RESEARCH ARTICLE

DOI: 10.4274/ijca.2024.85856
Int J Cardiovasc Acad 2024;10(3):62-69

Assessment of Endothelial Dysfunction in T2DM: A Doppler Ultrasound Study Correlated with CRP Levels, Glycemic Control, and BMI

Surya Prakash Sabapathi[1], Karthikeyan Selvaraj[2], Amirtha Ganesh Balasubramaniyan[3]

[1]Department of Cardiology, Panimalar Medical College Hospital and Research Institute, Chennai, India
[2]Department of Cardiology, Mahatma Gandhi Medical College and research Institute, Puducherry, India
[3]Department of Cardiology, All India Institute of Medical Sciences, Mangalagiri, India

Abstract

Background and Aim: Endothelial dysfunction is a crucial precursor to atherosclerosis and cardiovascular complications, particularly prevalent in individuals with type 2 diabetes mellitus (T2DM). This study aimed to assess endothelial impairment in T2DM using flow-mediated dilatation (FMD) and to determine its correlation with body mass index (BMI), duration of diabetes, C-reactive protein (CRP) levels, and glycemic control.

Materials and Methods: A total of 100 T2DM patients aged thirty to sixty participated. Doppler ultrasonography was used to measure brachial artery FMD, while blood samples were used to assess glycosylated hemoglobin A1c (HbA1c) and CRP levels. Correlations were evaluated using the Pearson correlation coefficient.

Result: The duration of diabetes r value is negative 0.866, p-value less than 0.001, CRP levels as "r value" is negative 0.724, "*P*-value" less than 0.001, and HbA1c levels "r value" is negative 0.722, "*P*-value" less than 0.001 were observed to have negative relationships with FMD. Additionally, there was a significant association r value was negative 0.342, "*P*-value" less than 0.001 between BMI and FMD. These results were corroborated by subgroup analyses, which highlighted the intricacy of "endothelial dysfunction" in T2DM and the significance of comprehensively addressing several risk variables. This study elucidates the intricate interplay of metabolic, inflammatory, and vascular factors contributing to "endothelial dysfunction" in T2DM patients. Elevated HbA1c and CRP levels, prolonged diabetes duration, and high BMI were linked to impaired endothelial function, underscoring the importance of holistic risk factor management.

Conclusion: For patients with T2DM, maintaining endothelial function and reducing cardiovascular risk require comprehensive treatment plans that target inflammation, obesity, and glycaemic management. Timely intervention and vigilant monitoring of risk factors are crucial to prevent vascular complications in high-risk populations.

Keywords: Endothelial dysfunction, cardiovascular risk, glucose regulation, flow-mediated dilatation, T2DM, CRP

To cite this article: Sabapathi SP, Selvaraj K, Balasubramaniyan AG. Assessment of Endothelial Dysfunction in T2DM: A Doppler Ultrasound Study Correlated with CRP Levels, Glycemic Control, and BMI. Int J Cardiovasc Acad. 2024;10(3):62-69

Address for Correspondence: Surya Prakash Sabapathi, Department of Cardiology, Panimalar Medical College Hospital and Research Institute, Chennai, India
E-mail: dr.suriya01@gmail.com
ORCID ID: orcid.org/0000-0002-0095-0853

Received: 18.06.2024
Revised: 29.08.2024
Accepted: 03.09.2024
Published Online: 18.09.2024

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a widespread health issue characterized by insulin resistance, elevated blood sugar levels, and increased susceptibility to cardiovascular issues.[1] "Endothelial dysfunction" is a notable risk factor for atherosclerosis and cardiovascular disease among the various complications associated with T2DM. The equilibrium between vasodilation and vasoconstriction is crucial, and when "endothelial dysfunction" occurs, characterized by reduced vascular endothelial cell function, it disrupts this balance, leading to vascular damage and organ dysfunction.[2] Understanding the mechanisms and implications of "endothelial dysfunction" in T2DM is imperative for devising effective preventive and therapeutic strategies to mitigate cardiovascular risk in this population.

One layer of endothelial cells that lines the inside of blood arteries is called the vascular endothelium, and it is essential for preserving vascular homeostasis. Through the production of numerous vasoactive chemicals, including as nitric oxide (NO), prostacyclin, and endothelin-1, endothelial cells control vascular tone, inflammation, thrombosis, and angiogenesis. NO exhibits the most potent vasodilatory properties by enhancing leukocyte adherence and platelet aggregation while simultaneously relaxing vascular smooth muscle cells.[3] "Endothelial dysfunction" in the context of T2DM leads to a reduction in the availability of NO and an increase in the synthesis of endothelium-derived molecules that promote vasoconstriction.[4]

One of the main characteristics of T2DM is elevated blood glucose, which is important in the development of "endothelial dysfunction". Extended exposure to elevated glucose concentrations triggers a sequence of metabolic and biochemical alterations in endothelial cells, culminating in compromised endothelial performance.[5] High blood sugar causes oxidative stress, inflammation, and advanced glycation end products , all of which worsen vascular damage and impede endothelial cell function.[6] Consequently, individuals with T2DM exhibit increased susceptibility to "endothelial dysfunction", predisposing them to accelerated atherosclerosis and cardiovascular events.

The assessment of endothelial function is a valuable tool for evaluating cardiovascular risk and disease progression in T2DM.[7] To evaluate endothelial function, Doppler ultrasonography is commonly used to quantify flow-mediated dilation (FMD) in the brachial artery. This non-invasive technique determines the artery's ability to expand in response to heightened blood flow, primarily induced by the endothelial cells' release of NO.[8,9] Impaired FMD reflects "endothelial dysfunction" and predicts adverse cardiovascular outcomes in T2DM.

Circulating biomarkers provide insights into the underlying causes of "endothelial dysfunction" in diabetes mellitus second type, complementing the information obtained from FMD. C-reactive protein (CRP), an acute-phase reactant and indicator of systemic inflammation, correlates with endothelial activation and dysfunction.[10] Elevated CRP levels in patients with T2DM indicate increased cardiovascular risk and reflect the inflammatory milieu conducive to "endothelial dysfunction".[11] Thus, correlating CRP levels with FMD offers a comprehensive assessment of the inflammatory component of "endothelial dysfunction" in T2DM.

Moreover, glycemic control, as reflected by glycosylated hemoglobin A1c (HbA1c) levels, plays a pivotal role in modulating endothelial function in T2DM. Prolonged hyperglycemia exacerbates endothelial damage, impairs NO bioavailability and promoting atherosclerosis. Consequently, optimizing glycemic control represents a cornerstone in the management of T2DM to mitigate "endothelial dysfunction" and reduce cardiovascular risk.

Considering these factors, the purpose of this study was to evaluate "endothelial dysfunction" using Doppler ultrasonography for FMD of the brachial artery in patients with T2DM. Evaluation and comparison of the degree of "endothelial dysfunction" will be conducted with systemic inflammation, as indicated by CRP levels, and glycemic management, as indicated by HbA1c levels. Furthermore, the relationships between diabetes length, body mass index (BMI), and the degree of "endothelial dysfunction" will be investigated in this study. In order to improve understanding of cardiovascular pathogenesis and direct targeted therapeutic approaches to reduce morbidity and mortality in this at-risk population, this study aimed to shed light on the intricate interactions between inflammatory, vascular, and metabolic factors that contribute to "endothelial dysfunction" in second type diabetes.

MATERIALS AND METHODS

Study Design

To gain insight into "endothelial dysfunction" among individuals with second type diabetes mellitus, this study adopted an observational methodology. Conducted jointly by the Institute of "Internal Medicine and the Institute of Diabetology", affiliated with Madras Medical College and Rajiv Gandhi Government General Hospital in Chennai, the research spans a duration of six months.

Sample Size and Selection Criteria

A total of 100 patients who met specific inclusion and exclusion criteria were enrolled in the study. The inclusion criteria were individuals aged between 30 and 60 years with a confirmed

diagnosis of T2DM. The exclusion criteria include individuals aged over 60 years, those with type 1 diabetes mellitus, pregnant women, critically ill patients, current smokers, and individuals with systemic hypertension or dyslipidemia under treatment.

Data Collection

Before being included in the study, participants must submit a comprehensive history taking, clinical assessment, and informed consent. Serum CRP and HbA1c levels, which are biomarkers of systemic inflammation and glycemic control, are assessed using blood samples.

Assessment of Endothelial Function

The procedure involves placing a pneumatic cuff on the forearm distal to the image site and inflating it to a suprasystolic pressure for 5 min. Upon deflation, shear stress causes the release of vasodilators like NO, which diffuses into the vascular smooth muscles, causing relaxation and vasodilatation. The change in brachial artery diameter from baseline to its maximum increase was used to calculate FMD, expressed as a percentage. However, for accurate data comparison between centers, standardization of this method is crucial.

There are five critical elements in FMD methodology that require standardization. The position of the probe is essential, with the cuff placed distal to it. The shear stress induced by cuff occlusion should last for 5 min to optimize reactive hyperemia. High-quality stereotactic images should be captured using a stereotactic apparatus. Environmental factors such as room temperature, time of day, and consumption of fatty foods or caffeine must be controlled. Additionally, reactive hyperemic stimuli, including cuff position, shear stress duration, and ischemia, should be standardized. Physiological variables like arterial stiffness, flow pattern, and blood viscosity should also be considered for consistency across studies (Figure 1).

Statistical Analysis

The demographic details and laboratory results of the participants were compiled using descriptive statistics. The association between FMD, CRP levels, HbA1c levels, illness duration, and BMI was evaluated using Pearson correlation analysis. Multivariate regression analysis can be used to account for possible confounders. "$P < 0.05$ is the threshold for statistical significance". The Institutional Ethics Committee of Madras Medical Collage approved the study protocol (study no: 07032016, date: 22.03.2016). Every participant provided informed consent, and study-wide participant data confidentiality was maintained.

RESULTS

This study assessed endothelial dysfunction in patients with T2DM using FMD and analyzed its correlation with various parameters, such as CRP levels, HbA1c, and BMI. The results of the present study provide significant insights into the relationship between endothelial function and these parameters.

The analysis of FMD in relation to age groups in Table 1 and Graph 1. demonstrated a clear pattern: younger individuals (31-40 years) exhibited a higher prevalence of FMD greater

Table 1: Distribution of age, HBA1c, CRP, BMI, and duration of DM based on FMD

Age wise distribution of flow-mediated diatation				
FMD group	**31-40 years**	**41-50 years**	**51-60 years**	**Total**
<5	0	10	32	42
	0.00%	31.20%	80.00%	42.00%
>5	28	22	8	58
	100.00%	68.80%	20.00%	58.00%
Total	28	32	40	100
	100.00%	100.00%	100.00%	100.00%
FMD with HBA1c				
HBA1c_group	**>5**	**<5**	**Total**	
<6.5	16	0	16	
	100.00%	0.00%	16.00%	
>6.5	42	42	84	
	50.00%	50.00%	84.00%	
Total	58	42	100	
	58.00%	42.00%	100.00%	
FMD with CRP levels				
FMD_group	**CRP group <5**	**CRP group >5**	**Total**	
>5	32	26	58	
	100.00%	38.20%	58.00%	
<5	0	42	42	
	0.00%	61.80%	42.00%	
Total	32	68	100	
	100.00%	100.00%	100.00%	
FMD with BMI				
FMD group	**<19**	**19-25**	**Above 25**	**Total**
>5	4	34	20	58
	100.00%	70.80%	41.70%	58.00%
<5	0	14	28	42
	0.00%	29.20%	58.30%	42.00%
Total	4	48	48	100
	100.00%	100.00%	100.00%	100.00%

FMD: Flow-mediated dilatation, BMI: Body mass index, HbA1c: Hemoglobin A1c, CRP: C-reactive protein, DM: Diabetes mellitus

than 5, with 100% of them falling into this category. In contrast, the prevalence of FMD >5 decreased notably with age, as evidenced by only 31.2% in the 41-50 years group and 20% in the 51-60 years group. This decline suggests that endothelial function deteriorates with advancing age, as indicated by the lower percentage of individuals with better FMD in the older age brackets.

In Table 2 and Graph 2, a strong correlation was observed between FMD and HbA1c levels. Patients with HbA1c levels >6.5% had a 50% prevalence of FMD >5, whereas none of the patients with HbA1c levels 6.5% exhibited FMD >5. This finding underscores the impact of poor glycemic control on endothelial function, indicating that higher HbA1c levels are associated with impaired endothelial performance.

The correlation between FMD and CRP levels also revealed significant findings. Patients with CRP levels exceeding 5 mg/L had an FMD >5 in only 38.2% of cases, whereas those with CRP levels below 5 mg/L had an FMD >5 in all cases. This suggests that elevated CRP levels, which are indicative of increased inflammation, are associated with reduced endothelial function.

BMI's relationship with FMD showed that a higher BMI was linked to poorer endothelial function. Specifically, individuals with a BMI >25 had only a 41.7% prevalence of FMD >5 compared with 70.8% in those with a BMI between 19-25 and 100% in those with a BMI 19. These data highlight the detrimental effect of high BMI on endothelial health.

Correlation analysis across the entire study cohort revealed strong negative correlations between FMD and HbA1c ($r = -0.724$, $P < 0.001$) and CRP ($r = -0.866$, $P < 0.001$), and a moderate negative correlation with BMI ($r = -0.342$, $P < 0.001$). These results indicate that worsening glycemic control, inflammation, and BMI are associated with impaired endothelial function.

Further analysis of the subgroups in Table 3 and Graph C, Table 4 and Graph D provides additional insights. In Group I, which consisted of patients with T2DM without other comorbidities, FMD showed very strong negative correlations with CRP levels ($r = -0.803$, $P < 0.001$) and duration of diabetes ($r = -0.802$, $P < 0.001$). However, HbA1c had a less pronounced correlation ($r = -0.397$, $P < 0.004$). Conversely, in Group II, which included patients with T2DM and other comorbidities, CRP levels ($r = -0.850$, $P < 0.001$) and duration of diabetes ($r = -0.767$, $P < 0.001$) showed strong negative correlations with FMD, while HbA1c had a similar strong correlation ($r = -0.759$, $P < 0.001$). BMI also demonstrated a moderate correlation ($r = -0.343$, $P = 0.015$) in this group.

In conclusion, the present study highlights a significant association between endothelial dysfunction and poor glycemic control, elevated inflammation, and high BMI in patients with T2DM. These findings suggest that addressing these factors could potentially improve endothelial function and overall vascular health in this population.

DISCUSSION

The results of this study provide valuable insights into the relationship between endothelial dysfunction and various factors in T2DM patients, with a focus on FMD and its correlation with HbA1c, CRP levels, and BMI.

Our findings indicate a notable decline in endothelial function with advancing age. Younger individuals (31-40 years) showed a significantly higher prevalence of FMD greater than 5 compared to older age groups. This decline is consistent with the existing literature on age-related endothelial dysfunction, possibly due to increased oxidative stress and reduced NO bioavailability. The deterioration of endothelial function in older adults is well documented and likely to be intensified in the context of T2DM.

Table 2: Correlation analysis of FMD and related variables

Variable	Pearson correlation	*P*-value	n
FMD	-0.722	<0.001	100
HBA1c	-0.724	<0.001	100
CRP	-0.866	<0.001	100
BMI	-0.342	<0.001	100

FMD: Flow-mediated dilatation, BMI: Body mass index, HbA1c: Hemoglobin A1c, CRP: C-reactive protein

Table 3: Correlation analysis of FMD and related variables in group I type 2 diabetes mellitus patients without other comorbidities

Variable	Pearson correlation	*P*-value	n
FMD	-0.927	<0.001	50
CRP	-0.803	<0.001	50
Duration	-0.802	<0.001	50
HbA1c	-0.397	<0.004	50

FMD: Flow-mediated dilatation, BMI: Body mass index, HbA1c: Hemoglobin A1c, CRP: C-reactive protein, DM: Diabetes mellitus

Table 4: Correlation analysis of FMD and related variables among patients with group II type 2 diabetes mellitus and other comorbidities

Variable	Pearson correlation	*P*-value	n
CRP	-0.850	<0.001	50
Duration	-0.767	<0.001	50
HbA1C	-0.759	<0.001	50
BMI	-0.343	0.015	50

FMD: Flow-mediated dilatation, BMI: Body mass index, HbA1c: Hemoglobin A1c, CRP: C-reactive protein, DM: Diabetes mellitus

The strong negative correlation between HbA1c levels and FMD underscores the impact of poor glycemic control on endothelial health. Patients with higher HbA1c levels, which are indicative of chronic hyperglycemia, showed markedly reduced endothelial function. This finding is consistent with previous studies that established a link between high HbA1c and endothelial dysfunction in patients with diabetes. Chronic elevated blood glucose levels contribute to endothelial damage through several mechanisms, including the formation of advanced glycation end products and increased oxidative stress, which impair endothelial NO production and function.

Similarly, the significant negative correlation between CRP levels and FMD highlights the role of inflammation in endothelial dysfunction. Elevated CRP levels, a marker of systemic inflammation, were associated with reduced FMD. This finding corroborates the hypothesis that inflammatory processes contribute to endothelial impairment in diabetes. CRP-induced endothelial dysfunction is thought to result from inflammatory cytokines that adversely affect endothelial cell function and promote atherogenesis.

The relationship between BMI and FMD suggests that obesity further exacerbates endothelial dysfunction. Higher BMI values were associated with poorer endothelial function, consistent with existing research that links obesity to endothelial impairment. Excess adiposity contributes to endothelial dysfunction through various mechanisms, including increased production of inflammatory cytokines, elevated oxidative

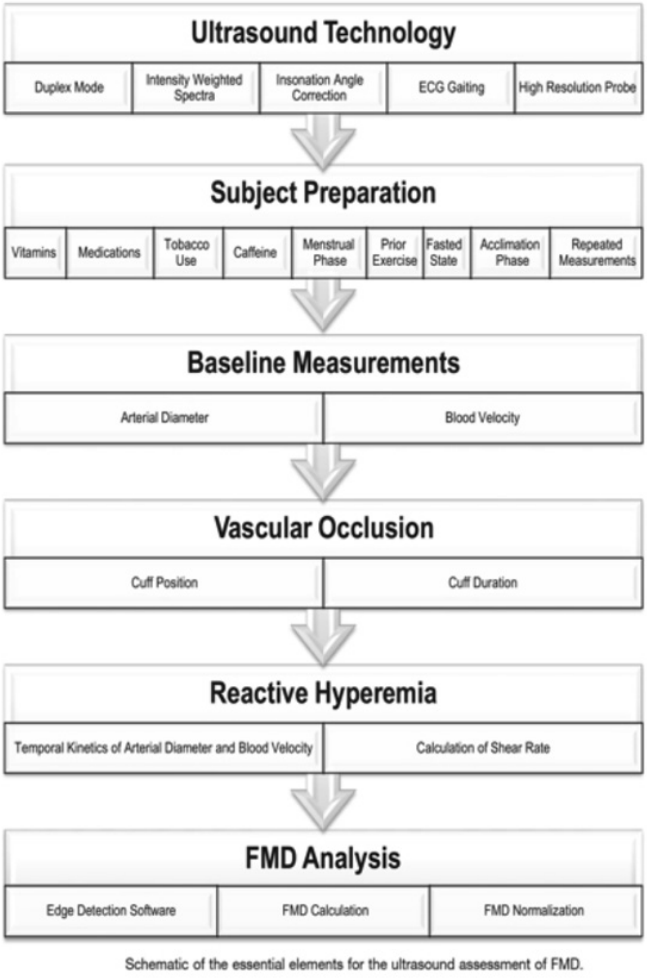
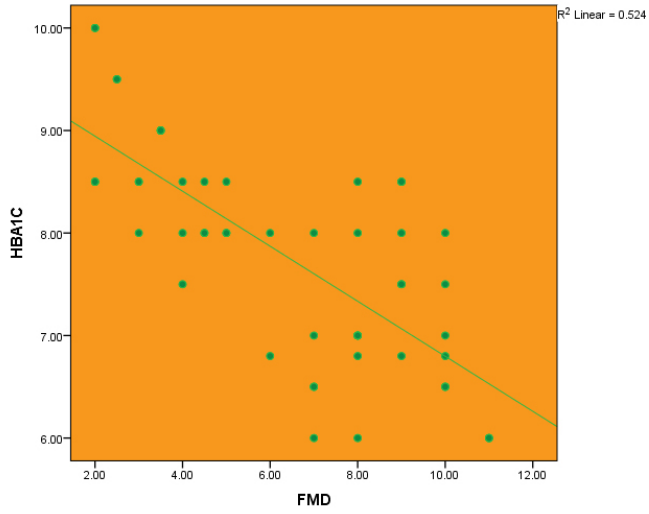


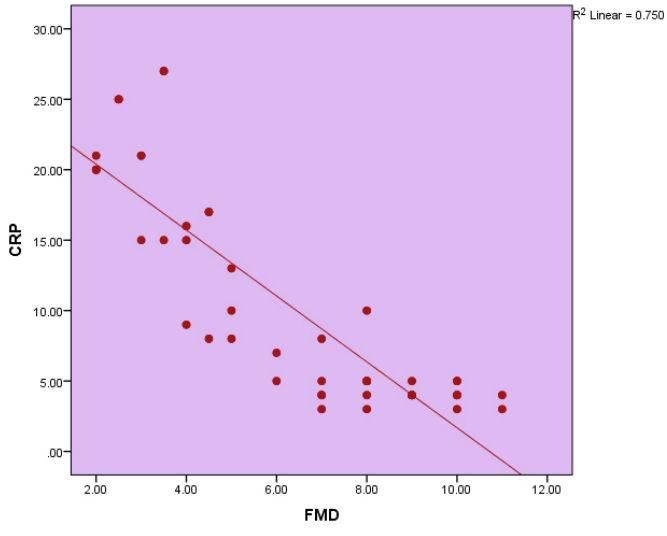
Figure 1: Essential elements for USG assessment of FMD

USG: Ultrasonography, FMD: Flow-mediated dilatation



Graph 1: The following diagram shows the correlation between FMD and HbA1c in 100 subjects

FMD: Flow-mediated dilatation, HbA1c: Hemoglobin A1c

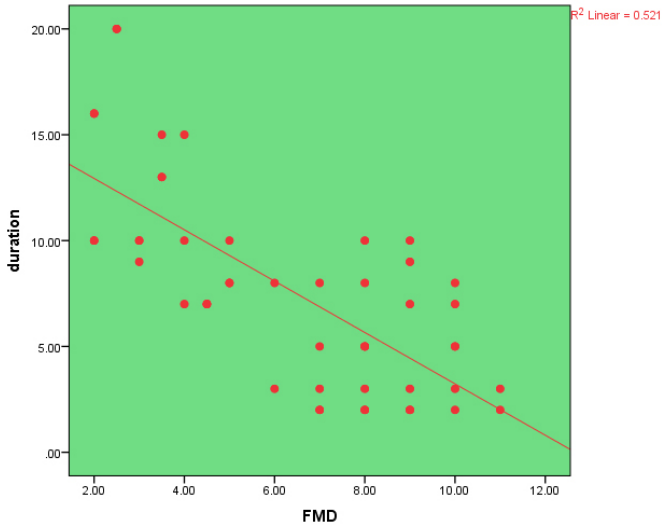


Graph 2: The following diagram shows the correlation between FMD and CRP in 100 subjects

FMD: Flow-mediated dilatation, CRP: C-reactive protein

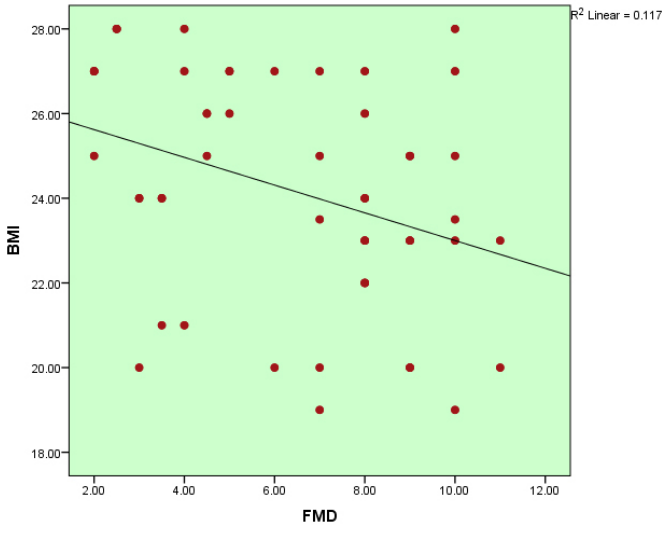
stress, and altered metabolic pathways. The findings indicate that managing BMI could be a crucial factor in improving endothelial function in patients with T2DM.

Subgroup analyses reveal additional nuances. In patients with T2DM without other comorbidities (Group I), FMD showed a strong negative correlation with CRP levels and diabetes duration. This suggests that systemic inflammation and longer duration of diabetes can significantly affect endothelial function. In contrast, patients with T2DM and other comorbidities (Group II) showed strong correlations between FMD and both CRP levels and HbA1c, with BMI also showing a moderate correlation. The presence of additional comorbidities can compound the effects of poor glycemic control and inflammation on endothelial function.



Graph 3: The following diagram shows the correlation between FMD and the duration of diabetes in the 100 subjects included in the study

FMD: Flow-mediated dilatation



Graph 4: The following diagram shows the correlation between FMD and BMI in 100 subjects

FMD: Flow-mediated dilatation, BMI: Body mass index

Overall, our study highlights the multifaceted nature of endothelial dysfunction in T2DM. The interplay between glycemic control, inflammation, and obesity underscores the need for comprehensive management strategies targeting these factors. Effective control of blood glucose levels, systemic inflammation reduction, and weight management are crucial for improving endothelial function and reducing cardiovascular risk in patients with T2DM. Future research should explore interventions targeting these factors to further elucidate their impact on endothelial health and overall cardiovascular outcomes in patients with diabetes.

The study "Association of Endocan, ischemia-modified albumin (IMA), and high-sensitivity CRP (hsCRP) Levels With Endothelial Dysfunction" in T2DM demonstrates the strong relationship that exists between "endothelial dysfunction" in T2DM and biomarkers, such as endocan, IMA, and hsCRP. T2DM patients with "endothelial dysfunction" showed higher levels of endocan, hsCRP, and IMA than those without, suggesting that these variables may operate as separate risk factors for vascular impairment in this group.[12]

The research titled "Association of Endocan, IMA, and hsCRP Levels With Endothelial Dysfunction" in T2DM demonstrated a strong correlation between biomarkers, such as endocan, IMA, and hsCRP, and "endothelial dysfunction" in individuals with T2DM. Elevated levels of endocan, hsCRP, and IMA were observed in T2DM patients with "endothelial dysfunction" compared with those without, suggesting that these biomarkers may serve as independent indicators of vascular impairment in this population.[13]

In contrast, the study "endothelial dysfunction" and platelet hyperactivity in T2DM; molecular insights and therapeutic strategies highlights the role of inflammation, insulin resistance, and hyperglycemia in promoting atherosclerotic vascular complications and offers molecular insights into the pathogenesis of these conditions in T2DM.[14]

Moreover, the article "Resistin levels and inflammatory and endothelial dysfunction" markers in obese postmenopausal women with T2DM clarifies the relationships between resistin levels and "endothelial dysfunction" and inflammatory markers in obese postmenopausal women with T2DM. A greater risk of coronary heart disease was shown to be independently

correlated with elevated resistin levels, highlighting resistin's potential as a biomarker for cardiovascular risk assessment in this group.[15]

Additionally, there is a correlation between resistin levels and markers of inflammation and endothelial dysfunction in obese postmenopausal women with T2DM. This was clarified in the paper "Resistin levels and inflammatory and endothelial dysfunction" in obese postmenopausal women with T2DM. Elevated resistin levels were independently linked with an increased risk of coronary heart disease, suggesting the potential of resistin as a biomarker for cardiovascular risk assessment in this population.[16]

Adiponectin, CRP, and endothelial function were evaluated in "Type II diabetic and non-diabetic individuals" after "acute myocardial infarction (AMI)" in the research "Persistent endothelial dysfunction" is related to elevated CRP levels in patients with type II diabetes after AMI. Patients with T2DM were shown to have persistent endothelium-dependent dysfunction and inflammatory activity, which may have an impact on their lower risk of coronary artery disease.[17]

In the article "T2DM mellitus worsens arterial stiffness in hypertensive patients through endothelial dysfunction", researchers looked at the relationship between arterial stiffness and endothelial function in hypertensive patients with and without T2DM. Results showed that T2DM, especially in hypertensive individuals with diabetes mellitus, increased arterial stiffness by exacerbating "endothelial dysfunction", as seen by higher pulse wave velocity and reduced FMD.[18]

All the factors considered advance our comprehension of the multifactorial nature of "endothelial dysfunction" in T2DM and underscore the importance of identifying biomarkers for risk assessment and therapeutic targeting in this population.

Study Limitations

As a cross-sectional study, data are captured at a single point in time, which prevents the establishment of causal relationships between endothelial dysfunction and the various metabolic, inflammatory, and anthropometric factors examined.

There may be unmeasured confounding variables that influence endothelial function in T2DM, such as lifestyle factors (diet, physical activity), medication adherence, and other comorbid conditions, which were not accounted for in the analysis.

Doppler ultrasonography is a widely accepted non-invasive method for assessing endothelial function, but it may be subject to operator variability and technical limitations.

CONCLUSION

This study sheds light on the intricate connection between "endothelial dysfunction" and various vascular, inflammatory, and metabolic factors in individuals diagnosed with T2DM. The observation of noteworthy associations between FMD and variables such as glycemic management, CRP levels, diabetes duration, and BMI underscores the complex characteristics of diabetic vasculopathy. Optimizing glycemic control, attenuating inflammation, addressing obesity, and promoting cardiovascular health are critical strategies to mitigate cardiovascular risk in individuals with T2DM. These findings emphasize the importance of comprehensive risk factor management for preventing vascular complications and underscore the need for targeted interventions aimed at preserving endothelial function and improving vascular outcomes in high-risk populations.

Acknowledgment

We extend our appreciation to all healthcare professionals involved in diagnosing and managing patients included in this study.

REFERENCES

1. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, *et al*. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020;21:6275.
2. Sandoo A, van Zanten JJ, Metsios GS, Carroll D, Kitas GD. The endothelium and its role in regulating vascular tone. Open Cardiovasc Med J. 2010;4:302-12.
3. Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, *et al*. The vascular endothelium and human diseases. Int J Biol Sci. 2013;9:1057-69.
4. Dhananjayan R, Koundinya KS, Malati T, Kutala VK. Endothelial Dysfunction in Type 2 Diabetes Mellitus. Indian J Clin Biochem. 2016;31:372-9.
5. Funk SD, Yurdagul A Jr, Orr AW. Hyperglycemia and endothelial dysfunction in atherosclerosis: lessons from type 1 diabetes. Int J Vasc Med. 2012;2012:569654.
6. Yamagishi SI, Matsui T. Role of Hyperglycemia-Induced Advanced Glycation End Product (AGE) Accumulation in Atherosclerosis. Ann Vasc Dis. 2018;11:253-8.
7. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, *et al*. The assessment of endothelial function: from research into clinical practice. Circulation. 2012;126:753-67.
8. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, *et al*. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol. 2011;300:2-12.
9. Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound assessment of flow-mediated dilation. Hypertension. 2010;55:1075-85.
10. Stanimirovic J, Radovanovic J, Banjac K, Obradovic M, Essack M, Zafirovic S, *et al*. Role of C-Reactive Protein in Diabetic Inflammation. Mediators Inflamm. 2022;2022:3706508.
11. Mugabo Y, Li L, Renier G. The connection between C-reactive protein (CRP) and diabetic vasculopathy. Focus on preclinical findings. Curr Diabetes Rev. 2010;6:27-34.

12. Balamir I, Ates I, Topcuoglu C, Turhan T. Association of Endocan, Ischemia-Modified Albumin, and hsCRP Levels With Endothelial Dysfunction in Type 2 Diabetes Mellitus. Angiology. 2018;69:609-16.

13. Ahmed TM, Nassar M, Mohamed HAA, Elhadidy KE, Farhan HM, El Basset ASA, *et al*. Evaluation of serum levels of Irisin as a marker of endothelial dysfunction in patients with type 2 diabetes mellitus. Endocrinol Diabetes Metab. 2023;6:403.

14. Kaur R, Kaur M, Singh J. Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. Cardiovasc Diabetol. 2018;17:121.

15. Giandalia A, Alibrandi A, Giorgianni L, Lo Piano F, Consolo F, Longo Elia G, *et al*. Resistin levels and inflammatory and endothelial dysfunction markers in obese postmenopausal women with type 2 diabetes mellitus. Diabetol Metab Syndr. 2021;13:98.

16. Hayashi M, Morioka T, Hatamori M, Kakutani Y, Yamazaki Y, Kurajoh M, *et al*. Plasma omentin levels are associated with vascular endothelial function in patients with type 2 diabetes at elevated cardiovascular risk. Diabetes Res Clin Pract. 2019;148:160-8.

17. Nyström T, Nygren A, Sjöholm A. Persistent endothelial dysfunction is related to elevated C-reactive protein (CRP) levels in Type II diabetic patients after acute myocardial infarction. Clin Sci (Lond). 2005;108:121-8.

18. Bruno RM, Penno G, Daniele G, Pucci L, Lucchesi D, Stea F, *et al*. Type 2 diabetes mellitus worsens arterial stiffness in hypertensive patients through endothelial dysfunction. Diabetologia. 2012;55:1847-55.