

## Evaluation of endothelial dysfunction in patients with type 2 diabetes mellitus - Analysis of 100 cases

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### Abstract

**Background:** Atherosclerosis which is a precursor for macro-vascular disease involves both functional as well as structural changes in the vasculature. Functional changes involve abnormalities in endothelium, vascular smooth muscle cells and platelet functions. Endothelial dysfunction was reported in type 2 DM cases. Ultrasound assessment of brachial artery FMD (Flow Mediated Dilatation) provides important information about vascular function in health and disease. Low FMD values predict independently an established atherosclerosis.

**Materials and methods:** A total of 150 individuals included in this study; 100 were diabetes mellitus patients and 50 were age matched persons without diabetes, hypertension or vascular disease. Of 100 diabetes patients 50 each were grouped in two groups with good (HbA1c <7.5%) and poor (HbA1C >7.5%) control of hyperglycemia. Brachial artery flow mediated vasodilatation (FMD) was studied in all patient after overnight fasting (8 hours) using 7.5 MHz phased array transducer. The medial epicondyle was used as anatomical landmark for brachial artery.

**Observations:** In this study it is observed that, the mean age for FMD <4.5% is 59.70±11.08 and FMD >4.5% is 56.53±8.37. Age is higher in diabetics with FMD % <4.5. Females who FMD <4.5% were 50.0% and 42.5% with FMD >4.5%. Smoking in diabetics with FMD <4.5% was 10.0% and FMD >4.5 % was 15%. Hypertension was equally found 40%. Family history of diabetes with FMD % <4.5 was 30% and FMD >4.5% were none.

**Summary and conclusions:** The data suggest that endothelial function is a useful prognostic marker in coronary artery disease patients. In these patients, it has been reported that endothelial dysfunction is an independent prognostic factor and may predict future events, irrespective of the angiographic severity of the disease. Moreover, blunted endothelial function may reflect early atherosclerosis, and should lead to a detailed evaluation.

**Keywords:** Type 2 Diabetes mellitus, ultrasound, flow mediated dilation.

### 1. Introduction

Diabetes mellitus has assumed important non-communicable disease all over especially developing countries like India. The American Diabetes Association recently designated type-2 diabetes mellitus as a major risk factor for cardiovascular diseases [1]. Atherosclerosis which is a precursor for macro-vascular disease involves both functional as well as structural changes in the vasculature. Functional changes involve abnormalities in endothelium, vascular smooth muscle cells and platelet functions [2,3]. Endothelial function is the earliest to be affected in this cascade of events leading to atherosclerotic plaque formation. Endothelial dysfunction was reported in type 2 DM cases [4-9].

Endothelial function has been largely assessed as impaired endothelium-dependent vasodilatation because

endothelium-derived nitric oxide, is a major mediator of endothelium-dependent vasodilatation, involved in the regulation of other protective properties of endothelium [10,11]. Common conditions such as dyslipidemia, hypertension, diabetes and smoking are associated with endothelial dysfunction, being in the same time risk factors which promote the development, progression, and complications of atherosclerosis. Ultrasound assessment of brachial artery FMD (Flow Mediated Dilatation) provides important information about vascular function in health and disease [12,13]. International Task Force on Brachial Artery Reactivity has laid guidelines for performance of FMD in the year 2002[14], thus standardizing the test for wider application. Sorensen *et al* proved this procedure was simple and easily reproducible [15]. FMD is considered to represent endothelium-dependent vasodilation[16]. Low FMD values

predict cardiovascular events independently of established atherosclerosis [17].

## 2. Materials and Methods

This study was an open, randomized and comparative study done between 1-8-2012 till 31-7-2014 at SVS Hospital, Mahabubnagar, and Telangana State. A total of 150 individuals included in this study; 100 were diabetes mellitus patients and 50 were age matched persons without diabetes, hypertension or vascular disease. Of 100 diabetes patients 50 each were grouped in two groups with good (HbA1c <7.5%) and poor (HbA1C >7.5%) control of hyperglycemia. Patients with history of myocardial infarction or acute coronary syndrome during preceding four weeks were excluded from the study. Clinical evaluation, electrocardiogram, biochemical tests and assessment of brachial artery flow-mediated vasodilatation were done in all subjects after taking informed consent for the study. Clinical examination included blood pressure recording, assessment of cardiovascular status and height and body weight measurements. Biochemical assessment included fasting and post-prandial blood sugar and fasting lipid profile. Patients were evaluated for presence and duration of conventional cardiovascular risk factors viz. hypertension, family history of premature CAD, dyslipidemia, current smoking and diabetes mellitus. Hypertension was defined as systolic blood pressure more than 140 mm Hg or diastolic blood pressure 90 mm Hg or more or those on hypertensive medications. Dyslipidemia was defined as LDL level greater than 130 mg/dl, or HDL less than 40 mg/dl, or TG more than 200 mg/dl. Family history was coded as positive if a first degree relative had a coronary event before the age of 55 years in males and 65 years in female relatives. In extension to the same study there was a group IV consisting of 24 patients who got converted to good control after treatment modification after 3 months of follow up; while 26 cases did not get good glycemic control various reasons.

### 2.1 Equipment

Colour Doppler ultrasonography of the brachial artery, by PHILIPS HD 7 XE Image point machine using 7.5 and 10 MHz linear probe was performed to assess FMD, which provide information regarding endothelial function.

### 2.2 Image acquisition

The subject is positioned supine with the arm in a comfortable position for imaging the brachial artery. The brachial artery is imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected for continuous 2D grayscale imaging.

### 2.3 Endothelium-dependent FMD

To create a flow stimulus in the brachial artery, a sphygmomanometric (blood pressure) cuff is first placed

either above the antecubital fossa or on the forearm. A baseline rest image is acquired, and blood flow is estimated by time-averaging the pulsed Doppler velocity signal obtained from a mid artery sample volume. Typically, the cuff is inflated to at least 50 mm Hg above systolic pressure to occlude arterial inflow for a standardized length of time. This causes ischemia and consequent dilation of downstream resistance vessels via auto-regulatory mechanisms. Subsequent cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress causes the brachial artery to dilate. The longitudinal image of the artery is recorded continuously from 30 s before to 2 min after cuff deflation. A mid-artery pulsed Doppler signal is obtained upon immediate cuff release and no later than 15 seconds after cuff deflation to assess hyperemic velocity. When the cuff is placed on the upper part of the arm, reactive hyperemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm [18-20].

Brachial artery flow mediated vasodilatation (FMD) was studied in all patient after overnight fasting (8 hours) using 7.5 MHz phased array transducer. The medial epicondyle was used as anatomical landmark for brachial artery. Flow mediated vasodilatation (FMD) was calculated as follows as per the guidelines laid by Corrette and others [16]:

$$\text{FMD (\%)} = \frac{d_2 - d_1}{d_1} \times 100$$

Where d1 - Base line brachial artery diameter d2 - Brachial artery diameter at 1 min post deflation

The cases were followed for 6 months for the control of diabetic state and repeat FMD was done in patients with poor glycemic control. It had been observed improvement in the values of FMD after good control.

### 2.4 Statistical Analysis

In the present study the data collected is analyzed statistically by computing the standard quantities namely mean, Standard deviation, Standard error of mean and percentages. The difference between different parameters based on quantitative variables is compared using student's t test for independent samples and the difference is considered statically significance groups whenever p value < 0.05. Paired "t" test was used for intra group comparison and unpaired "t" test was used for comparison between.

## 3. Results and observations

Basic and anthropological information of the patients is given in Table 1.

**Table 1: Comparison of baseline characteristics and biochemical parameters in diabetics and controls**

| Parameter                          | DM Group I (HbA1C < 7.5%) | DM Group II (HbA1C > 7.5%) | Control Group III | 'p' value  |
|------------------------------------|---------------------------|----------------------------|-------------------|--|
| Number                             | 50                        | 50                         | 50                |  |
| Mean Age                           | 48.88 ± 6.18              | 49.24 ± 7.12               | 47.14 ± 5.98      | 0.79   |
| Male                               | 24                        | 23                         | 24                | 0.92   |
| WHR                                | 0.93 ± 0.17               | 0.98 ± 0.12                | 0.89 ± 0.07       | 0.88   |
| BMI (KG/M <sup>2</sup> )           | 23 ± 2.6                  | 23 ± 3.1                   | 23 ± 2.9          | 0.95   |
| HbA1C                              | 7.33 ± 0.91%              | 8.7 ± 1.31%                | 5.78 ± 0.30%      | 0.072 Gr I vs Gr II<br>0.016 Gr I vs Gr III<br>< 0.001 Gr II vs Gr III |
| Mean FBS (mg%)                     | 98.56 ± 6.54              | 117 ± 12.42                | 87.56 ± 11.32     | 0.004 Gr I vs Gr II<br>0.012 Gr I vs Gr III<br>0.005 Gr II vs Gr III   |
| Mean brachial artery diameter (mm) | 4.68 ± 0.15               | 3.97 ± 0.24                | 10.63 ± 0.14      | 0.18 Gr I vs Gr II<br>0.001 Gr I vs Gr III<br>< 0.001 Gr II vs Gr III  |

**Table 2: Grouping of patients according to glycaemic control**

| Patient group | Number of patients | HbA1c level | Level of glycaemic control |
|---------------|--------------------|-------------|----------------------------|
| Group I       | 50 (24 Male)       | <7.5%       | Good                       |
| Group II      | 50 (23 Male)       | >7.5%       | Poor                       |
| Group III     | 50 (24 Male)       | <6.5%       | Non-diabetic               |
| Group IV*     | 24 (12 Male)       | <7.5%       | Good                       |

\*24 patients were re-examined after 3 months of treatment showed good glycaemic control re-examined for FMD

**Table 3: Comparison of FMD (%) with WHR in female diabetics**

| WHR (Female) | FMD % ± SD  |
|--------------|-------------|
| ≤ 0.9        | 4.68 ± 0.92 |
| ≥ 0.9        | 3.68 ± 0.84 |
| 'p' value    | 0.62 NS     |

**Table 4: Comparison of FMD (%) with WHR in male diabetics**

| WHR (Male) | FMD % ± SD  |
|------------|-------------|
| ≤ 1.0      | 5.32 ± 0.72 |
| ≥ 1.0      | 4.12 ± 0.68 |
| 'p' value  | 0.28 NS     |

**Table 5: Comparison of FMD (%) in the study group with relation to BMI**

| BMI Kg/M <sup>2</sup> | FMD % ± SD  |
|-----------------------|-------------|
| < 25                  | 5.68 ± 1.02 |
| 25– 30                | 4.24 ± 0.92 |
| 30 - 35               | 3.16 ± 0.94 |
| 'p' value             | 0.05        |

**Table 6: Analysis of FMD for lipid parameters**

| Lipid parameter                | FMD % ± SD  |
|--------------------------------|-------------|
| Serum cholesterol < 200 mg     | 5.84 ± 0.84 |
| Serum cholesterol 200 – 250 mg | 5.28 ± 0.95 |
| Serum cholesterol > 250 mg     | 4.03 ± 0.86 |
| 'p' value                      | 0.05        |
| HDL > 40 mg                    | 3.88 ± 1.06 |
| HDL < 40 mg                    | 4.46 ± 0.92 |
| 'p' value                      | 0.268 NS    |
| LDL < 130 mg                   | 4.82 ± 0.94 |
| LDL > 130 mg                   | 3.84 ± 0.86 |
| 'p' value                      | 0.156 NS    |
| Triglyceride < 150 mg          | 5.88 ± 1.02 |
| Triglyceride > 150 mg          | 3.78 ± 1.04 |
| 'p' value                      | 0.05        |

24 cases with poor showed remarkable improvement in the FMD values (3.97 ± 0.24 to 6.84 ± 0.14) though the values were still much less than that of control group.

**Table 7: Showing the significant improvement with good control**

| Parameter  | First visit | After 6 months | 'p' value           |
|------------|-------------|----------------|---------------------|
| HbA1c      | 9.86±1.02   | 7.12 ± 0.94    | 0.045 (significant) |
| FMD % ± SD | 3.97±0.24   | 6.84 ± 0.14    | 0.36 (significant)  |

In this study it is observed that, the mean age for FMD <4.5% is 59.70±11.08 and FMD >4.5% is 56.53±8.37. Age is higher in diabetics with FMD % <4.5. Females who FMD <4.5% were 50.0% and 42.5% with FMD >4.5%. Smoking in diabetics with FMD <4.5% was 10.0% and FMD >4.5% was 15%. Hypertension was equally found 40%. Family history of diabetes with FMD % <4.5 was 30% and FMD >4.5% were none. Duration of diabetes with FMD <4.5% was 8.2±6.32 and FMD >4.5% was 6.92±4.88. Duration was longer in patients with FMD <4.5%. BMI with FMD <4.5% was 26.46±2.82 and FMD >4.5% was 24.72±3.89. BMI was more in diabetics with FMD <4.5%. Total cholesterol in diabetics with FMD % <4.5 was 168.80±45.15 and FMD 4.5% was 166.20±42.50. Mean LDL was 98.20±35.69 in diabetics with FMD < 4.5% and 101.95±35.98 in diabetics with FMD >4.5%. HDL was 31.30±6.89 in diabetics with FMD < 4.5% and 35.20±9.90 in diabetics with FMD >4.5%. TG was 220.50±173.44 in diabetics with FMD < 4.5% and 161.72±65.80 in diabetics with FMD >4.5%. TG was high in diabetic with FMD <4.5%.

#### 4. Discussion

Endothelial dysfunction occurs much before the atherogenesis and diabetes is a major cause of the reduced flow mediated dilatation (FMD) [1, 2, 21, 22]. Using FMD% as a measure of endothelial function, there was a significant difference between the diabetic cohort (3.97 ± 0.24) and the controls (10.63 ± 0.14); (p value of 0.001). This finding shows the presence of impairment of endothelial dependent function in diabetics. This stage is reversible with good control of diabetes as reported by earlier [23-29] and the present study. Reddy and Yousuf opined India will be burdened by highest coronary artery disease burden in the

world by the end of 2015 [30]. Obesity and dyslipidemia in the present study showed mixed results. People with obesity showed a significant difference in FMD %. Serum total cholesterol and triglycerides showed a significant change while serum HDL and LDL levels did not show any significant change with FMD %. This finding was similar to that by Dosi *et al* [28].

Some studies have shown an independent inverse association between brachial FMD and CVD events [31-35], but Fathi *et al* and Frick *et al* did not find any relation of FMD with ischemic heart disease [36,37]. A recent large meta-analysis suggested that the association between FMD and the estimated 10-year risk of coronary heart disease, assessed using the Framingham risk score, was strongest in the low risk populations compared with medium or high risk populations [24,38]. There is, however, a paucity of data on the predictive value of brachial FMD for incident cardiovascular events in low-risk populations or subjects free of CVD at baseline. The study by Shimbo *et al* [15,39] attempted to address this question, but their findings were inconclusive due to small sample size and less ethnically diverse cohort compared with the USA population. In contrast, in the current study an inverse association between FMD and clinical CVD events remained significant after adjustment for multiple cardiovascular disease risk factors or for the FRS (Framingham risk score).

The data suggest that endothelial function is a useful prognostic marker in coronary artery disease patients [40]. In these patients, it has been reported that endothelial dysfunction is an independent prognostic factor and may predict future events, irrespective of the angiographic severity of the disease [40,41]. Individuals with endothelial dysfunction require better control of their lipid profile, C reactive protein, serum glucose, blood pressure, and smoking, since it is well known that all these factors affect endothelial function significantly. Moreover, blunted endothelial function may reflect early atherosclerosis, and should lead to a detailed evaluation (and control) of all the conventional and newer risk factors, such as infection/ inflammation, homocysteine. Persisting severe endothelial dysfunction in patients with advanced atherosclerosis may require a more aggressive control of risk factors and probably modification of current medication [42].

**Table 8: FMD% in different studies**

| Studies                      | Non-diabetics | Diabetics    | p value |
|------------------------------|---------------|--------------|---------|
| Present study                | 10.63 ± 0.14  | 3.97 ± 0.24  | 0.001   |
| Clarkson <i>et al</i> [44]   | 9.3 ± 3.8%    | 5.0 ± 3.7%   | 0.001   |
| Diptichand <i>et al</i> [45] | 6.8 ± 4.4%    | 3.6 ± 3.3%   | 0.01    |
| Ravikumar <i>et al</i> [46]  | 6.64 ± 4.38%  | 1.72 ± 2.8%  | 0.001   |
| Bhargava K <i>et al</i> [43] | -             | 5.50 ± 2.12% | -       |

FMD% in different studies

## 5. Limitations of the present study

The sample is relatively small in this study. Also, a comparison with other markers of atherogenicity including arterial stiffness and carotid intima thickness would be valuable. Moreover, long-term follow-up studies are needed to find out whether treatment modalities aimed at improving endothelial function can translate into a delayed progression of atherosclerosis.

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