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Core Components of the Metabolic Syndrome in Nonalcohlic Fatty Liver Disease

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Abstract:
Objectives: *The present study was undertaken to explore whether MetS and its individual components are associated with NAFLD.*
Materials and methods: *67 diagnosed NAFLD subjects and 50 healthy controls were included. NAFLD was diagnosed by ultrasound and serum C-peptide was measured by Immulite technique.*
Results: *The NAFLD and control subjects were age and BMI- matched (age in years, p=0.380 and BMI in kg/m2,p=0.763). On logistic regression analysis, taking NAFLD as the dependent variable a significant association was found with low HDL (p<0.001), WHR, PSG and dyslipidemia (p=0.019, p=0.031, p=0.001 respectively).*
Conclusions: *Nonalcohlic fatty liver disease (NAFLD) does not seem to be associated with MetS in Bangladeshi population as defined by the 3 major criteria provided by IDF, ATP III and WHO. Various components of MetS like central obesity, dyslipidemia, hypertension, and diabetes are associated with NAFLD. However, they do not seem to cluster in the manner as predicted by IDF, ATP III and WHO criteria.*
Key words: *Nonalcohlic fatty liver disease (NAFLD), Metabolic syndrome (MetS), Insulin resistance, ELISA*

I. Background:

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide. The estimated prevalence of NAFLD may be as high as one third in the general population.[1] NAFLD encompasses a spectrum of liver lesions occurring in the absence of significant alcohol intake[2]. The level of alcohol consumption in NAFLD definition, the maximum level of alcohol intake agreed by the National Institutes of Health (NIH) Clinical Research Network on NAFLD/NASH is one standard drink a day for women (70 g ethanol/week), and two standard drinks a day for men (140 g ethanol/week).[3]

Liver enzyme levels in NAFLD patients fluctuate, normal values being present in up to 78% of patients at any one time.[4] Liver enzyme levels do not reliably correlate with liver histology, and the full range of disease may be seen in patients with NAFLD who have normal transaminase level.[5]

Ultrasonography and CT and MRI scanning are reliable for detecting moderate to severe fatty changes in the liver. Hepatic fat causes increased echogenicity on ultrasound, compared with the lower echogenicity of the spleen or renal cortex.[6.7.8] Ultrasound is a powerful and useful diagnostic tool in the detection of NAFLD. It is an accurate and reliable method that can reduce the need for liver biopsy in the appropriate clinical setting.[9]

An association between NAFLD and MetS has been claimed finally in some retrospective and prospective studies.[10] As metabolic syndrome is a group of disorders centrally linked by insulin resistance, fatty liver is generally regarded as part of the metabolic syndrome.[11, 12] Current evidence suggests that both peripheral and hepatic insulin resistance are implicated in hepatic fat accumulation by a combination of increased peripheral lipolysis and increased visceral fat stores. In the last decade, several studies have demonstrated independently a strong association between NAFLD and each component of the metabolic syndrome, including central obesity, hypertriglyceridemia and mixed hyperlipidemia, type II diabetes mellitus and hypertension. Dyslipidemia and insulin resistance both are strongly associated with the presence of NAFLD. In 2005, Jimba et al.,[13]detected NAFLD by ultrasound in 62% of patients newly diagnosed with type 2 diabetes mellitus. Prevalence of MetS in Europe varies from 12-26% depending on geographical area, urbanisation and ethnic mix. Studies in Asia, suggest the prevalence is 5-20%, with an overall global prevalence of around 16% of the adult population.[14] Prevalence in India appears to be highest, at around 26% of the adult urban population and prevalence appears to be increasing as obesity rates and urbanisation increase.[14,15] A study on Prevalence of Metabolic Syndrome in Rural Bangladeshi Women done by Zaman et al., 2006 and was found that the

combined effect of good physical activity level and a very low prevalence of smoking may partly explain the low prevalence of metabolic syndrome in this rural population of Bangladesh. The association of NAFLD with MetS is still a debated issue and their causal relationship is yet unsettled. As the increasing prevalence of obesity coupled with diabetes, dyslipideamia, hypertension and ultimately metabolic syndrome put a very large population at risk of developing NAFLD in the coming decade.[10] However, ethnic variation and the multiple definition of MetS due to different criteria proposed by IDF, ATP III and WHO have complicated the interpretation of the interrelationship of these orders. So it is necessary to conduct a large scale study about association of MetS and its individual components with NAFLD which has not been done yet in Bangladesh. The present study has been undertaken in the above perspective.

II. Research Design and Methods

The study was conducted in the Biomedical Research Group and Department of Biochemistry & Cell Biology of the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka. Diagnosed patients of NAFLD (by ultrasonography) were collected from the Dept of Radiology & Imaging, BSMMU. This study was done during the period of January 2010 to December 2010. The inclusion criteria were as follows: (1) NAFLD with age ranging from 25-55 years by ultrasonogram, (2) Controls were also with age as NAFLD selected through negative finding after an ultrasonogram of hepatobilliary system by the same equipments. Exclusion criteria were as follows: (1) Alcohlic, (2) Persons with known renal disease, (3) Patients with serious co-morbid diseases (severe infection, stroke, myocardial infarction, major surgery, mal-absorption etc), (4) Persons having any other known liver disease other than fatty change, (5) Persons with a positive serological findings for hepatitis B or C virus, (6) Pregnant women and lactating mothers, (7) Smokers. Finally, 67 NAFLD and 50 control subjects were enrolled in this study. The laboratory evaluation included measurement of the serum fasting glucose by glucose-oxidase method and serum Triglyceride and serum HDL cholesterol by enzymatic-colorimetric method, serum total Cholesterol by enzymatic endpoint (Cholesterol Oxidase/Peroxidase) method. Insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were analyzed by Homeostasis Model Assessment. Serum C-peptide was measured by IMMULITE technique.

Statistical Analysis: Data were managed and statistical analyses were performed using Statistical Package for Social Science (SPSS) for Windows version 11.5.

III. Results:

Among 67 NAFLD and 50 control subjects were age- and BMI- matched (age in years, M±SD; 41±8 vs 39±7, p=0.380 and BMI in kg/m^2, M±SD; 20±3.1 vs 20±3.0, p=0.763) (Table: 1). As expected, patients with NAFLD were more likely to have insulin resistanc than controls (p=0.04), Serum TG was significantly higher in NAFLD subjects [Mean (±SD), mg/dl, 226.97±186.0 vs 144.70±91.60, p=0.02] and HDL cholesterol was significantly lower in NAFLD subjects [Mean (±SD), mg/dl, 38.92±8.45 vs 31.50±6.07, p=0.0001] (Table: 1). To further examine, no significant association was found between the metabolic syndrome and NAFLD in Z- test of proportion (Table: 2). Proportion of MetS according to IDF criteria was 40.29% in NAFLD and 34.0% in Control. However, Metabolic syndrome by ATP III criteria was 28.35% in NAFLD and 40.0% in control and by WHO criteria it was 22.38% in NAFLD and 26.0% in control (Table: 2). Compaired with the normal group, from 67 NAFLD patients, 41 of increased serum triglyceride and from 50 controls subjects, 20 were found with increased S TG level above normal. So, the percentage of increased S TG level above 61% NAFLD patients had higher TG but only 40% controls had higher value ($P < 0.05$) (Table: 3). In case of serum HDL, 95% male and 95% female NAFLD had HDL cholesterol value lower than the cut point (Table: 3). Regarding waist circumference, according to ATPIII criteria, 14.8% male NAFLD and none of the control males were above the cut of value ($P < 0.05$), but 77.5% female NAFLD and 67.7% female controls had higher levels (Table: 3). On logistic regression analysis, taking NAFLD as the dependent variable and age, sex, BMI, postprandial glucose, serum TG, total cholesterol, HOMA%S, HDL cholesterol as independent variables, a significant association was found with low HDL- cholesterol (p=0.0001) (Table: 4). In another logistic regression analysis taking NAFLD as the dependent variable and WHR, FSG, PSG, dyslipidemia, HTN, HOMA%S as independent variables a significant association was found with WHR, PSG and dyslipidemia (p=0.019, p=0.031, p=0.001 respectively)(Table: 5).

Table: 1 Anthropometric, clinical and biochemical parameters of study subjects related to metabolic syndrome

Variables	Control (n=50)	NAFLD (n=67)	t/p value
Age (yrs)	39.80±7.14	41.07±8.15	0.881/0.380
BMI (kg/m2)	20.20+3.10	20.37+3.07	0.303/0.763
WHR	0.90+0.04	0.93+0.05	2.95/0.004
Waist-C (cm)	90.16+ 9.4	94.55+10.00	2.41/0.017
SBP (mmHg)	114+12	114+10	0.146/0.884
DBP (mmHg)	74+9	75+8	0.367/0.714
FSG (mmol/l)	5.41+2.16	6.01+2.7	1.27/0.6014
PSG (mmol/ l)	7.90+4.38	10.13+5.35	2.4/0.015
HOMA%S	110.39+52.97	82.32+56.55	2.03/0.04
S TG (mg/dl)	144.70±91.60	226.97±186.07	03.14/.02
HDL-C (mg/dl)	38.92±8.45	31.50±6.07	5.27/0.0001

Results were expressed as mean ±SD. Unpaired students t test was performed to compare between groups. NAFLD=Non Alcoholic Fatty Lever Disease, n-Number of subjects, BMI=Body Mass Index, WHR=WaistHipRatio,SBP=SystolicBloodPressure,DBP=DiastolicBloodPressure,FSG=FastingSerumGlucose,PSG= Postprandial Serum Glucose, SGPT=Serum Glutamate Pyruvate Transaminase, HOMA%S=Insulin Sensitivity by homeostasis model assessment, S TG=Serum Triglyceride, T Chol=Total Cholesterol, HDL-C= High Density Lipoprotein Cholesterol, LDL-C=- Low Density Lipoprotein Cholesterol

Table 2: Proportion of Metabolic Syndrome in the study subjects according to IDF , ATP III and WHO criteria in study subjects.

Parameters	Control (n=50)	NAFLD (n=67)	Z/p Value
Frequency of MetS-IDF	17(34.0%)	27(40.29%)	Z=0.70 p > 0.05
Frequency of MetS-ATP III	20(40.0%)	20(40.0%)	Z=1.31 p > 0.05
Frequency of MetS-WHO	13(26.0%)	15(22.38%)	Z=0.45 p > 0.05

Data are expressed in No (%).

Table 3: Proportion of serum HDL, serum TG, PSG and Waist Circumference (according to ATPIII criteria) in study subjects

Parameter	Control (n=50)	NAFLD (n=67)	Z/p Value
Frequency of Serum HDL <40mg/dl in male (46)	16(82.4%)	25(95.00%)	Z=1.31.p>0.05
Frequency of Serum HDL <50mg/dl in female (71)	27(87.1%)	38(95.0%)	Z=0.16,p>0.05
Frequency of serum TG≥150 mg/d	20(40.0%)	41(61.2%)	Z=2.32,p<0.05
Frequency of PSG >7.8mmol/l	14(28.0%)	33(49.3%)	Z=2.41,p<0.05
Frequency of WC > 102 cm in male (46)	0.0001	4(14.8%)	Z=2.17,p<0.05

Data are expressed in No (%)

Table: 4 Logistic regression analysis taking NAFLD as dependent variable and AGE, SEX, BMI, PSG, S TG, T Chol, HOMA%S and HDL-C as independent variables

Variable	B Value	P Value
Age	0.014	0.655
Sex	0.232	0.649
BMI	0.013	0.882
PSG	0.094	0.104
S TG	0.000	0.924
T Chol	0.007	0.291
HOMA%S	0.003	0.364
HDL-C	0.157	0.0001

NAFLD=Nonalcohlic Fatty Liver Disease, WHR=Waist Hip Ratio, FSG=Fasting Serum Glucose, PSG=Postprandial Serum Glucose, HTN=Hypertension, HOMA%S=Homeostatic Model Assessment for Insulin Sensitivity

Table: 5 logistic regression analysis taking NAFLD as dependent variable and WHR, FSG, PSG, Dyslipidemia, HTN, HOMA%S as independent variables.

Variables	B Value	P Value
WHR	11.137	0.019
FSG	0.283	0.087
PSG	0.189	0.031
Dyslipidemia	1.750	0.001
HTN	20.712	0.998
HOMA%S	0.0001	0.929

NAFLD=Nonalcohlic Fatty Liver Disease, WHR=Waist Hip Ratio, FSG=Fasting Serum Glucose, PSG=Postprandial Serum Glucose, HTN=Hypertension, HOMA%S=Homeostatic Model Assessment for Insulin Sensitivity.

IV. Discussion:

In the present study all three major criteria (IDF, ATP III, WHO) were used to diagnose MetS and its association with NAFLD (as diagnosed by ultrasonography) was explored. Although liver biopsy is the gold standard for the diagnosis of fatty liver but it is an invasive and expensive tool.[16]
Ultrasonography represents a non-invasive, inexpensive and widely available method, useful for the detection of liver steatosis with a sensitivity of 60–94% and a specificity of 66–95%; it also leads to a subjective estimation of the entity of fatty infiltration on a three or more point scoring system (mild, moderate and severe).[17]

Although NAFLD did not show any significant association with MetS in the present study, the association of the syndrome with some individual components of MetS was quite obvious. On group difference analysis using means (Tables 1), the NAFLD group showed significant higher value regarding waist-hip ratio, waist circumference, serum triglyceride and it showed significant lower value regarding HOMA%S (an indicator of insulin sensitivity) and HDL-cholesterol. These all are in conformity with the findings in other populations and all these factors have been implicated in the pathophysiology of NAFLD.[3,18]

In the present study no significant difference was found between NFALD patients and the control subjects regarding fasting blood glucose levels, but 27% of NAFLD patients had higher fasting glucose level than the cut point defined by ATP III and 36% NAFLD subjects exceeded the cut point defined by IDF for fasting blood glucose level (Tables 3). In case of postprandial glycaemia the NFALD group had significantly higher postprandial glucose level than the controls. It is also evident by the percentage where 49% of NAFLD subjects had higher postprandial blood glucose above the cut point defined by WHO and only 28% controls had glucose intolerance (Tables 3). This finding suggests that the NAFLD patients of this study had postprandial glucose intolerance which is one of the important features of metabolic syndrome defined by WHO.

A study in 2005 by Fan et al., demonstrated that obesity, abdominal obesity, diabetes mellitus, dyslipidemia, hypertension and cholelithiasis increased the risk for fatty liver by 28.49-, 38.15-, 36.72-, 26.35-, 27.02- and 23.49-fold, respectively, indicating that abdominal obesity is more closely related with fatty liver than are the other factors.[10] However, patients with metabolic syndrome had the highest the risk for fatty liver (RR=39.33) and as many as 48.21% of patients suffering from metabolic syndrome also had fatty liver. The prevalence of abdominal obesity, impaired fasting glucose, low HDL-C, hypertriglyceridemia and hypertension in subjects with fatty liver were 71.71, 42.36, 34.03, 47.2 and 70.2%, respectively. They suggested that fatty liver is closely related with multiple metabolic disorders and that perhaps fatty liver (independent of obesity) should be regarded as one of the components of metabolic syndrome and that fatty liver patients with metabolic disorders may be at increased risk of cardiovascular events.[19]

The NAFLD patients had significantly higher insulin resistance than the controls. But no significant secretory defect was found in these patients. Since there are practical difficulties in accurately measuring insulin

resistance, it was omitted as a component of metabolic syndrome.The lack of association of NAFLD with MetS (as cluster of risk factors) raises the fundamental issue on the usefulness of the concept for clinical decision making.[20,21] Nevertheless, its association with individual components suggests the important role of prevention through a two-pronged approach for targeting both NAFLD and MetS.

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