Pathology Section

Incidence of Microalbuminuria and its Correlation with Ankle Brachial Index, Sugar Levels and Duration of Type 2 Diabetes Mellitus with Special Emphasis to Predict Vascular Complications

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is a metabolic disorder characterised by persistence hyperglycaemia resulting from the defects in insulin secretion, action or both. Diabetes prevalence is rapidly increasing, especially in urban India. The changing lifestyle has majorly contributed to the increasing prevalence of diabetes.

Aim: To determine the incidence of microalbuminuria and its correlation with age, sex, duration, Fasting Blood Sugar (FBS), Postprandial Blood Sugar (PPBS) and Body Mass Index (BMI). Also to detect the presence of vascular complications using Ankle Brachial Index (ABI), Pulse Wave Velocity (PWV), Augmentation Index (AI) and microalbuminuria.

Materials and Methods: The present cross-sectional study was conducted in Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka, India, from March to June 2018. The study included assessment of blood glucose level both FBS and PPBS, microalbumin levels in 100 known diabetic patients and also ABI, PWV and AI were measured using periscope instrument. Analytical test to measure the differences between two means of the two groups independent t-test was applied and multiple regressoon analysis was done.

Results: Out of total 100 subjects, 59% were males and 41% were females. The correlation of microalbuminuria with factors like, duration of diabetes, FBS, PPBS, PWV and AI was statistically significant with a p-value of <0.05 in this study. The correlation of duration of diabetes with factors like age, BMI, microalbuminuria was statistically significant with a p-value of <0.05.

Conclusion: This study confirmed that incidence of microalbuminuria increases with duration of diabetes, poorly controlled blood sugar levels. Microalbuminuria, ABI, PWV and AI are the reliable, non invasive and simple methods used for the screening of vascular complications.

Keywords: Arterial stiffness, Microalbumin, Poor glycaemic control, Screening method

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterised by persistence hyperglycaemia resulting from the defects in insulin secretion, action or both [1]. According to World Health Organisation (WHO), the global incidence of diabetes was 422 million in 2014. Diabetes affects more than 62 million Indians leading to one million deaths every year [2]. Diabetes prevalence is rapidly increasing, especially in urban India [3]. The major risk factors of DM are sedentary lifestyle, increased animal fat consumption, increased cholesterol levels, obesity, high blood pressure and smoking [4].

Increased glucose levels in diabetes may also be due to genetic predisposition which causes decreased insulin secretion. Insulin resistance causes compensatory B-cell hyperplasia to maintain normoglycaemia [5]. B-cell failure causes impaired glucose tolerance which progresses to diabetes mellitus.

Diabetic patients with uncontrolled glycaemic levels, longer duration of the diabetes, hypertension, high blood lipids, smoking, high BMI can develop various complications. The microvascular complications are retinopathy, nephropathy, and neuropathy. The macrovascular complications are cerebrovascular disease, Ischemic heart disease and peripheral arterial disease [4]. Diabetes reduces life expectancy by 5-10 years. The long term complications of diabetics are often associated with uncontrolled blood sugar level [5].

A strong association has been reported between microalbuminuria and cardiovascular outcomes in patients with type 2 DM [6]. Microalbuminuria is an early marker of nephropathy in diabetics. Microalbuminuria is defined as levels of albumin ranging from 30-300 mg in a 24 hour urine collection and 20-200 mg/L in a spot sample. The amount of urinary albumin excretion increases along the continuum from microalbuminuria to albuminuria and proteinuria, increasing the risk of cardiovascular events [7].

Detection of the complications early may prevent or delay the emergence of end stage disease such as blindness, the need for renal replacement therapy, or amputation. The methods which are currently used for the detection of nephropathy are urine albumin level and serum creatinine level [8]. But microalbuminuria is a more sensitive method for the early diagnosis. About 50% of the diabetic patients develop microalbuminuria and 30-50% of these develop into proteinuria which leads to end stage renal disease.

Ankle Brachial Index (ABI) is a simple method to screen Peripheral Arterial Disease (PAD) and to evaluate cardiovascular pronostics. ABI is the ratio of Systolic Blood Pressures (SBP) in the lower and upper extremities. PWV and AI gives the status of arterial stiffness of the vessels. Values of ABI between 0.9-1.4 were considered as normal. ABI values <0.9 are conventionally used as pathological threshold to define peripheral vascular disease and high cardiovascular risk. A high ABI of >1.4, which is a marker

of mitral annular calcification is often associated with neuropathy and/or chronic kidney disease [9].

Periscope is a computer based cardiovascular analysis system that uses simultaneous non invasive blood pressure measurements from four limbs and Electrocardiogram (ECG) waveform to calculate important parameters like ABI, PWV, AI, central aortic pressure values and ejection slope etc. Research has established these paramètres as independent predictors of atheroscelrosis, cardiovascular disease, end stage renal disease, hypertension, neuropathy, retinopathy and erectile dysfunction [9].

Mortality in diabetics is due to complications of cardiovascular, cerebrovascular and renal systems, which are associated with uncontrolled blood sugar level. Hence detection of vascular complications with the easily accessible methods can prevent the mortality secondary to diabetes mellitus.

The objectives of the present study were:

-To study the incidence of microalbuminuria and its correlation with duration of diabetes and sugar levels in patients with type 2 DM.

-To study the values of ABI for the detection of peripheral vascular disease in patients with type 2 DM.

-To study the incidence of arterial stiffness by using PWV and AI and its correlation with duration of diabetics.

MATERIALS AND METHODS

The present cross-sectional study was conducted in Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka, India, among 100 diabetic patients from March to June 2018. Informed consent was obtained from all the participation in the study. Approval for the study was taken from the Institutional Ethical Committee of Shri B.M. Patil Medical College, Hospital and Research Centre, BLDE University, India, as per the guidelines (2006) of Indian Council of Medical Research. Declaration of Helsinki was followed during the entire study.

Inclusion criteria: Patients who were diagnosed with type 2 DM without complications visiting weekly diabetic clinic/Department of Medicine, BLDEU Shri B.M. Patil Medical College, Hospital and Research centre for regular follow-up were included in the study.

Exclusion criteria: Patients who had a past history of a predisposition to hypercoagulability, including thrombocytosis; a history of venous thromboembolism; known inherited coagulation disorders; cancer; pregnancy; recent surgery; hyperthyroidism or patients who were taking standard anticoagulant treatment with either coumarin derivatives or heparins. Patients with type 1 diabetes and liver disorders were excluded from the study.

Sample size calculation: Sample size was calculated using following formula, $n=Z^2 \times SD^2/d^2$ where, Z=Z value at α level=95%, SD: Standard Deviation, d=allowable error is 5% of the mean. Calculated sample size=60, Dropout rate=10%. Hence, included 100 patients of type 2 DM in this study.

Study Procedure

The study included assessment of blood glucose level both FBS and PPBS, microalbumin levels, ABI, PWV and AI in the same patient.

1. **Glucose level:** Blood samples were collected and tested for Fasting Blood Sugar (FBS) level and Postprandial Blood Sugar (PPBS) levels. According to American Diabetes Association (ADA) recommendations goal of diabetes care is to maintain the sugar levels with FBS ≤130 mg/dL, PPBS ≤200 mg/dL. Hence, patients with FBS levels <130 mg/dL and PPBS <180 mg/dL were considered in the glycaemic controlled group and FBS levels >130 mg/dL and PPBS >180 mg/dL were considered

in the poor glycaemic control group [10]. Study did not include Glycated Haemoglobin (HbA1c) as ADA recommendation was followed and due to cost factor.

2. **Microalbuminuria:** Albumin to creatinine ratio was done on first morning urine sample to detect the concentration of microalbumin. Concentration of microalbumin in sample above 30 mg/L was considered as patients having microalbuminuria [11].

3. **Ankle brachial index, PWV and AI:** ABI is the ratio of SBP in the lower and upper extremities. PWV and AI gives the status of arterial stiffness of the vessels. These three values were measured using periscope. Values of ABI between 0.9-1.4 was considered as normal. ABI <0.9 and ABI >1.4 were considered as abnormal [12].

STATISTICAL ANALYSIS

Analytical test to measure the differences between two means of the two groups independent t-test was applied. Multiple linear regressions was applied to find out the correlation between the factors. The p-value <0.05 was considered statistically significant.

RESULTS

A total of 100 diabetic patients were included in the study. Out of 100 patients, 59% were males and 41% were females.

The age of the patients ranged from 31-82 years, with an average of 60 years. Majority of the patients were in the age range of 61-70 years (38%) followed by 51-60 years age group of 61-70 years (38%) followed by 51-60 years (23%), 41-50 years (20%), >70 years (13%) and only 6% patients were in the age group of 31-40 years [Table/Fig-1].

Age group (in years)	Frequency
31-40	06
41-50	20
51-60	23
61-70	38
>70	13
Total	100

[Table/Fig-1]: Distribution of patients according to age (years).

The BMI of the patients ranged from 15.2 kg/m² to 34.13 kg/m², with an average of 24.18 kg/m². Out of 100 patients 29% of patients had normal BMI (<18.9 to 24.9 kg/m²) followed by 45% of the patients being preobese (25-29.9 kg/m²) and 26% were obese (class1) with a BMI of ≥30 kg/m². Majority of the patients were in the category of preobese [Table/Fig-2].

BMI (in Kg/m²)	Frequency
<18.9	03
19-24.9	26
25-29.9	45
≥30	26
Total	100

[Table/Fig-2]: Distribution of patients according to Body Mass Index (BMI).

The duration of diabetes in this study ranged from one to 20 years, with an average of 10 years. Most of the patients were with 10-15 years of duration of diabetes (57%) , followed by total of 42% of patients with <10 years duration, which had 21% of patients in each group of one to five years and 5-10 years of duration respectively [Table/Fig-3].

In this study, out of 100 patients, 68% of patients had uncontrolled sugar levels and 32% of patients had controlled sugar level. All patients

Range of duration (years)	Frequency
1-5	21
6-10	21
10-15	57
>15	1
Total	100

[Table/Fig-3]: Distribution of patients according to duration of diabetes.

were on medication with either oral antidiuretic drugs or insulin. A 62% of the patients in the present study had microalbuminuria and 38% of patients had normal albumin levels [Table/Fig-4].

Microalbuminuria	Frequency
Absent	38
Present	62
Total	100

[Table/Fig-4]: Distribution of patients according to microalbuminuria.

Arterial stiffness measured by PWV and AI using periscope, showed 60% of the patients with abnormal values indicating that they had arterial stiffness and normal values in 40% of the patients [Table/Fig-5].

Arteriosclerosis	Frequency
Normal	40
Abnormal	60
Total	100

[Table/Fig-5]: Distribution of patients according to arteriosclerosis.

Ankle branchial index which gives the atherosclerotic condition of the vessels ranged from 0.82-1.22. A 7% of the patients had abnormal ABI with <0.9 ABI and 93% of the patients had normal ABI [Table/Fig-6]. The average mean±standard deviation of ABI in diabetic men and women patients was 1.126±0.0.16, at 95% confidence level and 5% error.

ABI range	Frequency
<0.9	07
0.9-1.4	93
>1.4	00
Total	100

[Table/Fig-6]: Distribution of patients according to Ankle Brachial Index.

Microalbuminuria was compared with the other continuous variables like age, BMI, duration of diabetes, FBS, PPBS, PWV, AI and ABI values. To find out the correlation between the factors, multiple linear regressions was applied. The p-value <0.05 was considered statistically significant.

The correlation of microalbuminuria with factors like age, duration of diabetes, FBS, PPBS, PWV and AI was statistically significant with a p-value of <0.05 [Table/Fig-7,8]. The correlation of duration of diabetes was statistically significant with a p-value of <0.05 with microalbuminuria, age, BMI and factors like FBS, PPBS, PWV, AI, and ABI were not significant [Table/Fig-9].

Variable	Microalbuminuria (Mean±standard deviation)		t-test value	p-value
	≥30 (n=62)	<30 (n=38)		
Age (years)	63.5±9.204	53.16±11.441	4.715	<0.001
Body mass index (kg/m²)	27.3482±3.81812	25.7289±3.69205	2.101	0.039
Duration of diabetes mellitus (years)	11.9±3.248	6.16±3.545	8.118	<0.001
Fasting blood sugar (mg/dL)	286.1±79.063	162.24±76.607	7.752	<0.001
Postprandial blood sugar (mg/dL)	449.77±118.084	242.82±100.181	9.359	<0.001
Brachial artery pulse wave velocity	1726±746.8471	1415±244.9691	3.021	<0.001
Augmentation index	24.32±13.333	15.87±9.555	3.683	<0.001
Ankle brachial index right	1.07±0.0825	1.05±0.0898	1.106	0.272
Ankle brachial index left	1.105±0.08022	1.0682±0.08167	2.204	<0.001

[Table/Fig-7]: Comparison of variables with two groups (with and without microalbuminuria).
p-value <0.05 was considered statistically significant

Characterstics	Odds ratio	p-value
Age	-0.106	0.335
Body mass index	-0.027	0.715
Duration of diabetes mellitus	0.392	<0.001
Fasting blood sugar	0.428	<0.001
Postprandial blood sugar	0.437	<0.001
Augmentaion index	-0.23	<0.001
Brachial artery pulse wave velocity, Augmentaion index	0.6	<0.001
Ankle brachial index right	-0.038	0.686
Ankle brachial index left	-0.049	0.622

[Table/Fig-8]: Correlation between microalbuminuria with other variables.
p-value<0.05 was considered statistically significant

Characterstics	Odds ratio	p-value
Age	0.52	<0.001
Body mass index	0.117	<0.001
Fasting blood sugar	0.106	0.44
Postprandial blood sugar	0.181	0.216
Microalbuminuria	0.237	<0.001
Brachial artery pulse wave velocity	-0.134	0.192
Augmentaion index	0.038	0.69
Ankle brachial index right	-0.063	0.396
Ankle brachial index right	0.126	0.101

[Table/Fig-9]: Correlation between duration of diabetes with other variables.
p-value<0.05 was considered statistically significant

DISCUSSION

Diabetes mellitus is an extending metabolic disorder in the world with microvascular and macrovascular complications that if not being diagnosed and properly treated leads to debility, increased mortality, and morbidity. Diabetes mellitus leads to acceleration of atherosclerotic changes in vessels and thus involvement of different organs. In this study, authors have evaluated values of sugar levels and duration of diabetes with microalbuminuria, ABI, PWV and AI values in type 2 diabetic patients. Thus, authors studied the utility of these parameters in detecting the patients who are at risk for vascular complications [10,11].

Hence, the methods chosen in this study are microalbuminuria, and measurement of ABI, PWV and AI by periscope. These methods are simple, reliable, cost-effective and non invasive techniques for the early detection of nephropathy, arterial stiffness and atherosclerosis in patients with type 2 DM. Early detection and treatment of vascular complication in patient with type 2 DM reduces the mortality rate.

A total of 100 diabetic patients were included in the study. The study showed slight predominance of males (59%) and (41%) were females The age of the patients ranged from 31-82 years, with an average of 60 years. Majority of the patients were in the age range of 61-70 years followed by 51-60 years age range (23%).

The BMI of the patients ranged from 15.2-34.13 kg/m², with an average of 24.18 kg/m². Out of 100 patients 29% of patients had normal BMI followed by 45% of the patients being preobese, and 26% were obese (class 1) with a BMI of ≥30 kg/m². Majority of the patients were in the category of preobese.

The duration of diabetes in the study ranged from one to 20 years, with an average of 10 years. Most of the patients were with 10-15 years of duration of diabetics (57%), followed by total of 42% of patients with <10 years duration, which had 21% of patients in each group of one to five years and 5-10 years of duration respectively.

In this study, all patients were on medication with either oral antidiuretic drugs or insulin. According to the ADA recommendations goal of diabetes care is to maintain the sugar levels with FBS <130 mg/dL, PPBS ≤180 mg/dL. Hence, patients with FBS levels <130 mg/dL and PPBS <2000 mg/dL were considered in the controlled group and FBS levels >130 mg/dL and PPBS >200 mg/dL were considered in the uncontrolled group.

In this study, out of 100 patients, 68% of patients had poorly controlled glycaemic levels and 32% of patients had controlled glycaemic level. A 62% of the patients in this study had microalbuminuria and 38% of patients had normal levels.

Correlation between the duration of diabetes and microalbuminuria of the patients showed that, 40% of the patients with microalbuminuria were in the group with 10-15 years duration of diabetes. Total 15% of the patients having microalbuminuria had diabetes since 15 years, 5% of patients had diabetes in the range of 5-10 years. The study showed significant statistical correlation between duration of diabetes and microalbuminuria. These findings were similar to the findings of Idowu AA et al., study, in which microalbuminuria was correlated with the duration of diabetes in 50 diabetic patients. They also observed that significant number of patients with microalbuminuria had diabetes for >10 years duration [12]. Previous studies also reported in their study that, microalbuminuria was associated with duration of diabetes which has been again confirmed by the present study data [13-16].

Correlation of the sugar levels and the microalbuminuria showed that 96% of the patients with microalbuminuria had poor glycaemic control. There was significant statistical correlation between sugar levels and microalbuminuria with a p-value of <0.001.

Baig JA et al., studied 60 patients to compare duration of Type 2 Diabetes Mellitus (T2DM) with microalbumin levels, concluded that in patients with T2DM, long duration of diabetes and poor glycaemic control significantly correlated with high level of microalbumin. Similar to Baig JA et al., in present study also, patients with poor glycaemic control had higher level of microalbumin [17].

Ankle brachial index which gives the atherosclerotic condition of the vessels ranged from 0.82-1.22 in this study. A 7% of the patients had anormal ABI with <0.9 ABI and 93% of the patients had normal ABI. Makhdoomi K et al., correlated ABI and microalbuminuria in 206 patients with type 2 DM. In their study, 20% of the patients had abnormal ABI and 29% had microalbuminuria. Correlation between ABI less than 0.9 and microalbuminuria was significant [18]. In this study, 60% of patients had microalbuminuria and out of these 60 patients only five patients had abnormal ABI levels. Statistically significant correlation was not found between ABI and microalbuminuria in this study. Correlation between abnormal ABI and duration of diabetes was not statistically significant in this study. As many other risk factors like hypertension, dyslipidemia, LDL can lead to abnormal ABI levels.

Arterial stiffness measured by PWV and AI using periscope, showed 60% of the patients with abnormal values indicating that they had arterial stiffness and normal values en 40% of the patients.

In this study, the duration of diabetes was correlated with arterial stiffness i,e., with AI and observed that, abnormal values were found in 35% of the patients in the group of 10-15 years of duration with diabetes followed by 14% patients in the group >15 years and 6% in 5-10 years duration. There was no significant statistical correlation between duration of diabetes and arterial stiffness.

Limitation(s)

Study included only 100 patients with type 2 DM patients without controls, findings of the study can be extended to larger sample size for the reliability of results. One more limitation of the study was HbA1C was not included in the study to assess the glycaemic control.

CONCLUSION(S)

Considering vascular complications as the most important risk factor in the mortality of diabetic patients, screening test for these complications is recommended in type 2 DM patients. Microalbuminuria is the predictor of later development of diabetic nephropathy. The correlation of microalbuminuria with duration of diabetes, FBS, PPBS, PWV and AI showed statistical significance. Hence the study confirmed that, incidence of microalbuminuria increases with duration of diabetes, poorly controlled blood sugar levels and there is no association of age, BMI and sex on the prevalence of microalbuminuria in type 2 DM. Microalbuminuria, ABI, PWV and AI (measured with periscope) are the reliable and simple methods used for the screening of vascular complications. The attempt of detecting microvascular complications with simple, easily available, non invasive and reliable techniques like microalbuminuria, ABI, PWV and AI can reduce the mortality rate in diabetics.

REFERENCES

[1] Gupta M, Singh JP. Correlation of microalbuminuria with glycosylated hemoglobin in patients of diabetes having nephropathy. Int J Adv Med. 2017;4(3):805-08.

[2] Joshi SR, Parikh RM. India- diabetes capital of the world: Now heading towards hypertension. J Assoc Physicians India. 2007;55:323-24.

[3] Kumar A, Goel MK, Jain RB, Khanna P, Chaudhary V. India towards diabetes control: Key issues. Australias Med J. 2013;6(10):524-31.

[4] Marshall SM, Flyvbjerg A. Prevention and early detection of vascular complications of diabetes. BMJ. 2006;333(7566):475-80.

[5] "Diabetes Complications". Diabetes.co.uk. Retrieved. 2012.

[6] Anavekar NS, Pfeffer MA. Cardiovascular risk in chronic kidney disease. Kidney Int. 2004;66(Suppl 92):S11-15.

[7] Basi S, Fesler P, Mimran A, Lewis JB. Microalbuminuria in type 2 diabetes and hypertension: a marker, treatment target, or innocent bystander? Diabetes Care. 2008;31(Suppl 2):S194-201.

[8] Dabla PK. Renal function in diabetic nephropathy. World J Diabetes. 2010;1(2):48-56.

[9] Panel L, Potier C, Khalil A, Mohammedi K, Roussel R. Use and utility of Ankle brachial index in patients with Diabetes. Eur J Vasc Endovasc Surg. 2011;41(1):110-16.

[10] Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australasien Medical Journal. 2014;7(1):45-48.

[11] Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy. Phys Ther. 2008;88(11):1322-35.

[12] Idowu AA, Ajose AO, Adedeji AT, Adegoke AO, Jimoh KA. Microalbuminuria, other markers of nephropathy and biochemical derangementsin type 2 diabetes mellitus: Relationships and determinants. Ghana Med J. 2017;51(2):56-63.

[13] Indriani V, Lestari T, Dewantari V. Duration of diabetes as an important risk factor of microalbuminuria in type 2 diabetes. Universa Medicina. 2020;39(1):42-46.

[14] Thakur SK, Dhakal SP, Parajuli S, Sah AK, Nepal SP, Paudel BD. Microalbuminuria and its risk factors in type 2 diabetic patients. J Nepal Health Res Counc. 2019;17:61-65.

[15] Liu Z, Fu C, Wang W, Xu B. Prevalence of chronic complications of type 2 diabetes mellitus in outpatients-a cross-sectional hospital based survey in urban China. Health and Quality of Life Outcomes. 2010;8(1):62.

[16] Khan NJ, Farid MI, Hameed S, Aziz M. Frequency of microalbuminuria in newly diagnosed type 2 diabetics at Nishtar Hospital, Multan. Ann King Edward Med Coll. 2004;10(4):374-75.

[17] Baig JA, Asif N, Sarfaraz A, Alam JM. Correlation of microalbuminuria with glycosylated hemoglobin (HbA1c) and duration of type 2 diabetes mellitus (T2DM) in male and female patients. Middle-East J Sci Res. 2016;24(9):2900-03.

[18] Makhdoomi K, Mohammadi A, Yekta Z, Aghasi MR, Zamani N, Vossughian S. Correlation between ankle-brachial index and microalbuminuria in type 2 diabetes mellitus. Iran J Kidney Dis. 2013;7(3):204-09.

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