DOI: 10.7860/JCDR/2024/61444.18899

Original Article

Internal Medicine Section

Endothelial Dysfunction in Type 2 Diabetes Patients and its Association with Microvascular Complications: A Cross-sectional Study

AMEYA SANJAY CHOGLE[1], ARUN PRASATH[2], CHANDNI CHANDRAMOULI[3], SIVA RANGANATHAN GREEN[4]

ABSTRACT

Introduction: The prevalence of Type 2 Diabetes Mellitus (T2DM) is increasing worldwide. Microangiopathic and macroangiopathic complications are the main causes of morbidity and mortality in diabetes. Endothelial dysfunction is important in the early pathophysiology of vascular complications. Screening is very important in order to prevent these complications.

Aim: To investigate endothelial dysfunction, as measured by Flow Mediated Vasodilation (FMD) of the brachial artery, in diabetic individuals. It also aims to explore the correlation between FMD and Glycated Haemoglobin (HbA1c), as well as the association between FMD and microvascular complications such as diabetic neuropathy, diabetic retinopathy, and albuminuria.

Materials and Methods: This cross-sectional study was conducted at Mahatma Gandhi Medical College and Research Institute, Puducherry, India, between 2020 and 2021 on 160 patients. All patients diagnosed with T2DM, attending the Outpatient Department (OPD) or admitted to the ward, were enrolled in the study using consecutive sampling. FMD was measured for each patient, and Urine Albumin Creatinine Ratio (UACR) was also assessed. Diabetic retinopathy was evaluated through fundus examination, and diabetic neuropathy was screened using the monofilament test. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 27.0 to determine the correlation between FMD and these variables.

Results: A total of 160 patients were enrolled in this study, including 104 (65%) males and 56 (35%) females. The median age was 52 years, and the participants had a median Body Mass Index (BMI) of 29.5 kg/m^2. The median HbA1c was 8.7%. The median Fasting Blood Sugar (FBS) and Postprandial Blood Sugar (PPBS) levels were 145 mg/dL and 242 mg/dL, respectively. The median FMD among all patients was 5.6%, with an Interquartile Range (IQR) of 4.9% to 6.6%. There was an inverse correlation between HbA1C and FMD values, with a correlation coefficient of -0.718 (p-value <0.01), indicating a strong and significant inverse correlation. Patients with significant macroalbuminuria had a lower median FMD compared to non albuminuric patients, and this difference was statistically significant. The median FMD was also lower in patients with diabetic neuropathy and retinopathy.

Conclusion: The study findings suggest that endothelial dysfunction, as measured by FMD, is significantly impaired in patients with elevated HbA1c and microvascular complications of diabetes mellitus.

Keywords: Endothelium, Hyperglycaemia, Nitric oxide, Vasodilation

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder resulting from improper intermediary metabolism due to an absolute or relative deficiency of insulin, leading to hyperglycaemia. The initial phase of hyperglycaemia, which is commonly asymptomatic, accounts for the majority of long-term complications [1]. Endothelial dysfunction is considered the initial step in the progression of atherosclerosis and microvascular complications of diabetes mellitus [2]. The endothelium, which lines the intimal surface of blood vessels, forms the largest endocrine organ in the human body [3]. It plays a crucial role in regulating vascular smooth muscle tone, thrombosis, platelet aggregation, and blood vessel permeability. The endothelium releases vasoactive substances such as prostacyclin, endothelin, endothelial growth factors, interleukins, plasminogen inhibitors, and Nitric Oxide (NO) [4]. Altered endothelial function plays a central role in the pathogenesis of vascular complications. The term "endothelial dysfunction" refers to an impairment of the endothelium's ability to maintain vascular homeostasis properly. NO serves as an important marker of endothelial dysfunction. It is considered the initial step in the progression of atherosclerosis [5].

The endothelial cells sense the laminar stress on the endothelium caused by hyperemia, leading to increased activity of endothelial Nitric Oxide Synthase (eNOS). This, in turn, converts L-arginine to NO. NO activates guanylate cyclase, which stimulates the conversion of guanosine triphosphate to monophosphate. Consequently, this causes smooth muscle relaxation, leading to vasodilation. FMD reflects endothelial function [3]. The most widely used non invasive technique for assessing endothelial function is called "FMD". FMD measures the dilation or widening of a blood vessel in response to increased blood flow, which is a normal response of healthy endothelial cells. This non invasive method is based on the principle that physiological increases in blood flow and endothelial shear stress induce vasodilation, which is mediated by the release of endothelial NO [6]. However, FMD is currently classified as class III evidence and is not routinely recommended for screening cardiovascular risk [7,8]. Screening is crucial for preventing chronic complications. FMD can be performed using ultrasound equipment, which is commonly available in most tertiary care hospitals in India [7,9].

Only a few recent studies in India have combined all these parameters [10,11]. In a study by Ravikumar R et al., conducted on 50 diabetic and non diabetic individuals in the Chennai Urban Population study, it was found that FMD was impaired in diabetic subjects compared to non diabetics [10]. Another study by Bhargava K et al., showed that the mean FMD in patients with diabetes only was 5.51% compared to 7.03% in the non diabetic group. Thus, the presence of endothelial dysfunction is considered an early risk

factor for coronary artery disease as well [11]. The present study aims to correlate endothelial dysfunction, as measured by FMD, in T2DM patients with glycaemic control measured by HbA1c, and to study its association with microvascular complications such as diabetic retinopathy, neuropathy, and albuminuria.

MATERIALS AND METHODS

This cross-sectional study was conducted at a tertiary care institute in Puducherry, India, from March 2020 to March 2021. The approval of the Institutional Human Ethics Committee was obtained (letter no MGMCRI/Res/01/2019/41/IHEC/088).

Inclusion criteria: All patients diagnosed with T2DM who attended the OPD and were admitted to the ward were included in the study.

Exclusion criteria: Patients who were on Angiotensin Converting Enzyme Inhibitors (ACEI), had known cases of type 1 diabetes mellitus, end-stage renal disease, chronic liver disease, thyroid disorders, any proven case of peripheral neuropathy other than diabetic neuropathy, stroke, and pregnant or lactating women were excluded from the study.

Sample size: The study population consisted of 160 individuals selected from patients attending the OPD and inpatients using consecutive random sampling. The prevalence of diabetes mellitus was taken as 10.3% based on a previous study [12]. The required sample size (n) was calculated using the formula:

d=0.05

Formula:

$$\frac{Z^2_{1-\frac{\alpha}{2}}p(1-p)}{d^2}$$

$Z_{1-\alpha/2}$=1.96 for a 95% of confidence level with α=0.05

p=10.3%=0.103 (from previous study)

1-p=0.897

d=5%=0.05 (absolute precision)

$$n_1=\frac{(1.96^2\times(0.103)(0.897)}{(0.05)^2}$$

n_1=141.9≅142

Considering a 10% attrition rate, the final sample size was calculated as follows:

$n=n_1+n_1$*10%=142+14.2=156.2

n≅160

Procedure

After obtaining written informed consent, demographic details and previous clinical and medical history regarding other co-morbid conditions were recorded. The recent (within two months) HbA1c value was also recorded. Patients were instructed to visit the clinic after an overnight fast, abstaining from smoking, drinking coffee, or taking antioxidant vitamins for atleast 12 hours prior to testing. They were examined in the supine position, following 15 minutes of rest in a dark, quiet, air-conditioned room. Brachial artery FMD was measured during the examination. The right arm was cuffed with a standard blood pressure cuff positioned 5 cm below the antecubital fossa, and the artery was imaged 5 to 9 cm above the antecubital fossa. The cuff was inflated to 50 mmHg above the systolic blood pressure for five minutes. Imaging of the artery was performed before cuff inflation (baseline) and for 1.5 minutes after cuff deflation [5]. Normal FMD was considered to be in the range of 7.1-10% [13]. Microalbuminuria was diagnosed from concentrations in a spot sample using ACR (Albumin-to-Creatinine Ratio). Microalbuminuria is defined as concentrations ranging from 30 to 299 mg/g [14]. Diabetic neuropathy was screened using the monofilament test [15,16]. For the detection of diabetic retinopathy, participants underwent a fundus examination conducted by an ophthalmologist.

STATISTICAL ANALYSIS

The distribution of variables was initially analysed using SPSS version 27.0, and the data was found to have a non normal distribution based on the Kolmogorov-Smirnov test. Therefore, non parametric tests, specifically the Mann-Whitney U test, were used to make comparisons between two groups. To compare two quantitative variables, correlation coefficient was utilised.

RESULTS

The median age of the patients was 52 years (IQR 45-61), indicating that the majority of subjects belonged to the age group of 45 to 61 years. Of the enrolled subjects, 104 were males and 56 were females. The median BMI was 29.5 kg/m^2. The median HbA1c was 8.7%, while the median FBS and PPBS were 145 mg/dL and 242 mg/dL, respectively. The median ACR was 21.6 mg/g [Table/Fig-1]. The median systolic and diastolic blood pressure was 128 mmHg and 84.6 mmHg, respectively. Diabetic retinopathy and neuropathy were observed in 63 (39.37%) and 53 (33.12%) individuals, respectively. A total of 58 patients had albuminuria [Table/Fig-2].

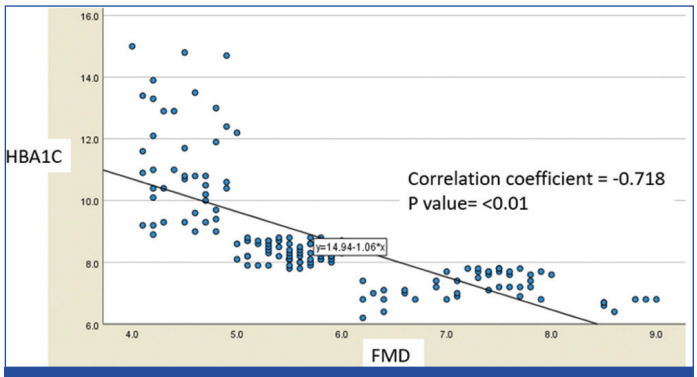
Parameters	Median	IQR	Mean	Range
BMI (kg/m^2)	29. 5	25.6- 31.8	28.5	20.3-34.2
HbA1c (%)	8.7	7.7- 9.0	8.7	6.2-15.0
FBS (mg/dL)	145	130-167	153	79-329
PPBS (mg/dL)	242	213-270	243	26-600
ACR (mg/g)	21.6	15.3-146.0	85.9	10.0-882.4

[Table/Fig-1]: Baseline characteristics.
BMI: Body mass index; HbA1c: Glycated haemoglobin; FBS: Fasting blood sugar; PPBS: Post prandial blood sugar; ACR: Albumin creatinine ratio

Parameters	n (%)
Hypertension	73 (45.62)
Diabetic retinopathy	63 (39.37)
Diabetic neuropathy	53 (33.12)
Macroalbuminuria	9 (5.62)
Microalbuminuria	49 (30.6)
Obesity (BMI >25 kg/m^2)	128 (80)

[Table/Fig-2]: Proportion of patients with diabetic complications and other comorbidities.

The median FMD among all patients was 5.6%. Females had a slightly higher FMD compared to males (5.8% vs 5.5%), but this difference was not statistically significant (p-value=0.537). There was an inverse correlation between HbA1c and FMD values, with a correlation coefficient of -0.718 and a p-value of <0.01, indicating a strong and significant inverse correlation [Table/Fig-3].



[Table/Fig-3]: Correlation plot of Flow-Mediated Dilation (FMD) with HbA1c.

The median FMD was lower in patients with diabetic neuropathy compared to those without neuropathy, and this difference was

statistically significant. In this study, the median FMD in patients with diabetic retinopathy was 5.1% compared to those without diabetic retinopathy, who had a median FMD of 6.0%. Patients with significant macroalbuminuria, as defined by the above ACR cut-off, had a lower median FMD compared to non albuminuric patients, and this difference was statistically significant [Table/Fig-4].

Microvascular complication	Median FMD (%) (IQR)	p-value
Diabetic retinopathy present (n=63)	5.1 (4.6-5.5)	<0.001
Diabetic retinopathy absent (n=97)	6.0 (5.5-7.4	
Diabetic neuropathy present (n=53)	5.0 (4.5-5.6)	<0.001
Diabetic neuropathy absent (n=107)	5.8 (5.4-7.3)	
Non albuminuric (n=102)	6.0 (5.5-7.4)	<0.001
Microalbuminuria (n=49)	5.1 (4.7-5.5)	
Macroalbuminuria (n=9)	4.5 (4.2-4.9)	

[Table/Fig-4]: Microvascular complication and Flow-Mediated Dilation (FMD). (Kruskal-Wallis test and Mann-Whitney U test)

DISCUSSION

Global literature has provided valuable data on FMD and the correlation between microvascular and macrovascular complications in diabetes mellitus. Most of the data available is from foreign studies, with only a few studies conducted in the Indian population [10,11,17,18]. This cross-sectional study was conducted in Puducherry, India aimed to contribute sufficient data on the effect of impaired FMD on the development of chronic complications in patients with T2DM. In this study, the population had a median FMD of 5.6%, which was lower than the normal values reported in recent studies (7.1% to 9%) [13]. This suggests that endothelial function, as measured by FMD, was altered in patients with T2DM. The median FMD in males and females was 5.5% and 5.8%, respectively, indicating that FMD was less impaired in females compared to males in present study population, although this difference was not statistically significant (p-value=0.537). There was a significant inverse correlation between FMD and HbA1c. This indicates that endothelial function was affected by poor glycaemic control, resulting in impaired FMD in these individuals. Other studies have also concluded that poor glycaemic control is associated with impaired FMD [17,18]. This can be attributed to vascular endothelial injury caused by increased formation of advanced glycation end products, activation of protein C kinase, and increased flux through the polyol pathway due to hyperglycaemia. These factors lead to increased oxidative stress and reduced levels of NO and eNOS, ultimately resulting in endothelial dysfunction [19-21].

In the study population, 63 (39.37%) patients had diabetic retinopathy. It was found that patients with diabetic retinopathy had lower FMD values, and this difference was statistically significant (p-value <0.001). Chronic hyperglycaemia leads to the formation of advanced glycation end products and activation of protein C kinase, causing endothelial dysfunction in retinal vessels. This results in altered retinal vascular permeability, damage to pericytes, and ultimately, retinal ischaemia. Similar studies have also concluded that impaired FMD, indicating endothelial dysfunction, is associated with diabetic retinopathy in patients with poor glycaemic control [22,23]. Patients with macroalbuminuria and microalbuminuria had a median FMD values of 4.5% and 5.1%, respectively, indicating that FMD was lower in patients with macroalbuminuria compared to those with microalbuminuria. Yokoyama H et al., conducted a study in 158 diabetics in Japan to assess the relationship between FMD and microalbuminuria, independent of cardiovascular risk factors. They demonstrated that impaired FMD was associated with increased age, albuminuria, BMI, and decreased eGFR. Similar findings were reported in studies conducted by Ito H et al., and Stehouwer CDA et al., [22,24,25]. These studies suggest that patients with albuminuria have endothelial dysfunction. Initially, there is a stage of hyperfiltration due to hyperglycaemia, followed by vascular endothelial injury caused by increased formation of advanced glycation end products, activation of protein C kinase, and increased flux through the polyol pathway due to hyperglycaemia, which ultimately leads to nephropathy [25,26].

Among the 160 patients, 53 (33.12%) had diabetic neuropathy. Individuals with diabetic neuropathy had a median FMD of 5.0%, which was significantly lower compared to patients without diabetic neuropathy, who had a median FMD of 5.8% (p-value <0.001). Additionally, a similar cross-sectional study conducted by Roustit M et al., found that endothelial dysfunction is strongly associated with Diabetic Peripheral Neuropathy (DPN) and may serve as a potential mediator between cardiovascular risk factors and DPN. FMD of the brachial artery, a measure of endothelial function, was significantly associated with DPN. These findings suggest that early endothelial dysfunction may contribute to the development of DPN in individuals with diabetes [27].

Limitation(s)

This study did not consider the duration of diabetes and the effect of dyslipidaemia on FMD, both of which are observed in patients with diabetes. Therefore, further studies should be conducted to investigate the impact of diabetes duration and dyslipidaemia on FMD. Additionally, it is important to note that this was a cross-sectional study, and further follow-up studies are needed to establish a correlation between improvements in FMD and better glycaemic control.

CONCLUSION(S)

Patients with T2DM exhibit endothelial dysfunction, as evidenced by impaired FMD. Endothelial dysfunction increases the risk of microvascular complications of diabetes, such as diabetic retinopathy, neuropathy, and albuminuria. Therefore, achieving good glycaemic control and implementing lifestyle changes are crucial in preventing these complications. Consequently, FMD holds prognostic significance and can be utilised to assess the response to interventions.

REFERENCES

[1] Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? Indian J Endocrinol Metab. 2016;20(4):546-51.
[2] Roden M, Shulman GI. The integrative biology of type 2 diabetes. Nature. 2019;576(7785):51-60.
[3] Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: Effects on angiogenesis, vascular remodeling, and wound healing. Int J Vasc Med. 2012;2012:e918267.
[4] Tran J, Magenau A, Rodriguez M, Rentero C, Royo T, Enrich C, et al. Activation of Endothelial Nitric Oxide (eNOS) occurs through different membrane domains in endothelial cells. PLOS ONE. 2016;11(3):e0151556.
[5] Yilmaz O, Afsar B, Ortiz A, Kanbay M. The role of endothelial glycocalyx in health and disease. Clinical Kidney Journal. 2019;12(5):611-19.
[6] 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(8828):1111-15.
[7] Thijssen DHJ, Bruno RM, van Mil ACCM, Holder SM, Faita F, Greyling A, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. Eur Heart J. 2019;40(30):2534-47.
[8] Goff DC, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, et al. 2013 ACC/AHA Guideline on the assessment of cardiovascular risk. Circulation. 2014;129(25 Suppl 2):S49-73.
[9] Crasto W, Patel V, Davies MJ, Khunti K. Prevention of microvascular complications of diabetes. Endocrinol Metab Clin North Am. 2021;50(3):431-55.
[10] Ravikumar R, Deepa R, Shanthirani C, Mohan V. Comparison of carotid intima-media thickness, arterial stiffness, and brachial artery flow mediated dilatation in diabetic and nondiabetic subjects (The Chennai Urban Population Study [CUPS-9]). Am J Cardiol. 2002;90(7):702-07.
[11] Bhargava K, Hansa G, Bansal M, Tandon S, Kasliwal RR. Endothelium-dependent brachial artery flow mediated vasodilatation in patients with diabetes mellitus with and without coronary artery disease. J Assoc Physicians India. 2003;51:355-58.
[12] Anusuya GS, Ravi R, Gopalakrishnan S, Abiselvi A, Stephen T. Prevalence of undiagnosed and uncontrolled diabetes mellitus among adults in South Chennai. Int J Community Med Public Health. 2018;5(12):5200-04.
[13] Maruhashi T, Kajikawa M, Kishimoto S, Hashimoto H, Takaeko Y, Yamaji T, et al. Diagnostic criteria of flow-mediated vasodilation for normal endothelial function and nitroglycerin-induced vasodilation for normal vascular smooth muscle function of the brachial artery. J Am Heart Assoc. 2020;9(2):e013915.

[14] Guy M, Newall R, Borzomato J, Kalra PA, Price C. Diagnostic accuracy of the urinary albumin: Creatinine ratio determined by the CLINITEK Microalbumin and DCA 2000+ for the rule-out of albuminuria in chronic kidney disease. Clin Chim Acta. 2009;399(1-2):54-58.
[15] Feng Y, Schlösser FJ, Sumpio BE. The Semmes Weinstein monofilament examination as a screening tool for diabetic peripheral neuropathy. J Vasc Surg. 2009;50(3):675-82.e1.
[16] Zhang Q, Yi N, Liu S, Zheng H, Qiao X, Xiong Q, et al. Easier operation and similar power of 10 g monofilament test for screening diabetic peripheral neuropathy. J Int Med Res. 2018;46(8):3278-84.
[17] Chugh SN, Dabla S, Jain V, Chugh K, Sen J. Glycaemic Control (excellent vs poor/fair control) on endothelial function in uncontrolled type 2 diabetes mellitus. J Assoc Physicians India. 2010;58:478-80.
[18] Chen S, Shen Y, Liu YH, Dai Y, Wu ZM, Wang XQ, et al. Impact of glycaemic control on the association of endothelial dysfunction and coronary artery disease in patients with type 2 diabetes mellitus. Cardiovasc Diabetol. 2021;20(1):64.
[19] Odegaard AO, Jacobs DR, Sanchez OA, Goff DC, Reiner AP, Gross MD. Oxidative stress, inflammation, endothelial dysfunction and incidence of type 2 diabetes. Cardiovasc Diabetol. 2016;15(1):51.
[20] Dhananjayan R, Koundinya KSS, Malati T, Kutala VK. Endothelial dysfunction in type 2 diabetes mellitus. Indian J Clin Biochem. 2016;31(4):372-79.
[21] Maruhashi T, Higashi Y. Pathophysiological association between diabetes mellitus and endothelial dysfunction. Antioxidants (Basel). 2021;10(8):1306.
[22] Yokoyama H, Sone H, Saito K, Yamada D, Honjo J, Haneda M. Flow-mediated dilation is associated with microalbuminuria independent of cardiovascular risk factors in type 2 diabetes. J Atheroscler Thromb. 2011;18(9):744-52.
[23] Suetsugu M, Takebayashi K, Aso Y. Association between diabetic microangiopathy and vascular endothelial function evaluated by flow-mediated vasodilatation in patients with type 2 diabetes. Int J Clin Pract. 2007;61(6):920-26.
[24] Ito H, Nakashima M, Meguro K, Furukawa H, Yamashita H, Takaki A, et al. Flow mediated dilatation is reduced with the progressive stages of glomerular filtration rate and albuminuria in type 2 diabetic patients without coronary heart disease. J Diabetes Res. 2015;2015:e728127.
[25] Stehouwer CDA, Henry RMA, Dekker JM, Nijpels G, Heine RJ, Bouter LEXM. Microalbuminuria is associated with impaired brachial artery, flow-mediated vasodilation in elderly individuals without and with diabetes: Further evidence for a link between microalbuminuria and endothelial dysfunction—The Hoorn Study. Kidney Int Suppl. 2004;66(92):S42-44.
[26] Stehouwer CDA, Gall MA, Twisk JWR, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: Progressive, interrelated, and independently associated with risk of death. Diabetes. 2002;51(4):1157-65.
[27] Roustit M, Loader J, Deusenbery C, Baltzis D, Veves A. Endothelial dysfunction as a link between cardiovascular risk factors and peripheral neuropathy in diabetes. J Clin Endocrinol Metab. 2016;101(9):3401-08.

PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of General Medicine, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.
2. Associate Professor, Department of Cardiology, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.
3. Assistant Professor, Department of General Medicine, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.
4. Professor, Department of General Medicine, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Siva Ranganathan Green,
1C, Type-I Staff Quarters, MGMCRI Campus,
Pillaiyarkuppam, Puducherry-607402, India.
E-mail: srgreen@mgmcri.ac.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 15, 2022
- Manual Googling: Apr 19, 2023
- iThenticate Software: Nov 17, 2023 (8%)

ETYMOLOGY: Author Origin

EMENDATIONS: 9

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Nov 13, 2022**
Date of Peer Review: **Mar 02, 2023**
Date of Acceptance: **Nov 20, 2023**
Date of Publishing: **Jan 01, 2024**