Association between Non-alcoholic Fatty Liver Disease and Left Ventricular Diastolic Dysfunction in Patients of Type 2 Diabetes

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Abstract

Objective: The purpose of the study was to assess if non-alcoholic fatty liver disease (NAFLD) in diabetic patients increases the risk and/or severity of diastolic dysfunction

Research design and methods: We studied 70 type 2 diabetic individuals without a history of ischemic heart disease, hepatic diseases, or excessive alcohol consumption, in whom NAFLD was diagnosed by ultrasonography. All patients had normal left ventricular systolic function and blood pressure values under medication. Left ventricular diastolic dysfunction was assessed by pulsed wave Doppler and tissue Doppler imaging, studying mitral inflow patterns and E wave, E’ wave velocities, E/A and E/E’ ratios.

Results and Conclusions: Fifty seven patients (81.43%) had NAFLD, and when compared with the other 13(18.57%) patients, age, sex, BMI, waist circumference, hypertension, smoking, diabetes duration, microvascular complication status, and medication use were not significantly different. In addition, the left ventricular (LV) mass and volumes, ejection fraction, systemic vascular resistance, arterial elasticity, and compliance were also not different. NAFLD patients had lower E’ (8.42±0.89 vs.9.72±0.54, P <0.0001) tissue velocity, higher E-to-E’ ratio (9.64±1.83 vs. 7.78±0.89, p<0.001), higher LV–end diastolic pressure (EDP) (15.52 ± 0.69 vs. 14.40±0.9 P <0.0001), higher LV EDP/end diastolic volume LV EDP/EDV (mmHg/mL) (0.19 ±0.15 vs. 0.17±.02 p < 0.001) and higher glycosylated haemoglobin (HbA1C) (8.53±1.02 vs.7.65±0.66 p<0.01) than those without steatosis.

All of these differences remained significant after adjustment for hypertension and other cardio metabolic risk factors. Our data show that in patients with type2 diabetes and NAFLD, even if the LV morphology and systolic function are preserved, early features of LV diastolic dysfunction detected. The frequency of diastolic dysfunction was significantly higher in diabetic patients with NAFLD versus controls.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is recognized as the hepatic expression of the metabolic syndrome, a cluster of cardiovascular risk factors, including type 2 diabetes mellitus. Observational studies have connected NAFLD with insulin resistance, suggesting that the presence of fatty liver and especially that of non-alcoholic steatohepatitis, promotes the development of glycoregulation disorders.² The addition of NAFLD to glycoregulation disorders may increase the cardiovascular risk of these patients, independently of the presence of other components of the metabolic syndrome.² Diastolic dysfunction in diabetic patients is the main characteristic of diabetic cardiomyopathy and represents a prognostic factor for the development of diastolic heart failure with preserved ejection fraction.³ The prevalence of diastolic dysfunction is higher in patients with metabolic syndrome compared with the general population, reaching 60% in diabetic patients.⁴ Diastolic dysfunction impairs life quality, reducing exercise capacity and accounting for up to 50% of acute heart failure hospitalizations in patients with preserved ejection fraction.⁵ Recently it was proven that diastolic dysfunction is a frequently met in patients with NAFLD and type2 diabetes or arterial hypertension.⁶

Diabetic cardiomyopathy may induce changes in cardiac structure such as myocardial hypertrophy, fibrosis, and fat droplet deposition. Early changes in cardiac function are also manifested as abnormal diastolic function that with time can lead to loss of contractile function.⁷ In parallel, it is recognized that non-alcoholic fatty liver disease (NAFLD) is largely prevalent in subjects who are obese or have type2 diabetes.⁸ Nowadays, growing evidence suggests that NAFLD is linked to increased risk of CVD (Cardio Vascular Disease) events in non diabetic and type 2 diabetic individuals.⁹ Several investigators have examined the association of NAFLD with markers of subclinical CVD (e.g., carotid artery intima-media thickness) or clinical CVD.¹⁰ Conversely, the information regarding abnormalities in cardiac function among NAFLD patients is limited and controversial. It has been shown that non diabetic, normotensive patients with NAFLD have echo-cardiographic features of early left ventricular (LV) diastolic dysfunction as measured by tissue Doppler echocardiography¹¹,¹² and impaired LV energy metabolism, as measured by cardiac 31P-magnetic resonance spectroscopy (MRS), compared with control subjects without steatosis.¹³ In a recent study involving type 2 diabetic men, Rijzewijk et al.¹⁴ found that, compared with those with lower intrahepatic fat content, patients with higher intra hepatic fat content, as measured by 1H-MRS, had impaired myocardial perfusion and lower high energy phosphates but similar values.
of LV function and morphology (as detected by cardiac magnetic resonance imaging [MRI]). Since two-dimensional echocardiography using tissue Doppler imaging is the most simple and reliable imaging method to evaluate early, subclinical changes in LV function, we wanted to apply this technique to test whether subtle cardiac abnormalities could be detected in type 2 diabetic individuals with NAFLD in comparison with those without steatosis.

**Material and Methods**

The study was conducted in type 2 diabetes mellitus patients in year August 2016-August 2018 after obtaining informed consent.

**Inclusion Criteria**

1. The patients of Diabetes Mellitus, who wishes to participate in the study. ADA (American Diabetes Association) criteria for diagnosis of diabetes mellitus:
   i. Random plasma glucose concentration >11.1 mmol/L (200 mg/dl) accompanied by classic symptoms of Diabetes Mellitus (polyuria, polydipsia, polyphasia, weight loss)
   ii. Fasting plasma glucose >7.0 mmol/L (126 mg/dl) on two separate occasions.
   iii. Two hours plasma glucose >11.1 (200 mg/dl) during an oral glucose tolerance test with 75 grams of oral glucose.
2. Diabetic patients who were on lifestyle modification and treatment (on oral medication or insulin) with an ejection fraction of more than 50% (Normal E/A) on echocardiogram with no evidence of cardio-respiratory illness.

**Exclusion Criteria**

1. Patients who had a prior history of ischemic heart and valvular disease, chronic heart failure, cirrhosis, or overt nephropathy.
2. Those who had known causes of chronic liver disease (i.e., alcohol or drug-induced liver disease, hemochromatosis, or autoimmune or viral hepatitis). All women were of postmenopausal status and did not take hormonal replacement therapy
3. Abnormal thyroid function test

**Method**

We studied 70 type 2 diabetic patients who attended the MBS Hospital, Kota. A 12-lead standard resting electrocardiogram, 24-h Holter monitoring, bicycle ergometry and cTMT were performed in all patients to exclude the presence of silent myocardial ischemia or significant disturbances of sinus rhythm; no patients had any abnormal test results. Of the 70 participants included in the study, 57(81.43%) patients met the clinical criteria for a diagnosis of NAFLD (i.e., hepatic steatosis on ultrasound among persons who did not drink alcohol and who did not have viral hepatitis, drug-induced liver disease, iron overload, or other known causes of liver disease) and 13(18.57%) patients did not. All participants gave written informed consent for participation in medical research. BMI was calculated by dividing weight in kilograms by height in meters squared BMI=18.25 – Normal, 25-29.9 – Overweight, 30 –Obese (WHO).

Waist circumference was measured midway between the lower-rib margin and the superior anterior iliac crest. Blood pressure was measured in duplicate by a physician with a mercury sphygmomanometer (at the right upper arm using an appropriate cuff size) after participants had been seated quietly for at least 5 min.

In all participants, the presence of microvascular complications such as sensory neuropathy (by biothesiometer) and nephropathy (by serum creatinine and albumin urea measurements) were also recorded. Information on smoking and alcohol consumption was obtained from all participants by a validated questionnaire. Blood samples were drawn in the morning after an overnight fast. Serum liver enzymes, ferritin, creatinine, thyroid function test and other biochemical blood measurements were determined by standard laboratory procedures. Fasting and PP plasma glucose (Glucose peroxidase enzyme) Serum total cholesterol, HDL, LDL, and triglycerides were determined by enzymatic kinetic assay method, using semi autoanalyzer (Transasia model no, erba – chem 5.1) after overnight fasting. Blood urea (Berthelot method based on urease enzyme) Serum creatinine (Joffe’s alkaline picrate method), HbA1c was measured by a high-performance liquid chromatography analyzer. All patients had negative hepatitis B and C viral markers.

Conventional echocardiography was used to measure LV diameters, wall thickness and mass according to standard criteria.7 LV end diastolic (EDV) and end systolic volumes and ejection fraction at rest were measured at the apical two chamber and four-chamber views (by modified Simpson rule).7 Left atrial maximal volume was measured at the end of LV systole from the apical two-chamber and four-chamber views (by modified Simpson rule).7 Measurements were indexed to body surface area when appropriate. Pulsed-wave Doppler was used to measure trans mitral peak early diastolic velocity (E), peak late diastolic velocity (A), and E-wave deceleration time (Dte). Isovolumetric relaxation time (IVRT) was also calculated.14 Each value was obtained from the average of three measurements.

**2D-Echocardiography (pulse wave) grading**

1. Impaired relaxation (grade I diastolic dysfunction).
   1a. Normal filling pressure -
   DT- 160-240ms, IVRT- 70-90 ms; E/A- 1-2,
   Mitral A duration >PVa duration,
   PVs2 >PVd (PVs2 can be <PVd in young person),
   No anatomical abnormalities.

   1b. Increase filling pressure -
   DT- >240 ms, IVRT- >90 ms, E/A- < 1.0,
   PVs2 > PVd,
   Mitral A duration > or <PVa duration.

2. Pseudonormal pattern (grade II diastolic dysfunction) –
   HT- 160-200ms, IVRT < 90 ms; E/A- 1-1.5
   PVs2 < PVd,
   Mitral A duration <PVa duration,
   PVa velocity increase (> 35 cm/s),
   2D echocardiographic evidence of structural heart disease (EF, LA andLVH)
   Reversal of E/A ratio (<1.0) with Valsalva maneuver.
3. Restrictive pattern (grade iii and iv diastolic dysfunction) –
   DT<160 ms, IVRT <70 ms; E/A >1.5
   PVs2<<PVd
   Mitral A duration <PVa duration,
   PVa velocity increase (>35 cm/s, usually but not always),

2D echocardiographic evidence of structural heart disease, decreased E/A ratio with preload reduction (Valsalva maneuver). PVa, PVd, PVs2, velocity components pulmonary vein).19

**Tissue Doppler imaging**

Tissue Doppler imaging was performed in all patients by a single experienced cardiologist, who was blinded to NAFLD details of participants. Systolic function was evaluated measuring the left ventricular ejection fraction and the systolic myocardial velocity (S’) by tissue Doppler imaging. Diastolic function was assessed by establishing the mitral inflow pulsed-wave Doppler pattern and by measuring the early diastolic myocardial velocity by tissue Doppler (E’), as well as the E/E’ ratio. The mitral inflow was interrogated in apical four chamber view, with the Doppler probe positioned at the tip of the mitral valve in diastole. IVRT was measured in apical five chambers view, with pulsed-wave Doppler probe positioned between the left ventricular outflow tract and the mitral valve, from the aortic valve closing signal to the beginning of the mitral inflow. The main limitation in using mitral inflow for the assessment of diastolic dysfunction is the difficulty to differentiate between the normal aspect of left ventricular relaxation and the pseudo-normalized pattern in moderate diastolic dysfunction, in which left ventricular relaxation is altered and filling pressures are elevated.

LV end diastolic pressure(EDP) was estimated as follows: LVEDP = 11.96 + 0.596.E-to-E’ ratio.20 The time interval between the QRS complex and the onset of mitral E-wave velocity was subtracted from the time interval between the QRS complex and E’ onset to derive T_{E-E’} which strongly depends on the time constant of LV relaxation and minimal pressure. The ratio of IVRT to T_{E-E’} was then calculated; this ratio provides incremental information as to the E/E’ ratio on LV filling pressure in subjects with normal ejection fraction and E-E’ ratio between 8 and 15(21). Global longitudinal strain and strain rate curves were obtained including all six LV myocardial segments from four-chamber, two-chamber, and long-axis apical views. The average values of peak systolic longitudinal strain and peak systolic strain rate from the three apical views were calculated as global longitudinal strain (LSSYS) and global strain rate (SRSYS), respectively. Similarly, the global diastolic strain rate during the early (SRE) and late (SRL) phase of diastole was also calculated. Standard echocardiographic views were obtained using frequency, depth, and sector width adjusted for frame-rate optimization (between 60and 100 frames per second). Tissue Doppler imaging was obtained by placing the Doppler probe at the level of the lateral mitral ring or at the level of the inter-ventricular septum, in four apical chamber view; the obtained velocities are similar, but inverted compared with the traditional mitral inflow by pulsed-wave Doppler, as illustrated in Figure 1.

Diastolic flow is represented by two negative waves: E’ corresponding to diastolic relaxation, simultaneous with the T wave on the ECG, a parameter relatively independent of the filling pressures, and A’ wave – the maximal late annular velocity, corresponding to atrial contraction. E’/A’ ratio is normally >1. The E’ velocity decreases with age, from 9 cm s-1 to 6 cm s-1 in subjects over 60 years old at the level of the inter-ventricular septum, and from 11 cm s-1 to 7 cm s-1 at the level of the lateral mitral ring.

The differentiation of the normal pattern from the pseudo-normal one was based on the Valsalva maneuver, which, in patients with diastolic dysfunction decreases the E/A ratio<1 or at least by 50%. The E’ wave velocities was <8 cm s-1 and the E/E’ ratio>15 in the pseudo-normal pattern, whereas in normal subjects the E’ wave velocity was >10 cm s-1 and the E/E’<8 cm s-1. We used the Data Analysis module of Microsoft Excel 2007 ® for the descriptive statistics and the Student T test, as well as the Fischer test).

**USG abdomen**

Hepatic ultrasonography was performed in all patients by a single experienced radiologist, who was blinded to the participants’ details. Hepatic steatosis was diagnosed on the basis of characteristic sonographic features, i.e., evidence of diffuse hyperechogenicity of the liver relative to the kidneys, ultrasound beam attenuation, and poor visualization of intrahepatic vessel borders and diaphragm.22 It is known that ultrasonography has a good sensitivity and specificity for detecting moderate and severe hepatic steatosis (90–95%), but its sensitivity is reduced when the hepatic fat infiltration upon liver biopsy is, 30%.4,10,20 A semi quantitative ultrasonographic scoring for the degree of steatosis (absent, mild, moderate, and severe) was also performed. The degree of steatosis was assessed by
Table 1: Clinical and biochemical characteristics of type 2 diabetic patients grouped NAFLD status

<table>
<thead>
<tr>
<th></th>
<th>Without NAFLD</th>
<th>With NAFLD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>13</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>7/6</td>
<td>31/26</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.61±11.21</td>
<td>55.10±10.38</td>
<td>0.28</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>22.85±5.48</td>
<td>29.12±5.35</td>
<td>0.44</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.46±10.74</td>
<td>95.05±8.85</td>
<td>0.57</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>9.80±6.38</td>
<td>8.44±9.05</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Hypertension

- Systolic blood pressure (mmHg): 138±12 vs. 139±13, 0.53
- Diastolic blood pressure (mmHg): 81±5 vs. 79±6, 0.19
- Mean blood pressure (mmHg): 100.81±10 vs. 98.9±11, 0.31
- Pulse pressure (mmHg): 59.8±11 vs. 59.4±10, 0.85
- Heart rate (bpm): 75.9±10 vs. 72.2±9, 0.20
- Current smokers: 0.0 vs. 0.0, NS
- Microvascular complications: 32 vs. 30, 0.78
- Carotid stenosis >30%: 42 vs. 58, 0.41
- Oral hypoglycemic agents (%): 58 vs. 48, 0.46
- Insulin (%): 26 vs. 28, 0.68
- ACE inhibitors (%): 54 vs. 64, 0.66
- Calcium channel blockers (%): 38 vs. 42, 0.72
- Diuretics (%): 14 vs. 18, 0.42
- Statins (%): 42 vs. 44, 0.36
- Bl. Urea (mg/dL): 25.06±37 vs. 23.71±17.6, 0.57
- Sr. Creatinine (mg/dL): 0.80±0.34 vs. 0.81±0.25, 0.94
- Fasting glucose (mg/dL): 114.38±19.65 vs. 129.91±34.90, 0.13
- HbA1c (%): 8.53±1.02 vs. 9.72±0.54, 0.0007
- Total cholesterol (mg/dL): 181.69±35.45 vs. 195.05±32.22, 0.23
- LDL cholesterol (mg/dL): 104.46±26.41 vs. 109.85±22.04, 0.46
- HDL cholesterol (mg/dL): 45.3±8.32 vs. 43.5±8.46, 0.43
- Triglycerides (mg/dL): 107.74±27.60 vs. 142.06±55.95, 0.15
- Alanine aminotransferase (units/L): 39.38±11.36 vs. 41.05±14.56, 0.70
- Gamma-glutamyltransferase (units/L): 41.07±11.36 vs. 43.57±14.79, 0.56

Data are means ± SD or percentages unless otherwise indicated.

The fall in echo amplitude with depth (rate of posterior beam attenuation), increasing discrepancy of echo amplitude between liver and kidney, and loss of echoes from the walls of the portal veins. The reproducibility of steatosis scores provided by our single blinded radiologist was very good.

Statistical Methods

Statistical methods used were unpaired student’s t-test and determination of correlation coefficient (r value) between E/A and other variables by using Graph Pad InStat Version 3.10.

A value of p>0.05 is considered as not significant, p<0.05 as mildly significant, p<0.01 as significant, p<0.001 as highly significant, p<0.0001 as very significant.

Results

In this study 70 type-2 diabetes cases of both sexes between 35-65 years of were included.

Subjects were assigned to two groups:

- Group 1: Diabetes patients with NAFLD.
- Group 2: DIABETES patients without NAFLD.

Clinical and biochemical characteristics of participants stratified by NAFLD status are summarized in Table 1.

NAFLD patients had higher plasma triglycerides and HbA1C than those without steatosis. The glycemic control of participants was not so good (mean HbA1C 8.53±1.02%). As expected, they also had higher serum liver enzymes, although the vast majority of our NAFLD patients, i.e. 90%, had serum alanine aminotransferase (ALT) and gamma glutamyl transferase (GTT) concentrations within the reference ranges (normal ranges for alanine amino transference and gamma glutamyl transferase, in our laboratory, were 10–40 units/L for women and 10–50 units/L for men, respectively). Fifty seven patients (81.43%) had NAFLD, and when compared with the other 13 (18.57%) patients, NAFLD patients had lower E’ (8.42±0.89 vs.9.72±0.54, P<0.0001) tissue velocity, higher E’ to E ratio (9.64±1.83 vs. 7.78±0.89, p<0.001), higher LV–end diastolic pressure (EDP) (15.52±0.69 vs. 14.40±0.9, P<0.0001), higher LV EDP/EDV (mmHg/mL) (1.44±0.10 vs. 1.46±0.10, 0.96 NS).

Arterial elasticity (mmHg/mL): 0.66±0.30 vs. 0.64±0.10, 0.96 NS.

SAC index: 247±149.84 vs. 247±149.84, 0.88 NS.

LV systolic pressure (mmHg): 15.52±0.69 vs. 14.40±0.9, P<0.0001.

LV EDP (mmHg): 15.52±0.69 vs. 14.40±0.9, P<0.0001.

LV EDP/EDV (mmHg/mL): 1.44±0.10 vs. 1.46±0.10, 0.96 NS.

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SAC index: 247±149.84 vs. 247±149.84, 0.88 NS.
found in LV EDVs and end systolic volumes and ejection fraction, indexed LV mass, indexed left atria 1 volume. Thus, the final results of this study can be applied to the general population.

Conclusions and discussions

In the metabolic syndrome and type 2 diabetes mellitus diabetic dysfunction is the consequence of metabolic abnormalities and structural remodeling, which result in abnormal relaxation and increased myocardial rigidity (Boudina and Dale 2007). The multiple metabolic abnormalities are the result of insulin signaling, glycol-lipotoxicity and increase in interstitial fat deposits, affecting consequently the myocardial function at tissue level.6 Endothelial dysfunction is associated as well to these abnormalities, resulting in vascular remodeling and systemic and coronary atherosclerosis. This translates in an increase in arterial rigidity, high blood pressure and pulse pressure. There is an increase in afterload and myocardial oxygen demands, with a decrease in myocardial perfusion, thus diminishing cardiac efficacy. As a consequence, myocardial hypertrophy, autonomic dysfunction and diastolic dysfunction develop, as the first stage of diabetic cardiomyopathy. In late stages, diastolic dysfunction worsens as a result to structural myocardial abnormalities, as cardiac steatosis, interstitial fibrosis and the alterations in extracellular matrix.15 NAFLD is defined as the fatty infiltration of the liver in patients without significant alcohol consumption, with a spectrum ranging from simple steatosis to steatohepatitis, histologically similar to alcoholic hepatitis, with a possible progression to end stage liver disease (Ludwig et al 1980). Considered at first an incidental finding, NAFLD is accepted today as a component of the metabolic syndrome, associated with significant cardiovascular risk factors like obesity, hypertension, dyslipidemia, diabetes mellitus and insulin resistance.16 Moreover, NAFLD may be an independent cardiovascular risk factor as well as a detrimental factor for the severity of the other elements of the metabolic syndrome associated with it.16

Our study showed that NAFLD in type 2 diabetes mellitus was associated with a higher frequency of diastolic dysfunction compared to diabetic patients without NAFLD.

These data suggest the fact that NAFLD, regardless of the presence of diabetes, further impairs the stiffness and relaxation of the left ventricle. This translates in earlier development of diastolic heart failure and a higher cardiovascular risk.

Data from studies performed on small groups of patients have shown that patients with NAFLD, in the absence of other cardiovascular risk factors included in the metabolic syndrome, present alterations in left ventricular geometry and early diastolic dysfunction.5 Other studies have shown that NAFLD is associated with a significant decrease in E’ wave velocity, this parameter being described as the only one to correlate with NAFLD.37 In another study, diastolic dysfunction and insulin resistance were independently associated with NAFLD, in multivariate analysis (Brea et al 2005). Poanta et al showed that patients with type 2 diabetes and NAFLD did not present an abnormal intima-media thickness, a known risk factor for cardiovascular events, but their study was performed on a small number of subjects (Poanta et al 2011).

In conclusion, in patients diagnosed with NAFLD the assessment of diastolic dysfunction by tissue Doppler imaging detects early changes in myocardial stiffness and compliance, which precede the late stages of myocardial dysfunction.

Acknowledgment

We have not received substantial contributions from non-contributors and no contributor has been omitted.

Ethical Clearance

This study has been conducted in M.B.S. and associated group of hospitals of Govt. Medical College, Kota. This study had been done originally by us after consent from patient and approval of ethics committee. Written informed consent was obtained from the patient and parents for publication of this case, reports and any accompanying images. We are ensuring that, this study manuscript has not been submitted and published elsewhere.

References