Insulinemic Status and Nonalcoholic Fatty Liver Disease in Type 2 Diabetes Mellitus

Authors
Dr Azmeri Alam¹, Professor Dr Liaquat Ali², Professor Dr Rahelee Zinnat³, Zebunnessa Zeba⁴
¹Associate Professor, Department of Biochemistry, Green Life Medical College, Dhaka, Bangladesh
²Ex-Professor and Head, Department of Biochemistry and Cell Biology, Biomedical Research Group, BIRDEM, Bangladesh
³Department of Biochemistry and Cell Biology, BUHS, Bangladesh
⁴Assistant Professor, Department of Public Health and Informatics, Jahangirnagar University, Savar, Bangladesh

Corresponding Author
Dr Azmeri Alam

Abstract
Introduction: In this article, non-alcoholic fatty liver disease (NAFLD) is discussed in detail. The epidemiology of NAFLD nonalcoholic steatohepatitis and the relationship of NAPLD with type 2 diabetes mellitus insulinemic resistance are broadly discussed. NAFLD incorporates histologically and clinically different non-alcoholic entities fatty liver (NAFL, steatosis hepatis) and steatohepatitis (NASH characterized by hepatocyte ballooning and lobular inflammation + fibrosis) might progress to cirrhosis and rarely to hepatocellular cancer. Type 2 diabetes mellitus (T2DM), insulin resistance (IR), obesity and NAFLD are particularly closely related. Type 2 diabetes mellitus (T2DM) and nonalcoholic fatty liver disease (NAFLD) commonly exist together. Age is major determinant of NAFLD. As Body mass index and waist-to-hip ratio were not independently associated with nonalcoholic fatty liver diseases in our study, suggesting that obesity might also be effects of insulin resistance. Insulin resistance leads to foot accumulation in hepatocytes by lipolysis and hyperinsulinemia.

Objective: The objective of the study was to find out the association of NAFLD with insulinemic status in type 2 DM subjects.

Results: The present data leads to the suggestion that there is a strong association between insulin resistance and NAFLD. However, the question of whether hyperinsulinemia or insulin resistance is the primary disorder of NAFLD remains unangered. Further studies are needed to elucidate interrelationship between NAFLD, metabolic syndrome and insulin resistance.

Keywords: Cirrhosis, insulin resistance, metabolic syndrome, nonalcoholic fatty liver, type 2 diabetes mellitus.

Introduction
A heterogenous group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defeats in insulin secretion, insulin action or both is known as...
Diabetes mellitus of DM This disease can be recognized either during less overt stages of characterized by fasting hyperglycemia, mostly by the presence of glucose intolerance. DM includes long-term damage, dysfunction and failure of various organs such as the eyes, kidneys, heart and blood vessels. Compared with the general population diabetes mellitus confers a 2- to 4-fold increase in cardiovascular risk. Macro vascular complications, including coronary artery disease, often cause death, although micro vascular complications of diabetes result in increased rates of morbidity.

The majority of cases of diabetes can be classified into two broad etiopathogenetic categories, called type 1 and type 2 diabetes, but the extent of heterogeneity among these types remains uncertain because of the increasing number of types of diabetes for which a specific etiology can be recognized, the current clinical classification, proposed by the American Diabetic Association (ADA) in 1997 and adopted by the World Health Organization (WHO) in 1999 and that supersedes the previously internationally recognized 1985 WHO classification (WHO Study Group 1985) now classifies diabetes according to both clinical stages and etiologic types.

The pathophysiology of type 2 diabetes mellitus is a field of rigorous investigations. Type 2 diabetes is well characterized by defects in insulin secretion, insulin action, free fatty acids (FFA) and fat distribution. The progressive deterioration of pancreatic insulin secretion has been implicated as the proximate cause of the progressive increase in plasma glucose level. Thus decrease in insulin secretion is a major contributor to the development of the overt type 2 DM state.

In a landmark 1980 article, a “hitherto unnamed disease that mimics alcoholic hepatitis” was described by Ludwig and colleagues and was named nonalcoholic steatohepatitis. NASH has since been recognized as part of a spectrum of hepatic disease known as nonalcoholic fatty liver disease (NAFLD) that occurs in people who consume little or no alcohol. Including NAFLD ranks the fifth most common cause of death after heart disease, stroke, pulmonary disease and cancer.

Obesity is an important factor determining the severity of insulin resistance in type 2DM.

Obesity may trigger insulin resistance through the accumulations of excess lipid within liver and skeletal muscle. It is still unclear whether insulin resistance causes NAFLD. Though both insulin secretory defect and insulin resistance are present in our population but insulin secretory defect seems to be primary defect in Bangladeshi type 2 DM patients. As NAFLD has been found to be associated with insulin resistance in the studies on western population the present has been undertaken to find any association of NAFLD with insulin secretory defect as well as with insulin resistance and also to find to find the prevalence of NAFLD in Bangladeshi population.

Objective of the study
The general objective of the study was to find out the association of NAFLD with insulinemic status in type 2 DM subjects.

Subjects and Methods
Place and period of the study
Study Place: A group of 256 T2DM subjects were included recruiting from the Out-Patient and In-Patient, Departments of the BIRDEM hospital.

The Study subjects:
1) T2DM with Fatty Liver: 127 as study subjects.
2) T2DM without Fatty Liver: 129 as control subjects.
3) The two groups well matched for age.
4) Adult subjects with age ranging from 30-55 years.
5) Voluntarily agreed to include in this study by providing informed consent.

Data Collection Form
A Data Collection Form was developed to obtain relevant demographic and socio-economic data
such as age, educational status, and occupational status. The Form also included anthropometric data, drug and medical history and clinical information.

**Recruitment of the subjects**
Subjects were recruited on everyday of the week from 11-00 noon to 2-00 PM from the Out-Patient Department (OPD) and indoor of BIRDEM who came for checking their glycemic status and were advice to take unrestricted carbohydrate diet to do normal physical activities and to avoid drugs that significantly interfere with blood glucose level for three days.

**Methods**

**Anthropometric measurements**
Anthropometric measurements including height (m), body weight (kg), waist circumference (cm), hip circumference (cm), skin fold thicknesses (mm), mid arm circumference were measured using standardized techniques. Body mass indexes (BMI) of the subjects were calculated using standard formula. BMI= Weight (kg)/ [Height (m)]2.

**Body fat mass (%)**
Body fat mass percentage of body mass was measured by Omron Body Fat Monitor (Omron Corporation, Japan) with standard procedure.

**Recording of blood pressure**
Blood pressure was measured in sitting position, with calf at the level of the heart. After 10 minutes of rest a second reading was taken. Recorded Korotkoff sound I (the first sound) and V (the disappearance of sound) denoted the systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively (according to WHO-ISH).

**Diagnosis of NAFLD**
Patients were diagnosed by 4D ultrasonography

**Collection of blood samples**
Fasting blood was collected between 8.00-9.00 am. Venous blood (05 ml) was taken with the subject sitting comfortably in a chair.

**Analytical methods and lab analysis**
1) Glucose (fasting) was measured by Glucose Oxidase (GOD-PAP) method (Randox Laboratories Ltd., UK) (Appendix I).
2) Total cholesterol by enzymatic endpoint method (Cholesterol Oxidase/ Peroxidase) method (Randox Laboratories Ltd., UK) (Appendix II).
3) Triglyceride by enzymatic colorimetric (GPO-PAP) method (Randox Laboratories Ltd., UK) (Appendix III).
4) HDL cholesterol by enzymatic colorimetric (cholesterol CHOD-PAP) method (Randox Laboratories Ltd., UK) (Appendix IV).

**Variables**
5) Outcome variables: NAFLD
6) Independent variables: Serum glucose, Serum insulin, HOMA% B, HOMA% S.
7) Confounding variables: Age, BMI, Lipids.

**Statistical Analysis**
Data were expressed as mean ±SD and/ or number where appropriate. Comparison between two groups was done using Students unpaired ‘t’ tests and Mann–Whitney U test. To adjust the effects of confounder variable logistic regression analysis was done with NAFLD and without NAFLD as dependent variables.

**Results**

**Anthropometric and Clinical characteristics of the study subjects**

**Age(years)**
Mean (±SD) age of T2DM with fatty liver and T2DM without fatty liver were 43.33±6.36 and 42.89±5.85 respectively. The value did not show any statistically significant difference (p=0.559) (Table-1)

**Body mass index (BMI, kg/m2)**
Mean (±SD) BMI of T2DM with fatty liver and T2DM without fatty liver were 26.19±3.69 and 25.32±3.47 respectively. The value did not show any statistically significant difference (p=0.053) (Table-1)
Waist-hip ratio (WH ratio)
Mean (±SD) WH ratio of T2DM with fatty liver and T2DM without fatty liver were 0.923±.060 and 0.918±.074 respectively. The value did not show any statistically significant difference (p=0.589) (Table-1)

Systolic blood pressure (mm Hg)
Mean (±SD) SBP of T2DM with fatty liver and T2DM without fatty liver were 120.78±12.47 and 120.38±12.47 respectively. The value did not show any statistically significant difference (p=0.798) (Table-1)

Diastolic blood pressure (mm Hg)
Mean (±SD) DBP of T2DM with fatty liver and T2DM without fatty liver were 80.39±7.28 and 80.31±7.38 respectively. The value did not show any statistically significant difference (p=0.927) (Table-1)

Table 1: Anthropometric measurement of the study subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fatty Liver(n= 127)</th>
<th>Control (n= 129)</th>
<th>t/p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>43.33±6.36</td>
<td>42.89±5.85</td>
<td>0.585/0.559</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.19±3.69</td>
<td>25.32±3.47</td>
<td>1.945/0.053</td>
</tr>
<tr>
<td>W/H</td>
<td>0.923±.060</td>
<td>0.918±.074</td>
<td>0.540/0.589</td>
</tr>
<tr>
<td>SBP(mmHg)</td>
<td>120.78±12.47</td>
<td>120.38±12.47</td>
<td>0.256/0.798</td>
</tr>
<tr>
<td>DBP(mmHg)</td>
<td>80.39±7.28</td>
<td>80.31±7.38</td>
<td>0.091/0.927</td>
</tr>
</tbody>
</table>

Results were expressed as mean±SD. Unpaired students t test was performed to compare between groups.T2DM-Type2 diabetes mellitus, BMI-Body Mass Index, WHR- Waist Hip Ratio, SBP- Systolic Blood Pressure, DBP- Diastolic Blood Pressure, n-Number of subjects.

Biochemical parameters of study subjects

Fasting serum glucose (m mol /l)
Median (Range) value of fasting serum glucose of T2DM with fatty liver and T2DM without fatty liver were 6.70(3.60—23.0) and 5.10(4.0-5.90) respectively. The value did not show statistically significant difference (p=0.260)

SGPT (U/L)
Median (Range) value of SGPT of T2DM with fatty liver and T2DM without fatty liver were 36(10-150) and 23(10-324) respectively. The value shows statistically significant difference (p=0.00).

Fasting serum insulin (mmole/l)
Median (Range) value of fasting serum insulin of T2DM with fatty liver and T2DM without fatty liver were 19.8(3.0-81.6) and 16.67(3-100.60) respectively. The value shows statistically significant difference (p=0.005).

HOMA%B
Median (Range) value of HOMA%B of T2DM with fatty liver and T2DM without fatty liver were 108.9 (6.30-462.70) and 90.8(11.1-451.3) respectively. HOMA%B was significantly higher in control subjects compared to T2DM subjects (p=0.014).

HOMA%S
Median (Range) value of HOMA%S of T2DM with fatty liver and T2DM without fatty liver was 31.9(5.8-212.10) and 34(6.8-190.8) respectively. Significant difference was present in case of insulin sensitivity between diabetic and control subjects (p=0.058).

Table 2: Biochemical parameters of the study subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fatty Liver(n= 127)</th>
<th>Control (n= 129)</th>
<th>z/p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mmol/l)</td>
<td>6.70 (3.60-23.0)</td>
<td>7.1(4.0-18.70)</td>
<td>1.12/0.260</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>36 (10-150)</td>
<td>23 (10-324)</td>
<td>5.05/0.0</td>
</tr>
<tr>
<td>INSULIN (IU/L)</td>
<td>19.8 (3.0-81.6)</td>
<td>16.67 (3-100.60)</td>
<td>2.83/0.005</td>
</tr>
<tr>
<td>HOMAB%</td>
<td>108.9 (6.30-462.70)</td>
<td>90.8 (11.1-451.3)</td>
<td>2.46/0.014</td>
</tr>
<tr>
<td>HOMAS%</td>
<td>31.9 (5.8-212.10)</td>
<td>34 (6.8-190.8)</td>
<td>1.89/0.046</td>
</tr>
</tbody>
</table>
Results are expressed as median (range). Mann–Whitney U test was performed as the test of significance at 5% significance level. n= number of subjects, HOMA%B=B cell function assessed by homeostasis model assessment, HOMA%S= insulin sensitivity by homeostasis model assessment

**Serum lipid level**

*Triglyceride (TG mg / dl)*

Median (Range) value of TG of T2DM with fatty liver and T2DM without fatty liver was 165(60-998) and 170(50-787) respectively. The value did not show statistically significant difference (p=0.998).

*Total cholesterol (TC, mg/dl)*

Median (Range) value of TC of T2DM with fatty liver and T2DM without fatty liver was 181(50-351) and 179(80-242) respectively. The value did not show statistically significant difference (p=0.299).

*High density lipoprotein cholesterol (HDL-C, mg/dl)*

Median (Range) value of HDL-C of T2DM with fatty liver and T2DM without fatty liver was 29(11-108) and 30(16-75) respectively. The value did not show statistically significant difference (p=0.16).

*Low density lipoprotein cholesterol (LDL-C, mg/dl)*

Median (Range) value of LDL-C of T2DM with fatty liver and T2DM without fatty liver was 115(26-269) and 109(11-242) respectively. The value did not show statistically significant difference (p=0.299).

### Table 3: Lipid profile of the study subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fatty Liver (n= 127 )</th>
<th>Control (n= 129)</th>
<th>z/p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>165 (60-998)</td>
<td>170 (50-787)</td>
<td>0.003/0.998</td>
</tr>
<tr>
<td>T cholesterol (mg/dl)</td>
<td>181(50-351)</td>
<td>179 (80-350)</td>
<td>0.76/0.44</td>
</tr>
<tr>
<td>HDL-c (mg/dl)</td>
<td>29 (11-108)</td>
<td>30 (16-75)</td>
<td>1.3/0.16</td>
</tr>
<tr>
<td>LDL-c (mg/dl)</td>
<td>115 (26-269)</td>
<td>109 (11-242)</td>
<td>1.03/0.299</td>
</tr>
</tbody>
</table>

Results were expressed as median (range). Mann-Whitney U test was performed and the test of significance at 0.05 significance level. n=number of subjects, T2 DM=Type 2 Diabetes Mellitus, TG =triglyceride, TC = Total Cholesterol, HDL-C= High Density Lipoprotein Cholesterol, LDL-C= Low Density Lipoprotein Cholesterol.

**Logistic regression analysis**

In logistic regression analysis taking Fatty liver as a dependent variable, Age, BMI= Body Mass Index, HOMA%S, HOMA%B, Age and Body Mass Index as independent variables a positive significant association was found with HOMA%B (p=0.032) and HOMA%S (p= 0.033).

### Table 4: Logistic regression analysis taking NAFLD/without NAFLD as a dependent variable and other parameters as independent variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>β value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.009</td>
<td>0.678</td>
</tr>
<tr>
<td>BMI</td>
<td>0.076</td>
<td>0.038</td>
</tr>
<tr>
<td>HOMA%S</td>
<td>0.010</td>
<td>0.033</td>
</tr>
<tr>
<td>HOMA%B</td>
<td>0.004</td>
<td>0.032</td>
</tr>
</tbody>
</table>

BMI= Body Mass Index, HOMA%B=B cell function assessed by homeostasis model assessment, HOMA%S= insulin sensitivity by homeostasis model assessment

**Discussion**

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function and is characterized by hepatic steatosis in individuals with little or no alcohol consumption. The spectrum of NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), which can progress to end-stage liver disease. NAFLD is commonly associated with obesity, type 2 diabetes, dyslipidemia, and insulin...
resistance, all of which are components of the metabolic syndrome, strongly supporting the notion that NAFLD is the hepatic manifestation of the syndrome (Angulo 2002 Day, 2006 and McCullough, 2006). The prevalence of NAFLD has been reported to be in the 15–30% range in the general population in various countries.

In a population-based study, waist circumference was found to be an independent risk factor for NAFLD. Rocha (2005) and Salgodo (2006), found that presence of NAFLD correlates significantly with both BMI and waist hip ratio. But In logistic regression analysis no correlation was observed between any parameters of lipid profile and presence of NAFLD.

The fasting glucose did not show any significant difference among NAFLD and non NAFLD group, and in logistic regression analysis FBG did not show any association with the presence of NAFLD.

Giulio Marchesini, et al. also found that nonalcoholic fatty liver disease was closely associated with insulin resistance, independent of body mass index and fat distribution. They suggest that in nonalcoholic fatty liver disease, insulin resistance may be a primary phenomenon, possibly in addition to obesity-associated insulin resistance, whereas in normal subjects sensitivity to insulin may depend primarily on obesity.

Insulin resistance leads to fat accumulation in hepatocytes by lipolysis and hyperinsulinemia. Recently, the cytokine–adipokine interaction related to NAFLD is increasingly drawing great attention to elucidate the underlying mechanism. Emerging lines of studies indicated that insulin resistance, abnormal lipid metabolism, and dysregulation of cytokines/adipokines (e.g., tumor necrosis factor-alpha, adiponectin, and leptin) are profoundly involved in the pathogenesis of NAFLD.

The present data leads to the suggestion that there is a strong association between insulin resistance and NAFLD. However, the question of whether hyperinsulinemia or insulin resistance is the primary disorder of NAFLD remains unanswered. Further studies are needed to elucidate the interrelationship between NAFLD, metabolic syndrome and insulin resistance.

**Conclusion**

From the present data it may be concluded that a greater degree of hyperinsulinemia and insulin resistance are present in T2DM subjects and this association is independent to obesity and glycemic lipiclimic status.

**References**