure 1). Forty eight

(65.75%) patients were having type 2 diabetes since less than or equal to five years whereas 27.39% patients had diabetes between five to ten years. Remaining patients (6.84%) had diabetes between 10 to 15 years. The mean duration of diabetes was 4.84±3.2 years. At baseline, 36 (49.31%), 20 (27.39%) and 17 (23.3%) patients had HbA1c level between 7-8%, 8-9% and >9% respectively.

Parameter	Result
Mean BMI (kg/m ²)	24.67±1.75
HbA1c (%)	8.39±1.48
Fasting Blood Sugar (mg/dL)	134.73±41.5
Postprandial Blood Sugar (mg/dL)	205.53±50.36
Total Cholesterol (TC) (mg/dL)	169.95±33.32
Triglycerides (TG) (mg/dL)	131.31±44.62
Low Density Lipoproteins (LDL) (mg/dL)	100.05±30.75
Very Low Density Lipoproteins (VLDL) (mg/dL)	26.34±9.03
High Density Lipoproteins (HDL) (mg/dL)	43.56±6.93

Table 2. Baseline Characteristics before Glycaemic Control (at the time of enrolment)

Baseline characteristics of patients before glycaemic control are shown in table 2.

	Before Treatment (n=73)	After Treatment (n=73)	P Value
HbA1c (%)	8.39±1.48	7.24±1.25	<0.05
Fasting Blood Glucose (mg/dL)	134.73±41.5	116.3±31.5	<0.05
Postprandial Blood Glucose (mg/dL)	205.53±50.36	171.3±36.7	<0.05

Table 3. Glycaemic Control Post-treatment with Anti-diabetic Medications

Significant difference was noted between glycaemic parameters i.e. HbA1c, fasting blood glucose and postprandial blood glucose after treatment (Table 3).

Mean %FAD before glycaemic control was 7.34±2.16 whereas mean %GTN was 14.60±2.96.

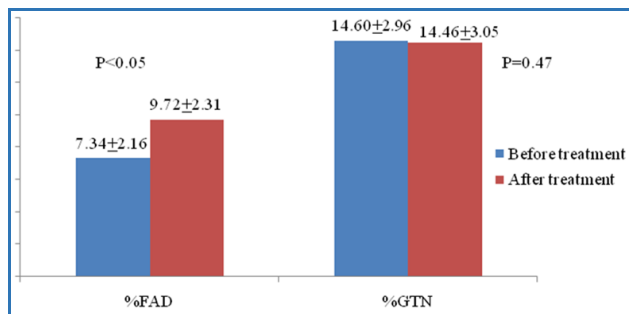


Figure 2. Endothelial Function before and after Glycaemic Control

Significant improvement was observed in endothelium-dependent dilation (%FAD) after glycaemic control (Figure 2; p<0.05) whereas %GTN did not show significant difference after glycaemic control (p=0.47). Before glycaemic control, 65

(89%) cases had endothelial dysfunction (%FAD <10%) which reduced to 39 (53.4%) cases after glycaemic control, showing endothelial function improvement in 26 cases after glycaemic control. After three months of treatment, 50 (68.5%) patients had HbA1c less than 7% whereas 23 (31.5%) patients still had more than 7%.

	Patients with HbA1c < 7%		
	Before Glycaemic Control	After Glycaemic Control	P value
%FAD	7.44±1.92	10.23±1.94	<0.05
%GTN	13.51±3.10	13.97±3.33	> 0.05
	Patients with HbA1c >7%		
	Before Glycaemic Control	After Glycaemic Control	P value
%FAD	7.13±2.65	8.64±2.70	> 0.05
%GTN	15.39±2.18	15.53±2.04	> 0.05

Table 4. Comparison of Endothelial Function in Patients with HbA1c less than and more than 7% before and after Glycaemic Control

Significant reduction in HbA1c was observed in patients with HbA1c <7% as well as those with HbA1c >7% (p<0.05). Percentage FAD significantly increased after glycaemic control in patients with HbA1c less than 7% (p<0.05). However, there was no significant difference in %GTN in patients with HbA1c less than or more than 7% (p>0.05). Percentage FAD did not improve in patients with baseline HbA1c more than 7% (Table 4; p>0.05). After three months of antidiabetic therapy, reduction in HbA1c (p<0.05) and percentage improvement in FAD (p<0.01) was significantly better in those with baseline value of less than 7% compared to those with more than 7%.

On correlating various variables with endothelium-dependent dilation (%FAD) at baseline, it was found that HbA1c (r value-0.266; p=0.023) and BMI (r value-0.263; p=0.02) had significantly negative correlation with %FAD. Age and duration of diabetes also had negative correlation with endothelium-dependent dilation but it was not statistically significant (age r value-0.130; p=0.27; duration of diabetes r value-0.206; p=0.79). After glycaemic control also HbA1c and BMI were found to have a significant negative correlation with % FAD (HbA1c r value - 0.403; p=0.00; BMI r value-0.240; p=0.041). Age and duration of diabetes also had negative correlation with endothelium dependent dilation but it was not statistically significant (age r value-0.097; p=0.414; duration of diabetes r value-0.221; p=0.061). No correlation was found between lipid profile and endothelial function before or after treatment.

DISCUSSION

Endothelial dysfunction, a common problem in patients with uncontrolled diabetes is a risk factor for multiple complications. Several studies have evaluated endothelial dysfunction in type 2 diabetes patients with control group¹⁰⁻¹³ or without control group.¹⁴ In this observational study, we evaluated endothelial dysfunction in uncontrolled type 2 diabetes mellitus and the influence of glycaemic control on endothelial function in these patients. For ensuring inadequately controlled diabetes, we enrolled cases with HbA1c more than 7%. The mean BMI of patients in our study i.e. 24.67±1.75 kg/m² was lower than others.^{10,12} Normal

weight in majority patients in our study shows that endothelial dysfunction is possible irrespective of obesity or overweight in diabetes patients. Similar to another study,¹⁴ we also recruited cases of type 2 diabetes with less than 15 years duration. The mean duration of diabetes in another study¹⁰ was 6.7 years compared to 4.84 years in our study.

Doppler study of brachial artery is an established method of non-invasive assessment of endothelial function.⁷ Diameters of the femoral and brachial arteries are measured during reactive hyperaemia and after sublingual administration of glyceryl trinitrate, both procedures inducing vasodilation; former mediated by endothelium dependent mechanism whereas the latter acts directly on smooth muscle cells (Endothelium-independent). Comparison of vasodilation produced by different mechanisms enables non-invasive assessment of endothelial function.

Endothelium dependent dilation (%FAD) is impaired in uncontrolled type 2 diabetes.¹⁰ Endothelial dysfunction was observed in 89% cases in our study as opposed to 78.6% in another study.⁹ In our study, at baseline, endothelium dependent dilation was significantly impaired as compared to endothelium-independent dilation in patients with inadequately controlled type 2 diabetes patients.

The mean percent increase in lumen diameter of brachial artery after stress i.e. reactive hyperaemia (%FAD) improved after glycaemic control suggesting improvement in endothelial-dependent dilation. Percentage FAD significantly increased after glycaemic control in patients with HbA1c <7%. However, there was no improvement in %FAD in patients with baseline HbA1c >7% indicating no significant improvement in endothelial function despite significant improvement in glycaemic status. In our study, %FAD was significantly impaired as compared to %GTN which suggest that endothelium-dependent vasodilation is significantly lower in poorly controlled diabetic cases than endothelium-independent vasodilation. Our findings are in accordance with previously reported findings.¹⁴ A comparative trial¹² studied endothelial function in diabetic patients invasively and reported that endothelial dysfunction is more common in diabetic patients compared to controls. However, they did not find difference in endothelium-dependent (%FAD) and endothelium-independent dilation (%GTN). According to them, blunted vasodilator response to glyceryl trinitrate suggests that dysfunctional endothelium is not solely responsible for the impaired vascular relaxation in diabetic patients. As glyceryl trinitrate and endothelium derived relaxing factor (EDRF) share a common final pathway for vasodilation, a selective defect in the diabetic vasculature might be responsible for the impaired responses to such nitrovasodilators.

Many studies¹⁵⁻¹⁷ demonstrated endothelial dysfunction in type 2 DM patients while some studies¹⁸⁻²¹ provide conflicting data regarding endothelium-independent dilation (%GTN). Our study also demonstrates impaired %FAD in uncontrolled diabetic cases. Percentage FAD is a marker of endothelium mediated dilation. It indicates release of endothelium EDRF or nitric oxide. It is contended that diabetes is associated with a deficiency of bioactive nitric oxide and accompanied by alteration in vessel architecture resulting in overall reduced dilatory capacity of resistance vessel. We observed significantly lower mean %FAD in

diabetes patients. The mean %FAD in our study was less than 10%. Our findings strongly support the presence of endothelial dysfunction in type 2 diabetes patients.

After glycaemic control there was statistically significant improvement in %FAD. A study¹⁰ evaluated endothelium-dependent vasodilation (%FAD) and endothelium-independent vasodilation (%GTN) after insulin therapy. Before glycaemic control %FAD and %GTN were 2.7±2.2 and 14.4±3.6 respectively. After glycaemic control, %FAD was 5.0±2.8 whereas %GTN was 14.1±2.8.

Another study evaluating endothelial dysfunction in uncontrolled diabetes cases demonstrated statistical difference between %FAD vs. %GTN before and after glycaemic control.¹⁴

The mean percentage of endothelium-dependent dilation was less than 10% in our study. After glycaemic control though mean %FAD improved, it was less than 10% which suggests that endothelial dysfunction was still present. These finding suggests that endothelial function may not become normal even after glycaemic control. Only relative improvement in endothelial function after glycaemic control suggests presence of other factors contributing to endothelial dysfunction in diabetes patients.

W. Bagg et al¹⁴ did not observe significant endothelial-dependent dilation after glycaemic control possibly because of the involvement of more advanced cases in the study. In addition to endothelial dysfunction, structural changes within the arterial wall could prevent an improvement of %FAD.

We observed significant negative correlation between endothelium-dependent dilation and glycated haemoglobin i.e. with increase in glycated haemoglobin, there was decrease in endothelium-dependent dilation. Gaenger et al¹⁰ also reported that absolute change in endothelium-dependent dilation has significant negative correlations with HbA1c ($r=-0.67$, $p<0.001$). However, another study¹² showed no correlation between endothelium-dependent dilation and HbA1c.

Overall observations indicate need of strict monitoring and control of glycaemic status of the patients to avoid development of endothelial dysfunction. Prevention of endothelial dysfunction would always be better than trying to treat it. Better glycaemic control will help in improvement of endothelial function and reducing further complications of diabetes. Prospective study with larger sample size is required to evaluate effect of intensive glycaemic control on endothelial function and assess correlation between endothelial dysfunction and glycaemic status.

Our study has some limitations. An observational study from single centre with defined age group limits generalisation of findings to overall population of diabetes mellitus. Absence of control group and short duration of therapy are the other limitations of study.

CONCLUSION

Uncontrolled type 2 diabetes mellitus is associated with impaired endothelial function which may be attributable to poor glycaemic status. There is negative correlation between endothelial dysfunction and glycaemic status of type 2 diabetes patients. Endothelial dysfunction improves after achieving glycaemic control.

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