

Assessment of Endothelial Function among Patients with Type 2 Diabetes Mellitus

Saadealdeen Majeed Hassoon, A. S. Kalid Mahdi Al Bermani¹

Department of Medicine, College of Medicine, Al-Mustansiriyah University, ¹Department of Internal Medicine and Cardiology, Al-Karama Teaching Hospital, Baghdad, Iraq

Abstract

Background: Endothelial dysfunction is an initial key step in the pathogenesis of atherosclerosis. Many tests developed to measure endothelial function, most of which are either invasive or semi-invasive. The unique nature of brachial artery reactivity test of being a noninvasive, quantitative, and repeatable procedure is gaining increasing interest as an index in assessing endothelial cell function. **Aim of Study:** The aim is to study the assessment of endothelial function among a group of patients with type 2 diabetes mellitus (DM), using brachial artery reactivity test. **Patients and Methods:** In a case control study enrolling 35 patients with type 2 DM matched against a similar number of nondiabetic participants of related genders and age groups to trace the brachial artery diameter both at rest and in response to reactive hyperemia using a commercially available U/S machine equipped with a high-resolution linear probe (7.5 MHz) following an already defined U/S protocol. **Results:** The diabetic participants revealed a statistically significant attenuation in brachial artery dilatation in response reactive hyperemia when compared with nondiabetic group (mean $\pm$ standard deviation) (4.63 ± 0.4 mm vs. 5.61 ± 0.47 mm). Comparing the two groups, a statistically significant impairment of the brachial artery dilatation is traced in relation to the atherosclerotic risk factors both in isolation and in clusters. Moreover, strict glycemic control ($HbA1c \leq 6\%$) significantly altered the response to vasodilatation (0.74 ± 0.17 mm vs. 0.43 ± 0.14 mm, $P = 0.0001$). Age, gender difference, and duration of diabetes did not significantly alter the results. **Conclusions:** Endothelial dysfunction is not uncommon among patients with type 2 DM. Brachial artery reactivity test is a well-accepted, noninvasive, repeatable, and a quantitatively reproducible index to trace endothelial function.

Keywords: Endothelial function, reactive hyperemia, type 2 diabetes

INTRODUCTION

Appreciation of the central role of the endothelium throughout the atherosclerotic disease process had led to the development of a range of methods to test different aspects of its function which include measures of both endothelial injury and repair.^[1] These tests have provided not only novel insights into pathophysiology of atherosclerosis but also a clinical opportunity to detect early disease, quantify risk, judge the response to intervention designed to prevent progression of early disease, and reduce later adverse events. Ideally, such tests should be safe, noninvasive, reproducible, repeatable, cheap, and standardized between laboratories; the results should also reflect the dynamic biology of the endothelium throughout the natural history of atherosclerosis, define the subclinical disease processes, as well as provide prognostic information for risk stratification in the later clinical phase.^[2]

Endothelium-dependent vasomotion has been the most widely used clinical endpoint for the assessment of endothelial function.^[3] Testing involves pharmacological and/or physiological stimulation of endothelial release of nitric oxide (NO) and other vasoactive compounds and often a comparison of vascular response to endothelium-independent vasodilators such as nitroglycerine. Determination of local (NO) bioavailability not only reflects its influence on the vascular tone but also traces other important functions of this molecule such as thromboregulation, cell adhesion, and proliferation.^[4]

Address for correspondence: Prof. Saadealdeen Majeed Hassoon, Department of Medicine, College of Medicine, Almustansyriyah University, Baghdad, Iraq.
E-mail: needlaedaas@gmail.com

Access this article online

Quick Response Code:



Website:
<http://www.mmjonline.org>

DOI:
10.4103/MJ.MJ_14_18

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Hassoon SM, Kalid Mahdi Al Bermani AS. Assessment of endothelial function among patients with type 2 diabetes mellitus. *Mustansiriyah Med J* 2018;17:57-61.

Aim of the study

The aim is to study the assessment of endothelial function among a sample of adult patients with type 2 diabetes mellitus (DM), using brachial artery reactivity test.

PATIENTS AND METHODS

An observational analytic case–control study is conducted in Al-Yarmouk Teaching Hospital/Department of Medicine with the assistance of Department of Radiology/Ultrasound Unit, in the period from April 2010 to October 2010, whereby (35) patients with type 2 DM were tested against (35) nondiabetic persons of related gender and age as a control group after getting their agreement to be enrolled in the study, proceeded by discussing the aim and the procedure for each volunteer. A brief history and physical examination were conducted looking for a previous or present cardiovascular disease defined as cardiac, coronary, cerebrovascular, peripheral arterial, and venous diseases and risk factors (smoking, arterial hypertension as is defined by JNC VII, dyslipidemia, and obesity). Body mass index as a measure of total body fat was extracted from participant weight in kilogram to his height in meter squared.^[5] Three-milliliter anticoagulated blood sample was aspirated from each participant to assess HbA1c fraction; a glycemic control represents HbA1c of 6% and less American association of clinical endocrinology(AACE).^[6] An extra fasting 10 ml was aspirated to measure plasma lipid profile. All the participants were tested using an already defined ultrasound protocol to trace brachial artery reactivity using a commercially available machine (Kontron/Voluson 530D of Kretz/Austria) equipped with a high-resolution linear probe (7.5MHz).^[8]

Ultrasound protocol

The participant is positioned supine with the arm extended in a comfortable position to measure the brachial artery diameter. The artery is imaged above the antecubital fossa in a longitudinal plane; a segment with clear anterior and posterior intimal interface between the lumen and the vessel wall is selected for continuous two-dimensional grayscale imaging [Figure 1].

A baseline resting image is acquired, and the brachial artery diameter is measured. To create a flow stimulus in the brachial artery, a blood pressure cuff is placed above the antecubital fossa and is inflated to at least 50 mmHg above the systolic pressure to occlude arterial blood flow for a standardized length of time (3 min), and this causes ischemia and consequent dilatation of downstream resistance vessels through autoregulatory mechanisms. Subsequent cuff deflation induces a brief high flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistant 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. At least 10 min of rest is needed after reactive hyperemia (flow-mediated diameter) before another image is acquired to reflect the reestablished baseline condition.

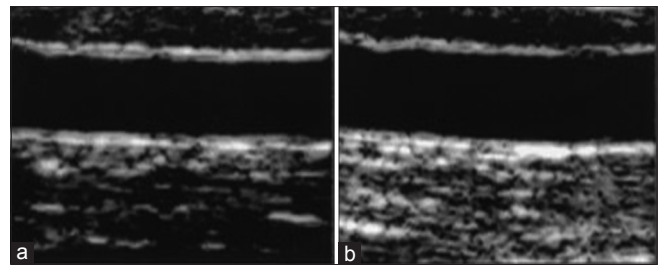


Figure 1: High-resolution ultrasound image of brachial artery at baseline (a), and 1 min. after upper-arm occlusion cuff inflation release and subsequent flow-mediated vasodilatation (b)

An exogenous (NO) donor (0.4 mg. GTN) sublingual tablet has been given to determine the maximum obtainable vasodilatation response which serves as a measure of endothelium-independent vasodilatation reflecting the vascular smooth muscle function.

Exclusion criteria

History and/or presence of cardiovascular disease defined as cardiac, coronary, cerebrovascular, peripheral arterial, and venovascular diseases.^[9]

Statistical analysis

Raw data from the two groups were collected and analyzed using software statistical package SPSS version 8, SPSS UK Ltd, St. Andrew's House, West Street, Woking, Surrey GU21 1EB, applying Pearson's Chi-square test and Student's *t*-test, and results were tabulated, blotted, and graphically represented in the form of mean and standard deviation, and the degree of difference between two measured variables was traced to be significant when $P < 0.05$ using Student's *t*-test.

RESULTS

Table 1 shows age- and gender-distribution among diabetic and nondiabetic groups in relation to the changes in mean values of brachial artery diameter at rest and in response to reactive hyperemia.

Table 2 is referring to mean brachial artery diameter among the two studied groups both at rest and in response to reactive hyperemia (intrinsic NO release) once and in response to GTN (extrinsic NO release) once again.

The mean difference in brachial artery diameter among both groups in relation to conventional risk factors for atherosclerosis is followed in Table 3. The nonalliance in relation to duration of diabetes is demonstrated in Table 4; on the other hand, Table 5 shows the influence of the strict diabetic control among diabetic group on the brachial artery diameter, whereby a significant difference in brachial artery diameter is traced, suggesting a better endothelial function under the umbrella of tight control.

DISCUSSION

Although is not yet clinically applicable tool, brachial artery vasomotion test as an index to trace endothelial function is

Table 1: Mean brachial artery diameter and the range in millimeters at rest, flow-mediated response, and in response to GTN among diabetics and nondiabetics

Brachial artery diameter (mm) in relation to	Diabetic group		Nondiabetic group		P
	Mean±SD	Range	Mean±SD	Range	
Baseline	4.02±0.32	3.4-4.4	4.20±0.44	3.6-5.0	0.057
Flow mediated	4.63±0.40	3.9-5.2	5.61±0.47	4.8-6.1	0.0001*
GTN mediated	5.83±0.46	5.1-6.4	5.96±0.30	5.3-6.3	0.157

*Significant difference between means using *t*-test for two independent means at 0.05 level of significance. SD: Standard deviation, GTN: Glyceril trinitrate

Table 2: Age and gender distribution among diabetic and nondiabetic groups in relation to mean degree increase in brachial artery diameter from baseline in response to reactive hyperemia

	Diabetic group		Nondiabetics		P
	n	Mean±SD	n	Mean±SD	
Age group					
<40	1	0.67±	1	1.10±	-
40-49	6	0.64±0.19	2	1.44±0.21	0.0001*
50-59	7	0.69±0.16	8	1.37±0.28	0.0001*
60-69	19	0.56±0.25	23	1.43±0.38	0.0001*
70 more	2	0.63±0.04	1	1.78±	-
Sex					
Male	23	0.53±0.21	20	1.33±0.38	0.0001*
Female	12	0.75±0.12	15	1.41±0.29	0.0001*
P		0.002*		0.322	

*Significant difference between means using *t*-test for two independent means at 0.05 level of significance. SD: Standard deviation

a well-known noninvasive research tool over the previous two decades,^[7] and is preferred on other techniques used for the same reason, which are either invasive or semi-invasive procedures and are dependent on the assessment of coronary endothelial cell function and/or assessing different biomarkers in blood plasma, reflecting the interaction between a shear stress and endothelial cell response,^[10] they are found to be subjected to variation in the level and/or interacting with other substances, besides they might be released from other sources other than endothelial cells, making them less sensitive and less specific index that would only add to the cost and complexity of the issue.^[11]

Brachial artery flow-mediated diameter measurement has been shown to correlate with the measures of coronary endothelial cell function with a high index of sensitivity and specificity.^[12] A unique advantage of this test is the noninvasive nature, being quantitative, and ability to repeat it in the same patient, making it more cost-effective and easy to handle,^[12] reasons that make it more rational to be adopted in our study.

When tested against a matched group of controls of related gender and age [Table 1], the diabetic patients had shown a significant impairment in the flow-mediated diameter (endothelial cell-dependent vasodilatation) ($P = 0.0001$), in contrast to the baseline and GTN-mediated (smooth muscle cell-dependent vasodilatation) brachial artery caliber, reflecting the role

of oxidative stress induced by diabetic state and associated metabolic derangement on a cellular level, a result which came consistent with many met analytic studies,^[13-15] suggesting impaired intrinsic NO release in response to shear stress of brachial artery hyperemia and pointing to an early endothelial cell dysfunction irrespective of the patient age [Table 2],^[16,17] a key step in initiating atherosclerosis,^[10,18] and providing an evidence based clue to the fact that DM is an atherosclerosis equivalent problem which starts at an earlier age than the nondiabetic subjects [Table 2].^[5,18]

Moreover, when adjusted for gender, a significant attenuation is traced in brachial artery diameter in response to reactive hyperemia between the two major tested groups (diabetic and control), suggesting that the unique protection provided by female gender probably through the positive add-on effect of estrogen on endothelial cell^[19] is omitted in the presence of diabetes and its detrimental metabolic effect on cellular biology. The unexpected relationship in gender variable within the same group may in part be related to the small size studied population sample.

Although diabetes carries a greater burden of additional cardiovascular risk factors,^[1,20] there still remains a significant risk in attenuating brachial artery reactivity when diabetes is the sole clinical characteristic [Table 3], suggesting that diabetes is independent and a powerful predictor for endothelial cell dysfunction and that the effects of other risk factors are augmented in its presence [Table 3].^[5]

On the other hand, duration of diabetes did not influence the flow-mediated brachial artery diameter significantly [Table 4], a step that came in agreement with results mentioned in many studies (Janka 1985; Hsueh and Anderson 1992; Bloomgarden 1998; De Mattia *et al.* 1998; Neri *et al.* 1998; Watts and Playford, 1998; and Gazis *et al.* 1999).^[17] Hyperglycemia whether acute or chronic is a major causal factor in the development of endothelial dysfunction in DM, and the mechanism underlying this phenomenon is likely to be multifactorial through influencing several metabolic pathways leading to increase oxidative stress contributing to endothelial dysfunction,^[17] while a tight control represented by HbA1c of 6% or less [Table 5] reverses the metabolic and cellular derangement induced by hyperglycemia and the associated released cytokines (e.g., monocyte adhesion molecules, interleukin-6, and tumor necrosis factor) which are negatively influencing cellular biology including

Table 3: The mean increase in brachial artery diameter in relation to clinical characteristics among the diabetics and controls

Risk factors for atherosclerosis	Degree of increase (mm)		P
	Diabetics (mean±SD)	Nondiabetics (mean±SD)	
No added risk factor	0.98±0.13	1.75±0.22	0.0001*
One added risk factor	0.55±0.15	1.43±0.37	0.0001*
Two or more added risks	0.55±0.17	1.22±0.22	0.0001*

*Significant difference between means using *t*-test for two independent means at 0.05 level of significance. SD: Standard deviation

Table 4: Impact of duration of diabetes on degree of mean increase in brachial artery diameter from baseline level in response to reactive hyperemia

Duration of diabetes (years)	Number of patients	Mean brachial artery diameter increase (mm) ± SD
5 years and more	13	0.58±0.17
<5 years	22	0.63±0.24
P*	0.525	

*independent sample *t*-test with 0.05 level of significance. SD: Standard deviation

Table 5: Effect of strict diabetic control on mean increase of brachial artery diameter from baseline in response to reactive hyperemia

Degree of glycemia control	Number of patients	Mean increase in brachial artery caliber (mm) ± SD
HbA1c 6% or more	15	0.43±0.11
HbA1c <6%	20	0.74±0.17
P	0.0001*	

*Significant difference between means using *t*-test for two independent means at 0.05 level of significance. SD: Standard deviation, HbA1c: Glycated hemoglobin

endothelial dysfunction;^[17] hence, strict control of diabetes will undoubtedly reverse the detrimental metabolic derangement of the disease.

Study limitations

The fluctuation in laboratory facilities concerning the availability of HbA1c kits had limited the size of the studied sample enrolled in the study. Inability to overcome the add-on effect of the drugs used by the patients and controls, which are known to influence the endothelial function, for example, angiotensin-converting enzyme inhibitors, angiotensin receptor blocker, statins, B-blockers, and salicylate.

CONCLUSIONS

Brachial artery reactivity test as an index to trace endothelial function is a noninvasive, easy to handle, acceptable, quantitative test which can be used repeatedly in the same patient and/or applied over a large number as a screening tool. Endothelial dysfunction is not uncommon among patients with type 2 diabetes and is traced irrespective of the associated cardiovascular risk factors and duration of diabetes. Significant changes were traced among the diabetics when compared with

the control group in respect to age, gender, and the degree of glycemic control. We recommend a more extended prospective study with larger sample to trace the influence of the previously mentioned variables on the clinical course of diabetes in order to risk stratify the patients and/or tracing the endothelial dysfunction in preclinical stage and to assess the influence of applying different pharmacological and nonpharmacological interventions to reverse it.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: Testing and clinical relevance. *Circulation* 2007;115:1285-95.
- Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, *et al.* Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: A Statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005;23:7-17.
- Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, *et al.* Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111-5.
- Vita JA, Keaney JF Jr. Endothelial function: A barometer for cardiovascular risk? *Circulation* 2002;106:640-2.
- Grasso AW. Non lipid cardiovascular risk factors. In: Griffin BP, Topol EJ, editors. *Manual of Cardiovascular Medicine*. 3rd ed. New York, USA: Lippincott Williams & Wilkins; 2009. p. 589.
- Unger J. Assessing glycemic control using glycated hemoglobin (A1c). *Diabetes Management in Primary Care*. New York, USA: Lippincott Williams & Wilkins; 2007. p. 330-3.
- Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, *et al.* Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the international brachial artery reactivity task force. *J Am Coll Cardiol* 2002;39:257-65.
- Title LM, Lonn E, Charbonneau F, Fung M, Mather KJ, Verma S, *et al.* Relationship between brachial artery flow-mediated dilatation, hyperemic shear stress, and the metabolic syndrome. *Vasc Med* 2008;13:263-70.
- Mackay J, Mensah GA. *The Atlas of Heart Disease and Stroke*. Geneva: World Health Organization; 2004. p. 18-9.
- Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. *J Am Coll Cardiol* 1999;34:631-8.
- Meyer AA, Kundt G, Steiner M, Schuff-Werner P, Kienast W. Impaired flow-mediated vasodilation, carotid artery intima-media thickening, and elevated endothelial plasma markers in obese children: The impact of cardiovascular risk factors. *Pediatrics* 2006;117:1560-7.

12. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrangé D, *et al.* Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 1995;26:1235-41.
13. Ametov AS, Demidova TI, Kosykh SA. NO synthesis in the vascular endothelium of patients with type II diabetes. *Klin Med (Mosk)* 2005;83:62-8.
14. Ammar RF Jr., Gutterman DD, Brooks LA, Dellsperger KC. Free radicals mediate endothelial dysfunction of coronary arterioles in diabetes. *Cardiovasc Res* 2000;47:595-601.
15. Avogaro A, Fadini GP, Gallo A, Pagnin E, de Kreutzenberg S. Endothelial dysfunction in type 2 diabetes mellitus. *Nutr Metab Cardiovasc Dis*. 2006;16 Suppl 1:S39-45.
16. Balletshofer BM, Rittig K, Enderle MD, Volk A, Maerker E, Jacob S, *et al.* Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 2000;101:1780-4.
17. Hadi HA, Suwaidi JA. Endothelial dysfunction in diabetes mellitus. *Vasc Health Risk Manag* 2007;3:853-76.
18. Cooke JP. Therapeutic interventions in endothelial dysfunction: Endothelium as a target organ. *Clin Cardiol* 1997;20:II-45-51.
19. Lieberman EH, Gerhard MD, Uehata A, Walsh BW, Selwyn AP, Ganz P, *et al.* Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. *Ann Intern Med* 1994;121:936-41.
20. Lloyd-Jones DM, Leip EP, Larson MG, D'Agostino RB, Beiser A, Wilson PW, *et al.* Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. *Circulation* 2006;113:791-8.