

# Echocardiographic Study of Left Ventricular Diastolic Dysfunction in Diabetes Mellitus

B.A.Shetty<sup>1</sup>, Vikram G.S<sup>2</sup>, Uttam U. Shettigar<sup>3</sup>

<sup>1</sup>Associate Professor, <sup>2</sup>Senior Resident, Postgraduate Student,  
Department of General Medicine, A.J. Institute of Medical Sciences, Kuntikana, Mangalore, Karnataka, India.

Corresponding Author: Uttam U. Shettigar

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## ABSTRACT

People with diabetes have an increased prevalence of atherosclerosis and coronary heart disease and experience higher morbidity and mortality after acute coronary syndrome and myocardial infarction than people without diabetes. This study intends to assess Left ventricular diastolic dysfunction in diabetes mellitus patients by an echocardiography with an intention to detect patients with left ventricular dysfunction at an early stage. This was a prospective study, conducted on 30 diabetic patients in A.J Institute of Medical Sciences, Mangalore who presented between July 2009 – July 2011. They were clinically evaluated and underwent relevant investigations. Echocardiography and Doppler study has been conducted on each patient as a part of screening for diastolic dysfunction. Among type 2 diabetes mellitus patients who were admitted without any history of myocardial infarction, hypertension, angina pectoris or any other cardiac disease, who were diabetic for at least 5 years. Out of 30 subjects 18 (60%) were found to have diastolic dysfunction with systolic function being normal. Among 30 type 2 diabetes mellitus patients, 20 (66.7%) were male and 10 (33.3%) were females. Out of 30 subjects 12 (40%) have family history of diabetes mellitus. Out of 30 subjects 9 (30%) were < 40 years of age, 12 (40%) were between 40 – 60 years of age, 9 (30%) were > 60 years of age. Significant differences in the parameters of left ventricular diastolic dysfunction were found. These findings suggest that relaxation properties deteriorate in diabetics more rapidly than in healthy subjects. These results contribute to better understanding of pathological process resulting in cardiac failure in diabetic patients and support the optimum therapeutic strategies. This concludes that early diagnosis, treatment is essential to prevent further complications.

**Keywords:** Diabetes Mellitus; Echocardiography; Diastolic dysfunction; Cardiomyopathy; Heart Failure.

## INTRODUCTION

The awarding of the Nobel Prize in Medicine in October 1923, 18 months after the first news of the discovery of the insulin, was part of a gripping tale of success, disappointment, and conflict. The story of Banting, Best, Collip, and Macleod brought to light the tensions of a 6-month period that began in the summer of 1921 and intensified

when the new extract corrected the metabolic acidosis in the first person to receive the substance in January 1922 (Leonard Thompson, age 14 years, at the Toronto General Hospital in Canada). In 1922 Banting and Macleod received a Nobel Prize for this historic discovery.<sup>[1]</sup>

The Hispanic population in the United States has grown by 61% in the past

10 years to the 25 million level, and there has been a 20% increase in the African-American population. These figures alone predict heavy pressure on healthcare budgets, as the diagnosis of diabetes is more sedentary, and older, which further increases the prevalence of type 2 diabetes. The increase in type 2 diabetes in children is an even more startling trend.<sup>[1]</sup> The reports of the World Health Organization (WHO) in the past decade have seen a march in prevalence of worldwide diabetes from 100 million a decade ago to 135 million in 1995, 151 million by 2000, and a projected number of 221 million by 2010.<sup>[2,3]</sup> Type 2 diabetes is the commonest form of diabetes constituting 90% of the diabetic population in any country. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025.<sup>[4]</sup>

A national survey of diabetes conducted in six major cities in India in year 2000 showed that the prevalence of diabetes in urban adults was 12.1%. Prevalence of impaired glucose tolerance (IGT) was also high (14.0%).<sup>[5]</sup> Prevalence of diabetes was found to be lower in the low socio-economic group living in urban areas compared with the high income group (12.6% vs. 24.6% in subjects >40 years).<sup>[6]</sup>

This was probably related to the physical activity of the low income group as most of them were involved in moderate to strenuous physical activity at work. However, due to inadequate control of diabetes, the long term complications such as coronary artery diseases were higher in the low socio economic group. This was to some extent related to the higher rates of risk factors such as uncontrolled diabetes, high cholesterol, hypertension, smoking and alcohol consumption.<sup>[7]</sup>

Mohan et al also found a lower prevalence of diabetes in the low income group compared with middle income group in Southern India.<sup>[8]</sup> In another study of the

socially deprived urban slum dwellers, in New Delhi, Misra et al also observed appreciable prevalence of obesity, dyslipidaemia, diabetes(10.3%) and increased body fat. High waist to hip (WHR) was observed, especially in women (51.1% vs. 9.4% in men) in the study.<sup>[9]</sup>

### ***Global prevalence of diabetes***

The World Health Organization has predicted that the major burden will occur in the developing countries. There will be a 42% increase from 51 to 72 million in the developed countries and 170% increase from 84 to 228 million, in the developing countries. The countries with the largest number of diabetic people are, and will be in the year 2025, India, China and United States.<sup>[10]</sup>

### ***Diabetes in Indians***

Epidemiological studies among migrant Asian Indians in many countries showed higher prevalence of type 2 diabetes compared with the host populations and other migrant ethnic groups. Studies conducted in India in the last decade have highlighted that not only is the prevalence of type 2 diabetes high, but also that it is increasing rapidly in the urban population. Over the next 30 years the global prevalence of diabetes mellitus is projected to increase by over 100 percent. This will raise the global burden of diabetes mellitus to 366 million by the year 2030.<sup>[11-16]</sup>

India shelters the most number of people with diabetes mellitus worldwide. From 31 million in the year 2000, the number of persons with diabetes mellitus in India would register a 2.5 fold increase over the next 30 years so as to reach an alarming level of estimated 80 million by the year 2030.<sup>[17]</sup>

The only published nationally representative study on burden of diabetes mellitus in India is Prevalence of Diabetes in India Study – PODIS (2002), a multi-centric study (49 urban and 59 rural centers) on

41,000 Indian people. PODIS has estimated the age and gender standardized prevalence of diabetes mellitus in India to be 3.3 percent. [18] The International Diabetes Federation (IDF) also reported that the total number of diabetic subjects in India is 41 million in 2006 and that this would rise to 70 million by the year 2025. [19]

The Prevalence of Diabetes in India Study (PODIS) was carried out in 108 centres (49 urban and 59 rural) in different parts of India to look at the urban-rural differences in type 2 diabetes and glucose intolerance. Diabetes was defined according to WHO and ADA criteria. According to ADA criteria, the prevalence of diabetes was 4.7% in the urban and 1.9% in the rural areas. The prevalence of diabetes according to WHO criteria was 5.6% and 2.7% among urban and rural areas respectively. [20,21]

## MATERIALS AND METHOD

This one year prospective, selectively analyzed, single-center pilot study was conducted in A.J. Institute of Medical Sciences, Mangalore between 2009 December to 2010 November.

In this hospital based study, patients with diabetes mellitus based on history, clinical examination and laboratory parameters (Hb, sugar levels, HbA1C, Sr. Creatinine and blood urea) were included. Patients with myocardial infarction, angina pectoris, heart disease and hypertension were excluded from the study.

Statistical analysis was done using, SPSS version 17.0 and  $P < 0.05$  was considered as significant.

## RESULTS

Out of 30 total subjects 20(66.7%) were male and 10 (33.3%) were females. (Table no. 1)

Out of 30 total subjects 12 (40%) have family history of diabetes mellitus. (Table no. 2)

**Table 1: Sex distribution.**

	Frequency	Percentage
Male	20	66.7
Female	10	33.3
Total	30	100

**Table 2: Family history of diabetes mellitus.**

	Frequency	Percentage
No	18	60.0
Yes	12	40.0
Total	30	100

Mean Hb g% (hemoglobin) was 11.38 with standard deviation of 2.10. Mean FBS (Fasting blood sugars) was 159.23 with standard deviation of 47.78. Mean PPBS (Post Prandial Blood Sugars) was 230.16 with standard deviation of 55.95. Mean HbA1C (Glycosylated Hemoglobin) was 7.95 with standard deviation of 0.54. Mean Serum Creatinine was 0.97 with standard deviation of 0.27. Mean Blood Urea was 30.93 with standard deviation of 12.76 (Table no. 3). Out of 30 total subjects 9 (30%) were <40 years of age, 12 (40%) were between 40-60 years of age, remaining 9 (30%) were more than 60 years of age. (Table no. 4)

**Table 3: Hematological parameters.**

	N	Minimum	Maximum	Mean	Std.Deviation
Hb gm%	30	7.90	16.40	11.3800	2.10490
FBS mg/dl	30	107.0	330.0	159.2333	47.78593
PPBS mg/dl	30	146.0	390.0	230.1667	55.95015
HbA1C	30	6.90	9.00	7.9500	0.54819
Serum Creatinine mg/dl	30	0.10	1.40	0.9767	0.27125
Blood Urea mg/dl	30	15.00	75.00	30.9333	12.76291

**Table 4:Age-wise distribution.**

Age(in years)	Frequency	Percentage
<40	9	30.0
40-50	6	20.0
50-60	6	20.0
>60	9	30.0
TOTAL	30	100.0

Mean VSWT of Group 1, Group 2 and Group 3 were 0.84, 0.90 and 0.81 respectively.

Mean LVPWT of Group 1, Group 2 and Group 3 were 0.82, 0.87 and 0.78 respectively.

Mean LVEDD of Group 1, Group 2 and Group 3 were 4.35, 4.10 and 4.21 respectively.

Mean LVESD of Group 1, Group 2 and Group 3 were 2.87, 2.74 and 2.74 respectively.

Mean FS of Group 1, Group 2 and Group 3 were 30.75, 31.8 and 33.2 respectively.

Mean EF of Group 1, Group 2 and Group 3 were 67.2, 67.0 and 69.4 respectively.

Mean AR dimension of Group 1, Group 2 and Group 3 were 2.81, 2.86 and 2.73 respectively. Mean LA dimension of Group 1, Group 2 and Group 3 were 3.02, 3.02 and 2.88 respectively. Mean MV area of Group 1, Group 2 and Group 3 were 4.46, 4.20 and 4.44 respectively. There was no statistically significant difference found in any of the variables between the groups. Out of 30 subjects none of the subjects were found to have RWMA. (Table no. 5)

**Table 5: Left ventricular systolic parameters.**

Variables	Groups	N	Mean	Standard Deviation	F	p-value
VSWT (cm)	G1	8	0.8438	0.07927	1.659	0.209
	G2	10	0.9000	0.12074		
	G3	12	0.8175	0.10997		
LVPWT (cm)	G1	8	0.8263	0.08484	2.126	0.139
	G2	10	0.8740	0.10865		
	G3	12	0.7850	0.10344		
LVEDD (cm)	G1	8	4.3575	0.41372	0.962	0.395
	G2	10	4.1070	0.32452		
	G3	12	4.2100	0.40170		
LVESD (cm)	G1	8	2.8700	0.30933	0.527	0.596
	G2	10	2.7460	0.35002		
	G3	12	2.7417	0.23889		
FS %	G1	8	30.7500	5.00714	0.504	0.610
	G2	10	31.8000	4.84883		
	G3	11	33.2727	6.34178		
EF %	G1	8	67.2500	4.97853	0.452	0.641
	G2	10	67.0000	5.29150		
	G3	12	69.4167	8-12917		
AR Dimension (cm)	G1	8	2.8187	0.40357	0.380	0.688
	G2	10	2.8650	0.29444		
	G3	12	2.7308	0.39583		
LA Dimension (cm)	G1	8	3.0213	0.21263	1.257	0.301
	G2	10	3.0280	0.24661		
	G3	12	2.8800	0.26354		
MV Area (sq.cm)	G1	8	4.4688	0.30815	1.884	0.171
	G2	10	4.2050	0.28230		
	G3	12	4.4458	0.38403		

Mean Velocity-E of Group 1, Group 2 and Group 3 were 95.25, 74.20 and 90.08 respectively.

Mean Velocity-A of Group 1, Group 2 and Group 3 were 42.00, 58.60 and 52.41 respectively.

Mean E/A Ratio of Group 1, Group 2 and Group 3 were 2.2, 1.2 and 1.7 respectively. Mean IVRT of Group 1, Group 2 and Group 3 were 72.12, 91.60 and 88.0 respectively. Mean DT of group 1, group 2 and group 3 were 136.50, 249.00 and 164.66 respectively.

The difference in mean values of all the variables between the groups was found to be very highly significant at p value 0.001. (Table no. 6)

**TABLE 6: Left Ventricular Diastolic Parameters.**

Variables	Groups	N	Mean	Standard Deviation	H	p-value
'E' Velocity (cm/sec)	G1	8	95.2500	5.33854	17.87	0.001
	G2	10	74.2000	8.71525		
	G3	12	90.0833	17.73265		
'A' Velocity (cm/sec)	G1	8	42.0000	3.38062	13.01	0.001
	G2	10	58.6000	8.11309		
	G3	12	52.4167	10.45734		
E/A Ratio	G1	8	2.2750	0.18701	19.47	0.001
	G2	10	1.2730	0.12737		
	G3	12	1.7008	0.37189		
IVRT (ms)	G1	8	72.1250	4.51782	18.73	0.001
	G2	10	91.6000	3.83551		
	G3	12	88.0833	4.71860		
DT (ms)	G1	8	136.5000	21.82397	22.29	0.001
	G2	10	249.0000	11.97219		
	G3	12	164.6667	22.90031		

## DISCUSSION

The present study, done in AJIMS, Mangalore among type 2 diabetes mellitus patients who were admitted without any history of myocardial infarction, hypertension, angina pectoris or any other cardiac disease, who are diabetic for at least 5 years. Echocardiography and Doppler study has been conducted on each patient as a part of screening for diastolic dysfunction. Out of 30 total subjects 20 (66.7%) were male and 10 (33.3%) were females. Out of 30 total subjects 12 (40%) have family history of diabetes mellitus.

Out of 30 total subjects 9 (30%) were < 40 years of age, 12 (40%) were between 40-60 years of age, remaining 9 (30%) were > 60 years of age. There was no

statistically significant difference found in any of the variables between the groups in evaluation of systolic functional parameters. Out of 30 subjects none of the subjects were found to have RWMA. The difference in mean values of all the variables between the groups was found to be very highly significant at p value 0.001 in assessment of diastolic functional parameters.

Out of 30 subjects 18 (60%) were found to have diastolic dysfunction with normal systolic function. Similar findings were found by Shresthe NR et al conducted a study on echocardiographic evaluation of diastolic function in asymptomatic type 2 diabetes, which included 100 asymptomatic patients in type 2 diabetes without evidence of coronary artery disease, congestive heart failure, thyroid or overt renal disease.

LVDD was found in 71 subjects (71%), of whom 60 had impaired relaxation and 11 had a pseudonormal pattern of ventricular filling. Systolic function was normal in all subjects. [22]

According to Rajput R et al conducted a study to assess cardiac function by echocardiography and Doppler in patients of NIDDM before and after the control of hyperglycemia. This study included 30 patients of uncomplicated type 2 diabetes mellitus and 30, age and sex matched healthy subjects. Systolic function of left ventricle was within normal range in all patients. Diastolic dysfunction of left ventricle was very common and was detected in 63% of patients. None of the control subjects had systolic or diastolic dysfunction. [23]

Congestive heart failure is a major public health problem in developed countries. Several epidemiological investigations have confirmed that up to half of patients in the community have heart failure due to diastolic dysfunction despite normal LV ejection fraction. [24]

Diastolic dysfunction as rigorously defined by comprehensive Doppler techniques is common, often not accompanied by recognized cardiac heart failure, and associated with marked increases in all-cause mortality. [25]

Diagnosis of diastolic heart failure, cardiomyopathies, and constrictive pericarditis – knowledge of the diastolic filling pattern and filling pressures allows the detection of cardiac diseases that are frequently missed or not suspected clinically, especially when the LVEF is normal. Although diastolic filling is affected by various factors, the direction of its change or progression is predictable in patients with known heart disease. Therefore assessment of the diastolic filling pattern allows LV filling pressures and LV compliance and relaxation to the estimated

and understood so that optimal treatment strategies can be offered to symptomatic and asymptomatic patients with diastolic dysfunction. [26]

Diastolic echocardiographic parameters, E, E/A, DT, E/Ea and LA volume, have been found to be powerful prognostic indicators for various conditions. [27-31] Even in asymptomatic patients, the presence of diastolic dysfunction portends a poor clinical outcome. [32]

## CONCLUSION

Out of 30 subjects 18 (60%) were found to have diastolic dysfunction with normal systolic function. The difference in mean values of all the variables between the groups was found to be very highly significant at p value 0.001 in assessment of diastolic functional parameters. There was no statistically significant difference found in any of the variables between the groups in evaluation of systolic functional parameters. Assessment of the diastolic filling pattern allows LV filling pressures and LV compliance and relaxation to the estimated and understood so that optimal treatment strategies can be offered to symptomatic and asymptomatic patients with diastolic dysfunction. [33]

Myocardial involvement in diabetes may occur early in the course of disease, initially impairing early diastolic relaxation and when more extensive, it causes decreased myocardial contraction. More frequent incidence of heart failure in diabetics even in the absence of any other underlying heart disease, leads to presumption that diabetes mellitus unfavorably affects the heart muscle by its complications.

Early diagnosis and treatment is important in preventing irreversible structural alterations and systolic dysfunction.

## REFERENCES

1. Barnett DM, Krall LP. The history of diabetes. In: Kahn CR, Weir GC, King GL, Jacobson AM, Moses AC, Smith RJ, editors. *Joslin's diabetes mellitus*. 14th ed. Noida: B.I.Publications; 2006. p. 1-17.
2. Ebbeling CB, Pawlak DB, Ludwig DS. Childhood obesity: public-health crisis, common sense cure. *Lancet* 2002;360:476.
3. Gan D, ed. *Regional estimates for diabetes for the year 2000*. Diabetes atlas 2000. Brussels: International Diabetes Federation, 2000;9,11.
4. Zimmet P, Albert KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001;414:782-787.
5. King H, Aubert RE, Herman WH. Global burden of diabetes 1995-2025; Prevalence, numerical estimates, and projection. *Diabetes Care* 1998;21:1414-31.
6. Ramachandran A, Snehalatha C, Kapur A, Vijay V, Mohan V, Das AK, et al. For the Diabetes Epidemiology Study Group in India (DESI). High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia* 2001;44:1094-1101.
7. Ramchandran A, Snehalatha C, Vijay V, King H. Impact of poverty on the prevalence of diabetes and its complications in urban southern India. *Diabetic Medicine* 2002;19:130-135.
8. Ramachandran A. Socio-Economic Burden of Diabetes in India. *Journal of the Association of Physicians of India*. July 2007;55:p. 10.
9. Mohan V, Shanthirani S, Deepa R, Premalatha G, Sastry NG, Saroja R. Intraurban differences in the prevalence of the metabolic syndrome in southern India- the Chennai Urban Population Study. *Diabetic Medicine* 2001;18:280-287.
10. Misra A, Pandey RM, Rama Devi J, Sharma R, Vikram NK, Khanna N. High prevalence of diabetes, obesity and dyslipidaemia in urban slum population in northern India. *Inter J of Obesity* 2001;25:1-8.
11. King H, Aubert RE, Herman WH. *Diabetic Care* 1998;21:1414-1431.
12. Zimmet PZ. *Diabetologia* 1999;42:499-518.
13. Mohan V, Shanthirani S, Deepa R, Premalatha G, Sastry NG, Saroja, R., *Diab. Med.*, 2001;18:280-287.
14. Kutty R, Soman CR, Joseph A, Pisharody R, Vijayakumar K. *Natl. Med. J. India*, 2000;13:287-292.
15. Misra A, Pandey RM, Rama Devi J, Sharma R, Vikram NK, Khanna N. *Int. J. Obesity*, 2001;25:1-8.
16. Verma NPS, Madhu SV. *Diab. Res. Clin. Prac.*, 2000;50(1):515.
17. Iyer R, Upasani S, Baitule MN. *ibid*, 2000;50(1):519.
18. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2004 and projections for 2030. *Diabetes Care* 2004;27:1047-53.
19. Sadikot SM, Nigam A, Das S, Bajaj S, Zargar AH, Prasannakumar K, et al. The burden of diabetes and impaired fasting glucose in India using the ADA 1997 criteria: prevalence of diabetes in India study (PODIS). *Diabetes Res Clin Pract* 2004;66:293-300.
20. Sicree R, Shaw J, Zimmet P. Diabetes and impaired glucose tolerance. In: Gan D, editor. *Diabetes Atlas*. International Diabetes Federation. 3rd ed. Belgium: International Diabetes Federation; 2006. p. 15-103.
21. Sadikot SM, Nigam A, Das S et al. The burden of diabetes and impaired glucose tolerance in India using the WHO 1999 criteria: prevalence of diabetes in India study (PODIS). *Diabetes Res Clin Pract* 2004;66:301-07.
22. Sadikot SM, Nigam A, Das S, Bajaj S, Zargar AH, Prasannakumar KM, et al. *Diabetes India*. The burden of diabetes and impaired fasting glucose in India using the ADA1997 criteria: prevalence of diabetes in India study (PODIS).

- Diabetes Res Clin Pract 2004;66:293-300.
23. Shrestha NR, Sharma SK, Karki P, Shrestha NK, Acharya P. Echocardiographic evaluation of diastolic function in asymptomatic type 2 diabetes. *J Nepal Med Assoc* 2009;48(173):20-3.
  24. Rajesh R, Jagdish, Siwach SB, Rattan A. Echocardiographic and Doppler assessment of cardiac functions in patients of non-insulin dependent diabetes mellitus. *JACM* 2002;3(2):164-8.
  25. Vasan RS, Larson MG, Benjamin EJ, Evans JC, Reiss CK, Levy D. Congestive heart failure in subjects with normal versus reduced LV ejection fraction: prevalence and mortality in a population-based cohort. *J Am Coll Cardiol*, 1999,33,1948-55.
  26. Redfield MM, Jacobsen SJ, Burnett JC Jr, Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA*, 2003,289,194-202.
  27. Connolly HM, Oh JK. Echocardiography. In: Libby P, Bonow RO, Mann DL, Zipes DP, Braunwald E, editors. *Braunwald's heart disease a text book of cardiovascular medicine*. 18th ed. New Delhi: Elsevier; 2008. p. 227-325.
  28. Zile MR, Brutsaert DL: New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. *Circulation* 2002; 105:1387.
  29. Tsang TS, Barnes ME, Gersh BJ, et al: Risks for atrial fibrillation and congestive heart failure in patients  $\geq 65$  years of age with abnormal left ventricular diastolic relaxation. *Am J Cardiol* 2004; 93:54.
  30. Temporelli PL, Giannuzzi P, Nicolosi GL, et al: GISSI-3 Echo Substudy Investigators: Doppler-derived mitral deceleration time as a strong prognostic marker of left ventricular remodeling and survival after acute myocardial infarction: Results of the GISSI-3 echo substudy. *J Am Coll Cardiol* 2004; 43:1646.
  31. Schillaci G, Pasqualini L, Verdecchia P, et al: Prognostic significance of left ventricular diastolic dysfunction in essential hypertension. *J Am Coll Cardiol* 2002; 39:2005.
  32. Hillis GS, Moller JE, Pellikka PA, et al: Noninvasive estimation of left ventricular filling pressure by E/e' is a powerful predictor of survival after acute myocardial infarction. *J Am Coll Cardiol* 2004; 43:360.
  33. Redfield MM, Jacobsen SJ, Burnett Jr JC, et al: Burden of systolic and diastolic ventricular dysfunction in the community: Appreciating the scope of the heart failure epidemic. *JAMA* 2003; 289:194.
  34. Raev DC. Which left ventricular function is impaired earlier in the evolution of diabetic cardiomyopathy? An echocardiographic study of young type 1 diabetic patients. *American Diabetes association. Diabetes care* July 1994 vol. 17; no.7: 633-639.

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