Int. J Med. Pharm. Res. (P-ISSN: 2958-3675 | E-ISSN: 2958-3683)
Available on: https://ijmpr.in/

International Journal
of Medical and Pharmaceutical Research

ORGINAL ARTICLE **OPEN ACCESS**

Endothelial dysfunction in type 2 diabetes mellitus compared to healthy subjects

Dr Dnyanesh N Morkar[1], Dr V Channaarya[2], Dr Anil Kumar Sharma[3], Dr Hima Morkar[4], Dr Saad Siddiqui[5]

[1]Professor, JNMC, Belagavi, department of general medicine
[2]Professor, KIMS, Bangalore, department of general medicine
[3]Professor, KIMS, Bangalore, department of radiology
[4]JNMC, Belagavi, department of general medicine
[5]Resident, JNMC, Belagavi, department of general medicine

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***Corresponding Author**
Dr. Saad Siddiqui,Resident, MD General Medicine, JNMC, Belagavi

Received: 10-12-2024
Accepted: 24-01-2025
Available online: 29-01-2025

A B S T R A C T

Background: Endothelial dysfunction serves as an early indicator of atherosclerosis, preceding structural changes. Its assessment offers insight into the preclinical phase of cardiovascular disease, particularly crucial in Type 2 Diabetes Mellitus (T2DM) due to its association with glycemic control and disease duration. Flow-mediated dilation (FMD), reliant on endothelial nitric oxide release in response to shear stress, reliably gauges endothelial function across various conditions.

Aim of the Study: This study aimed to evaluate endothelial dysfunction in T2DM patients compared to age- and sex-matched healthy controls. Additionally, it sought to correlate the duration of diabetes with the prevalence of endothelial dysfunction and examine its association with atherosclerosis risk factors in T2DM.

Methodology: Non-invasive assessment using high-resolution Duplex Doppler Ultrasound of the Brachial Artery was conducted on 50 T2DM cases with or without vascular complications and 20 healthy controls. FMD, calculated as percentage increase in brachial artery diameter with increased flow, was employed to quantify endothelial function.

Results: Endothelial dysfunction was observed in 20% of diabetics but absent in controls. Mean FMD values were significantly lower in diabetics (8.38 ± 12.32%) compared to controls (17.12 ± 10.53%; $p < 0.007$). FMD decline was noted across diabetes durations (<5 years: 0.28 ± 4.0%; 5-10 years: 2.12 ± 1.34%; >10 years: 3.50 ± 1.61%), though prevalence did not escalate with longer duration.

Conclusion: T2DM patients exhibit significantly impaired endothelial function compared to healthy counterparts, as evidenced by reduced FMD. The prevalence of endothelial dysfunction did not correlate with diabetes duration but was associated with hypertension, family history of diabetes, and smoking. Early intervention targeting these risk factors and optimizing glycemic control may mitigate vascular complications in T2DM.

KEYWORDS: Endothelial dysfunction, Diabetes mellitus, Cardiovascular disease, Brachial artery, Hypercholesterolemia

INTRODUCTION

Endothelial dysfunction is an early indicator of atherosclerosis, often manifesting well before structural changes occur in the arteries. Therefore, assessing endothelial function can offer critical insights into the preclinical phase of atherosclerosis and serve as an early predictor of future atherosclerotic disease. This dysfunction is not only associated with traditional risk factors but is also influenced by glycemic control and the duration of diabetes. Consequently, by modifying risk factors and improving glycemic control, we can potentially prevent the progression of the disease and subsequent vascular complications. Flow-mediated dilation (FMD) is a reliable method for evaluating endothelial function, as it reflects the endothelium's capacity to release nitric oxide (NO) in response to shear stress, making it a valuable tool for assessing endothelial function in various disease states.

Aims and Objectives of the Study

1. To investigate endothelial dysfunction in patients with Type 2 Diabetes Mellitus and compare it with age- and sex-matched non-diabetic healthy individuals.
2. To examine the correlation between the duration of Diabetes Mellitus and the prevalence of endothelial dysfunction.
3. To explore the relationship between atherosclerosis risk factors in Type 2 Diabetes Mellitus and endothelial dysfunction.

Methodology

This study was conducted at Kempegowda Institute of Medical Sciences and Research Centre, Bangalore, focusing on patients with Type 2 Diabetes Mellitus. The study is a case-control design, involving 50 subjects with Type 2 Diabetes Mellitus and 20 healthy individuals as controls.

Method of Data Collection

Data collection involved a comprehensive approach, including detailed patient histories, clinical examinations, and laboratory investigations. All data were gathered using a proforma specifically designed for this study.

Inclusion Criteria

•Age range: 30-75 years
•Both sexes
•Newly diagnosed patients with Type 2 Diabetes Mellitus or those under treatment with oral hypoglycaemic agents, insulin, or both
•Patients with or without microvascular or macrovascular complications

Exclusion Criteria

•Age below 30 years or above 75 years
•Patients who did not consent to participate in the study

Patients with Type 2 Diabetes and the control group were included in the study. Flow-mediated dilation (FMD) was assessed using colour Doppler ultrasonography of the brachial artery. The procedure was performed using a HELWLETT-PACKARD Image Point machine with a 7.5 and 10 MHz linear probe to evaluate endothelial function.

Total cholesterol, HDL, and triglycerides (TGs) were measured using an automated analyser with a reagent kit, employing enzymatic methods for cholesterol measurement. LDL cholesterol was calculated using the Friedewald's formula:

$$\text{LDL cholesterol} = \text{Total cholesterol} - (\text{HDL} - \frac{\text{TGs}}{5})$$

Dyslipidaemia was defined according to the ATP III guidelines as follows [37]:

•LDL levels ≥ 130 mg/dL
•HDL levels ≤ 40 mg/dL
•Triglycerides (TGs) ≥ 200 mg/dL

Investigations Conducted

Before being included in the study, all patients underwent the following investigations:

1. Haemoglobin (Hb)
2. Total count (TC), differential count (DC), and erythrocyte sedimentation rate (ESR)
3. Fasting blood sugar (FBS) and postprandial blood sugar (PPBS)
4. Urine routine examination
5. Blood urea and serum creatinine levels
6. Lipid profile
7. Electrocardiogram (ECG)
8. Fundoscopy

9. Colour Doppler ultrasonography of the brachial artery using a HEWLETT-PACKARD Image Point machine with a 7.5 and 10 MHz linear probe.

Assessment of Flow-Mediated Dilation in the Brachial Artery Using Color Doppler Ultrasonography
The assessment of brachial artery FMD was conducted once for all subjects using a 7.5 MHz phased array transducer connected to an HP Sonos 5500 echocardiography machine. This procedure was performed after an overnight fast. With the patient in a supine position, a sphygmomanometer cuff was placed on the right arm. The brachial artery was visualized in the antecubital fossa, and its diameter was measured manually from the intima-media using electronic callipers at end-systole. Both systolic and diastolic velocity time integrals (VTIs) were recorded using pulsed-wave Doppler.

Subsequently, the arm was occluded by inflating the sphygmomanometer cuff to at least 50 mm Hg above the systolic blood pressure for five minutes. The same measurements were repeated immediately (within 15 seconds) after the cuff was released. The brachial artery diameter was measured again one minute after cuff release to assess FMD. Ultrasound imaging of the brachial artery was continuously recorded on videotape before, during, and up to two minutes after the release of occlusion. Still images were also captured on the digital Enconcert system for post-procedure offline analysis.[4,33,34,35]

The blood flow in the brachial artery was calculated as follows:
Baseline Flow:

$$\text{Baseline flow} = \frac{\pi d_1^2}{4} \times HR_1 \times (\text{VTIS}_1 + \text{VTID}_1)$$

where d_1 is the brachial artery diameter, HR_1 is the heart rate, $VTIS_1$ is the systolic VTI, and $VTID_1$ is the diastolic VTI at baseline.

Reactive Hyperemia Flow:

$$\text{Reactive hyperemia flow} = \frac{\pi d_2^2}{4} \times HR_2 \times (\text{VTIS}_2 + \text{VTID}_2)$$

where d_2 is the brachial artery diameter, HR_2 is the heart rate, $VTIS_2$ is the systolic VTI, and $VTID_2$ is the diastolic VTI measured immediately after cuff release.
Percentage Increase in Brachial Artery Flow:

$$\%\text{Reactive hyperemia} = \left(\frac{\text{Reactive hyperemia flow} - \text{Baseline flow}}{\text{Baseline flow}}\right) \times 100$$

Flow-Mediated Dilation (FMD):

$$\text{FMD} = \left(\frac{d_3 - d_1}{d_1}\right) \times 100$$

where d_3 is the brachial artery diameter at one minute after cuff release.

Statistical Analysis
The collected data were statistically analyzed by calculating standard measures such as mean, standard deviation, standard error of the mean, and percentages. The differences between various parameters based on quantitative variables were compared using a student's t-test for independent samples. A difference was considered statistically significant if the p-value was less than 0.05.

Ethical Clearance
This study received approval from the Ethical Committee of Kempegowda Institute of Medical Sciences, Bangalore.

OBSERVATIONS AND RESULTS

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Table-1: **Distribution of subjects based on endothelial dysfunction.**

Endothelial Dysfunction FMD%*	**Study group**		**Total**
	Cases	**Controls**	
Absent (>4.5)	40 (80.0)	20 (100.0)	60 (85.7)
Present (<4.5)	10 (20.0)	-	10 (14.3)
Total	**50 (100.0)**	**20 (100.0)**	**70 (100.0)**

*Endothelial dysfunction defined as FMD% < 4.5

In this study, it is observed that endothelial dysfunction (FMD <4.5%) was present among 10 (20%) cases where none of the controls had endothelial dysfunction.

Table2: **Distribution of subjects having endothelial dysfunction by different risk factors.**

Evidence of endothelial dysfunction*	**Male**	**Female**	**Total**
Age (yrs)	5	5	10
▪Mean ± SD	61.2 ± 13.40	58.2 ± 9.52	59.80± 10.93
▪Range	(45-74)	(43-66)	(43-74)
Sex	5	5	10
DM	5	5	10
Hypertension	-	3	3
Peripheral neuropathy	1	3	4
Smoking	2	-	2
Duration of diabetes	5	5	10
▪ Mean ± SD	8.6 ± 6.67	7.90 ± 6.73	8.25 ± 6.32
▪ Range	6 mon-16yrs	3-20 yrs	6 mon-20yrs
Family history of diabetes	1	2	3
Obesity	2	4	6

Abnormal lipid profile			
▪ LDL	1	1	2
▪ HDL	5	4	9
▪ TG	3	2	5

* No cases of IHD, CVA, and Retinopathy were seen in endothelial dysfunction among the 10 cases

In this study, a comparison of various risk factors for endothelial dysfunction among diabetic patients revealed an equal prevalence of endothelial dysfunction in both male and female participants, with 5 cases each. Among female diabetics, there was a higher incidence of hypertension (3 cases), peripheral neuropathy (3 cases), obesity (4 cases), and a family history of diabetes (2 cases). In contrast, male diabetics showed a higher prevalence of smoking (2 cases), although the prevalence of abnormal lipid profiles was equal in both sexes.

The mean age among male diabetics was 61.2 ± 13.40 years, with a mean duration of diabetes of 8.6 ± 6.67 years, both of which were higher than those observed in female diabetics. The mean age for female diabetics was 58.2 ± 9.52 years, with a mean duration of diabetes of 7.90 ± 6.73 years. Notably, none of the male participants had hypertension, and no female participants reported a history of smoking.

Table-3: **Statistical inference based on Student's t-test for independent samples**

Study variables	**Study group**	**No. of subjects**	**Mean**	**Std. Deviation**	**Std. Error**	**t-value**	**df**	**p-value**
Age (yrs)	Cases	50	57.16	8.94	1.26	0.326	68	>0.745
	Controls	20	56.35	10.46	2.34			
Height (cms)	Cases	50	160.02	10.40	1.471	0.581	68	>0.563
	Controls	20	161.50	7.23	1.61			
Weight (kgs)	Cases	50	62.56	10.32	1.46	2.186	68	<0.032
	Controls	20	56.80	8.94	2.00			
BMI	Cases	50	25.073	3.74	.52	4.374	68	<0.0001
	Controls	20	21.04	2.68	.60			
Waist (cms)	Cases	50	83.98	17.28	2.44	0.554	68	>0.582
	Controls	20	81.75	7.69	1.72			
Hip (cms)	Cases	50	85.52	17.09	2.41	2.075	68	<0.042

	Controls	20	93.65	5.60	1.25			
ist/Hip ratio	Cases	50	.9812	.09	.01	4.913	68	<0.0001
	Controls	20	.8695	.04	.01			

**significant at p value<0.0001 n=50 for cases, n=20 for control
*significant at p value<0.05

In this study, it was observed that the mean age among the cases was 57.16 ± 8.94 years, while among the controls, it was 56.35 ± 10.34 years, with a p-value of >0.745, indicating no statistically significant difference between the two groups. This suggests that both cases and controls were well-matched in terms of age.

The mean height of the cases was 160.02 ± 10.40 cm, compared to 161.50 ± 7.23 cm in the controls. The mean weight was 62.56 ± 10.32 kg in the cases and 56.80 ± 8.94 kg in the controls, with no significant difference between the two groups. However, the Body Mass Index (BMI) was higher in the cases (25.07 ± 3.74) compared to the controls (21.04 ± 2.68), which was statistically significant. Despite this, when individual parameters such as height and weight were compared separately, no significant differences were found.

Regarding waist and hip measurements, the mean waist circumference among the cases was 83.98 ± 17.28 cm, compared to 81.75 ± 7.69 cm in the controls. The mean hip circumference was 85.52 ± 17.09 cm in the cases and 93.65 ± 5.60 cm in the controls. The waist-to-hip ratio was 0.981 ± 0.09 in the cases and 0.869 ± 0.04 in the controls. No significant differences were observed between the cases and controls when considering waist and hip measurements individually. However, the differences in BMI were statistically significant.

Table -4: **Comparison of measured parameters of FMD assessment in the study groups**

Study variables	**Study group**	**No. of subjects**	**Mean**	**Std. Deviation**	**Std. Error Mean**	**t-value**	**df**	**p-value**
Baseline diameter	Cases	50	3.800	0.51	.07	1.366	68	>0.176
	Controls	20	3.610	0.54	.12			
Baseline flow	Cases	50	676.58	196.46	27.78	0.830	68	>0.409
	Controls	20	631.62	224.40	50.17			
Reactive hyperemic flow	Cases	50	947.94	445.04	62.93	1.498	68	>0.139
	Controls	20	780.58	356.57	79.73			
Hyperemic flow%	Cases	50	81.65	74.94	10.59	2.229	68	**<0.014**
	Controls	20	122.97	55.39	12.39			
FMD%	Cases	50	8.38	12.32	1.74	2.788	68	**<0.007**
	Controls	20	17.12	10.53	2.35			

*significant at p value <0.05

In this study, it was observed that when assessing various parameters of Flow-Mediated Dilation (FMD), the hyperemic flow percentage in diabetic patients (81.64 ± 74.94) was significantly lower compared to non-diabetic controls (122.97 ± 55.39), with a p-value of <0.014. This indicates a substantial impairment in endothelial function among diabetic subjects.

Moreover, the FMD percentage, a direct measure of endothelial function, was also significantly reduced in diabetics (8.38 ± 12.32) compared to healthy subjects (17.12 ± 10.53), with a p-value of 0.007. This further highlights the diminished endothelial responsiveness in individuals with diabetes.

However, there was no significant difference observed in the baseline brachial artery diameter and the calculated brachial artery flow between diabetic and healthy subjects, suggesting that the observed differences in FMD parameters were not due to baseline disparities in arterial structure or flow but rather due to endothelial dysfunction specifically associated with diabetes.

Graph-1: **Distribution of subjects by FMD% in cases and controls**

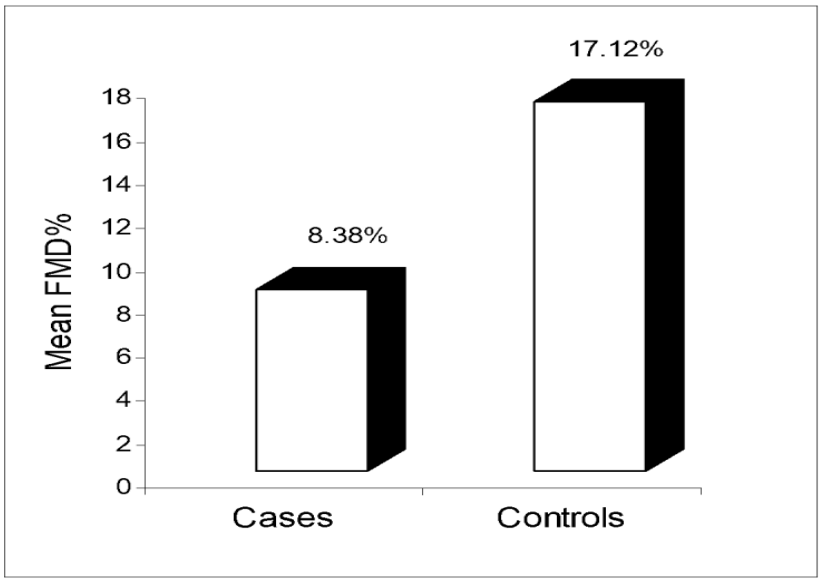

Table -5: Duration distribution of FMD in diabetic subject's descriptive statistics FMD%

Duration of diabetes	No. of subjects	Mean	Std. Deviation	Std. Error
<5	3	0.2800	4.01960	2.32072
5-10	4	2.1250	1.34505	0.67253
>10	3	3.5067	1.21825	0.70336
Total	10	1.9860	2.50457	0.79202

In this study, it was observed that mean FMD values were consistently lower in diabetic subjects across different durations of disease. Specifically, the mean FMD values in diabetic subjects were as follows: for durations of less than 5 years, the mean FMD was 0.28 ± 4.0%; for durations of 5-10 years, the mean FMD was 2.12 ± 1.34%; and for durations greater than 10 years, the mean FMD was 3.50 ± 1.2%.

Interestingly, while the prevalence of endothelial dysfunction did not show a significant increase with the duration of diabetes, the FMD values decreased in diabetic patients with longer durations (more than 10 years since diagnosis). This suggests that although endothelial dysfunction may not become more prevalent with longer diabetes duration, the severity of endothelial impairment, as measured by FMD, worsens over time among diabetic individuals.

Table 6: **Association of FMD with risk factors in diabetics**

Risk factors	**FMD <4.5 % (n=10)**	**FMD >4.5 % (n=40)**	**Significance By p value**
Age in years	59.70□11.08	56.53□8.37	0.320
Sex	Male=50.0% Female=50.0%	Male=57.5% Female=42.5%	0.228
Smoking	1 (10.0%)	6 (15.0%)	**p>0.05**
Hypertension	4 (40.0%	16 (40.0%)	**p>0.05**
Family history of DM	3 (30.0%)	-	**p>0.006****
Duration of DM	8.25□6.32	6.92□4.88	0.472
BMI kg/m^2 (Mean□SD)	26.46□2.82	24.72□3.89	0.194
T.Chol mg/dl (Mean□SD)	168.80□45.15	166.20□42.50	0.865
LDL mg/dl (Mean□SD)	98.20□35.69	101.95□35.98	0.769
HDL mg/dl (Mean□SD)	31.30□6.89	35.20□9.90	0.247
TG mg/dl (Mean□SD)	220.50□173.44	161.72□65.80	0.451
Inference	Smoking, hypertension and family history of diabetes was significant in diabetic patients with FMD<4.5%.		

In this study, it was observed that among diabetic subjects, those with FMD <4.5% had a higher mean age of 59.70 ± 11.08 years compared to those with FMD >4.5%, who had a mean age of 56.53 ± 8.37 years. Female representation was 50.0% among those with FMD <4.5% and 42.5% among those with FMD >4.5%. Smoking prevalence was 10.0% in diabetics with FMD <4.5% and 15% in those with FMD >4.5%. Hypertension was equally prevalent at 40% in both groups.

Regarding family history of diabetes, 30% of diabetics with FMD <4.5% had a family history, while none were observed among those with FMD >4.5%. The duration of diabetes was longer in patients with FMD <4.5% (8.2 ± 6.32 years) compared to those with FMD >4.5% (6.92 ± 4.88 years).

BMI was higher in diabetics with FMD <4.5% (26.46 ± 2.82) compared to those with FMD >4.5% (24.72 ± 3.89). Total cholesterol levels were 168.80 ± 45.15 mg/dL in diabetics with FMD <4.5% and 166.20 ± 42.50 mg/dL in those with FMD >4.5%. Mean LDL cholesterol levels were 98.20 ± 35.69 mg/dL in diabetics with FMD <4.5% and 101.95 ± 35.98 mg/dL in those with FMD >4.5%. HDL cholesterol levels were 31.30 ± 6.89 mg/dL in diabetics with FMD <4.5% and 35.20 ± 9.90 mg/dL in those with FMD >4.5%. Triglyceride levels were higher in diabetics with FMD <4.5% (220.50 ± 173.44 mg/dL) compared to those with FMD >4.5% (161.72 ± 65.80 mg/dL).

Overall, these findings indicate that diabetic subjects with lower FMD percentages tend to have older age, higher BMI, longer duration of diabetes, and less favourable lipid profiles compared to those with higher FMD percentages.

DISCUSSION

Age and Sex in Diabetics

In this study, the majority of diabetic cases (42%) were in the age group of 55-64 years, followed by 28% aged between 65-74 years. The mean age was 57.10 ± 8.94 years for cases and 56.35 ± 10.46 years for controls. This contrasts with Bhargava et al., who reported a lower mean age of 49.58 ± 8.72 years in their study. The sex distribution in our study showed 56% were male, whereas Bhargava et al. found a higher proportion of males at 89.5%. Another study by Ravikumar et al. observed similar age distributions in diabetics and non-diabetics.[4,41]

Smoking and Hypertension

In our study, 14% of diabetic cases were smokers and 40% were hypertensive. This is lower compared to Bhargava et al., who reported smoking prevalence at 36.8% and hypertension at 89.5% among their diabetic subjects.[4]

BMI

The mean BMI in diabetic subjects was 25.073 ± 3.74, significantly higher than non-diabetic controls (21.04 ± 2.68, $p < 0.001$). This contrasts with findings from Ramkumar et al., who reported lower BMIs in both diabetics (24.8 ± 4.0) and controls (23.9 ± 3.7) with no significant difference. Our study differed from Goodfellow and Ramsay, who reported a higher mean BMI of 26.9.[34,41]

Duration Distribution of FMD in Diabetic Subjects

We observed lower mean FMD values across all durations of diabetes: <5 years (0.28 ± 4.0%), 5-10 years (2.12 ± 1.34%), and >10 years (3.50 ± 1.61%). This contrasts with findings from Ramkumar et al., who noted decreasing FMD values with longer diabetes duration (e.g., <5 years: 2.14 ± 3.18%, 5-9 years: 1.83 ± 2.58%, >10 years: 1.60 ± 2.30%). This discrepancy may be attributed to our smaller sample size.

Comparison of Measured Parameters of FMD Assessment

•**Baseline Diameter (mm)**: Our study found mean baseline diameters of 3.800 ± 0.51 mm in cases and 3.610 ± 0.54 mm in controls, similar to Bhargava et al.'s findings (3.733 ± 0.729 mm). Goodfellow and Ramsay reported slightly higher baseline diameters in both cases and controls.[4,34]

•**Baseline Flow (ml/min)**: Mean baseline flows in our study were 676.58 ± 196.46 ml/min in cases and 631.62 ± 224.40 ml/min in controls, higher than Bhargava et al.'s findings (131 ± 71.5 ml/min). This discrepancy may reflect differences in study methodologies or participant characteristics.[4,34]

•**Percentage (%) Hyperemia**: Diabetic subjects exhibited a significantly lower percentage of hyperemic flow (81.64 ± 74.94%) compared to non-diabetic controls (122.97 ± 55.39%, $p < 0.014$). Bhargava et al. reported higher mean hyperemic flows (294.7 ± 165.1%) in diabetic cases, indicating a more severe impairment in endothelial function in their cohort.[4,34]

In this study, we observed that the mean FMD% in diabetic subjects was 8.38 ± 12.32%, whereas in controls it was 17.12 ± 10.53% ($p = 0.007$). This significant reduction in FMD% among diabetics compared to controls indicates impaired endothelial function in diabetes. Our findings align with previous studies that also reported diminished FMD in diabetic populations. For instance, Bhargava et al. found a mean FMD% of 5.506 ± 2.12%, which was lower than in our study but similarly highlighted impaired endothelial function in diabetics.

Clarkson et al. demonstrated a significant impairment in FMD in diabetics compared to controls (5.0 ± 3.7% vs. 9.3 ± 3.8%; $p < 0.001$), with the degree of impairment correlating directly with the duration of diabetes. Yu et al. and Dipti Chand et al. also reported significantly reduced FMD in diabetic groups compared to controls in their respective studies.

Furthermore, Ramkumar et al. observed a mean FMD% of 1.72 ± 2.8% in diabetics versus 6.64 ± 4.38% in non-diabetics, further underscoring the consistent finding of impaired endothelial function in diabetes across different populations and study settings.[4,38,41]

Overall, the collective evidence from these studies, including ours, consistently demonstrates that FMD% is significantly lower in diabetic individuals compared to non-diabetic controls, indicating a widespread endothelial dysfunction associated with diabetes mellitus.

CONCLUSION

In this study of 50 diabetic patients, endothelial dysfunction was identified in 20% of cases, affecting both male (5 cases) and female (5 cases) patients equally. The mean ages for males and females were 61.2 ± 13.40 years and 58.2 ± 9.12 years, respectively. Endothelial dysfunction was observed across all durations of diabetes, from initial diagnosis to over 10 years, although its prevalence did not correlate significantly with the duration of diabetes.

Several risk factors were associated with endothelial dysfunction among the diabetic patients studied. These included hypertension, a family history of diabetes, and smoking. Microvascular complications, particularly peripheral neuropathy, were prevalent, affecting 40% of the study population. However, no macrovascular complications were observed in any of the subjects.

In conclusion, this study underscores the significant prevalence of endothelial dysfunction in diabetic patients and highlights the importance of managing associated risk factors to potentially mitigate its development and progression. Further research with larger cohorts and longitudinal studies is warranted to better understand the trajectory of endothelial dysfunction in diabetes and to optimize preventive strategies and treatments.

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