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Prevalence and Predictor of Nonalcoholic Steatohepatitis (NASH) in Nonalcoholic Fatty Liver Disease (NAFLD)

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Summary:

Fatty liver is a common cause of chronic liver disease in developed as well as developing countries.We have designed this study to estimate the prevalence and predictors for non alcoholic steatohepatitis (NASH) in non alcoholic fatty liver disease (NAFLD). We have included 493 patients with sonographic evidence of fatty change in liver and 177 of them had done liver biopsy for histopathological study. Other causes of liver disease and alcohol consumption were excluded. Metabolic syndrome and biochemical and anthropometric evaluation was done. Females were predominating 250 (57.0 %). Centrally obese 422 (96.2 %) was more than over all obesity330 (75.1%). NASH was absent in 10 (5.6%) cases and diagnostic of NASH was 75 (42.4 %).Presence of diabetes could significantly (p = 0.001) predicted NASH. Age, sex, BMI, waist circumference, Serum HDL,triglyceride, insulin resistance index, hypertension, metabolic syndrome could not predict NASH. Serum GGT level was significantly (p = 0.05) higher in NASHwith a sensitivity of 45 % and specificity of 68 % only. Serum ALT and AST level could not detect NASH. Females were predominant sufferer of NAFLD in Bangladesh. Prevalence of NASH was much higher42.4%. Diabetes was the main predictor of NASH. GGT was the only biochemical indicator of NASH. We recommend liver biopsy in NAFLD with diabetes and raised GGT.

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Introduction:

Nonalcoholic fatty liver disease (NAFLD) is a clinico-histopathological entity with histological features that resemble alcohol-induced liver injury. By definition, occurs in patients with little or no history of alcohol consumption[1]. NAFLD is the most common liver disease in western countries, affecting 20-30% of the general population[2,3]. It encompasses a histological spectrum that ranges from fat accumulation in hepatocytes without concomitant inflammation or fibrosis (simple hepatic steatosis) to hepatic steatosis with a necro-inflammatory component (steatohepatitis) that may or may not have associated fibrosis. The latter condition, referred to as nonalcoholic steatohepatitis (NASH), may progress to cirrhosis in up to 20% of patients[4]. Reports have also suggested that the prevalence of NAFLD among Asian Indians is comparable to that seen in the West[5, 6]. Average age for NASH patients is 40-50 years and for NASH-related cirrhosis it is 50-60 years. NASH probably causes around 80% of cases of cryptogenic cirrhosis which accounts for 10-20% of all cirrhosis and progresses to advanced fibrosis in 32 to 37% of patients[7].

In parallel with the epidemic of obesity and metabolic syndrome worldwide, the prevalence of NAFLD in Asian countries has increased rapidly with a trend to younger patients during the last two decades. The prevalence of NAFLD was about 15% in adults in Shanghai and Hong Kong[8]. NAFLD has been associated with insulin resistance and hyperinsulinaemia, even in lean subjects with normal glucose tolerance [9]. Diabetes mellitus may be an independent predictor of NASH, including cirrhosis and hepatocellular carcinoma[10]. NAFLD is now recognized as the hepatic component of the metabolic syndrome, which includes hyperlipidemia, glucose intolerance, obesity, and systemic hypertension. Predictors of NASH increase with the number of components of the metabolic syndrome[11].The contrasting clinical course of NASH versus non NASH fatty liver (NNFL) indicates that these

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two conditions diverge early in the course of NAFLD although some patients probably transition from NNFL to NASH. Progression to cirrhosis is usually preceded by longstanding histological NASH and is infrequent in NNFL. Longitudinal studies with serial biopsies have shown that about one-third of NASH patients develop advanced fibrosis (stage 3 or 4 fibrosis) over 5-10 years from the time of the initial diagnosis[12]. Although usually relatively slow, progression to cirrhosis can occur in as little as 2–3 years. NASH is a common cause of 'cryptogenic' cirrhosis, which accounts for 10 – 20% of all cirrhosis[13]. Among patients diagnosed with NASH–related cirrhosis, the risk of developing a major complication of portal hypertension is 17, 23 and 52% at 1, 3 and 10 years, respectively. Among patients with early stage NASH, overall mortality over 10–15 years is about 10–12%, being significantly higher in NASH versus NNFL, compared to the general population. The risk of developing decompensated cirrhosis is 5–10% and for hepatocellular cancer it is 1–2%. There is a tenfold risk of cirrhosis relative to the general population[14].

A complete diagnosis of fatty liver disease ideally should define the histology, including the stage and grade of the disease as well as its etiology. In Bangladesh NAFLD is never been or insufficiently addressed in the field of medical research and practice. NASH is a potentially dangerous condition which requires medical intervention. The prevalence of NASH and potential risk factors for it is not yet explored here. We have designed this study protocol to estimate the prevalence of NASH in NAFLD and predictor of NASH in the perspective of Bangladeshwhich will be helpful future scientific knowledge and intervention.

Materials and Methods:

Study population:

We have included initially 439 patients at outpatient department of Hepatology in the University Hospital during the period of March 2010- December 2012 for fatty filtration in liver with ultrasonography. Exclusion criteria consisted of significant alcohol abuse (< 20g daily), evidence of hepatitis B and C and of drug induced fatty liver and other specific liver diseases: Hemochromatosis, Wilson's disease or autoimmune liver disease. These patients underwent clinical evaluation, anthropometric measurements, and blood tests. Liver biopsy was done after randomization in 190 patients but 4 biopsy samples were inadequate to comment for histopathology 4 patients withdrawn themselves from the study. The study was approved by the Institutional Review Board and all individuals provided written informed consent prior to enrollment in the study. Metabolic syndrome was defined according to Asian criteria,[15] and three of the five listed criteria were considered: waist circumference (WC) e"80 cm for women and e"90 cm for men, serum triglyceride ≥150 mg /dl (1.7 mmol/l) , serum HDL cholesterol <50 mg/dl (1.3 mmol/l) for women and <40 mg/dl (1 mmol/l) for men, elevated blood pressure (systolic blood pressure ≥130 and or diastolic blood pressure ≥85 mmHg or drug treatment for hypertension) and plasma glucose concentration e" 100 mg/dl (5.6 mmol/l) or drug treatment for diabetes.

Clinical and Biochemical evaluation:

All the patients were clinically evaluated: Blood pressure, Body mass index (BMI) and waist circumference was recorded for every patient. Liver function tests were performed prior to the liver biopsy. Blood samples were obtained under fasting conditions and the following tests were performed using standard laboratory methods: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, Gamma glutamyltranspeptidase (GGT) international normalized ratio (INR), blood glucose fasting and 2 hours after breakfast, lipid profile, Insulin level was assessed using the method of indirect chemiluminescence (MEIA). Insulin resistance was calculated according to the HOMA index (Homeostatic Metabolic Assessment).

Histological assessment

Liver biopsy specimens of 182 were analyzed by pathologist blinded to the patients' clinical and biochemical results. Histopathology was done in the department of Pathology BSMMU. The diagnosis of NASH was based on the Brunt et al criteria, [16]modified by Kleiner et al[17]. In this scoring system, the degree of disease activity in NAFLD was evaluated using the NAFLD Activity Score (NAS), which was calculated as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and hepatocyte ballooning (0–2) and thus ranged from 0 to 8. A NAS of 5 or more was diagnosed as "definitive NASH", NAS of 2 or

less as "non-NASH," and 3 or 4 as "borderline NASH." Other than NASH, was considered as NNFL. Hepatic fibrosis staging was as follows: 0 = no fibrosis; 1 = zone 3 fibrosis only; 2 = zone 3 and portal/periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis.

Statistical analysis

Results are presented as mean ± standard deviation (SD) for quantitative data and as numbers or percentages for categorical or qualitative data. Statistical differences in quantitative data were determined using t test or one way Anova test. Qualitative data were compared using the x^2 test. Multivariate regression analysis was done to explore the strongest predictor of NASH including the variables with significance in univariate analysis. For all tests, significance was achieved at $p < 0.05$.

Results:

Patient Characteristics:

Total of 439 patients wereincluded in this study. Females were 250 (57.0 %) and males were 189 (43.0 %). Mean age of the sample was 40.8 ± 10.2 years. Most of the population was house wife 217 (50.3 %), others were service holder 84 (19.5 %), business man 69 (16.0 %) and students were 59 (13.7 %). Hypertension and diabetes were prevailing in 83 (18.8 %) and 74 (16.8 %) respectively but metabolic syndrome was 188 (42.9 %). Triglyceride was high in 320 (72.8 %). BMI was normal in 51 (11.7 %), over weight 58 (13.2 %), Obese I 237 (53.9 %) and obese II 93 (21.2 %) according to criteria for Asian[18]. Most of the patients were centrally obese 422 (96.2 %) having waist circumference above normal. ALT, AST and GGT level were 54.1 ± 54.4, 45.1± 51.8 and 46.6 ± 33.7 u/l respectively. Insulin resistance index were higher than normal in 218 (49.6 %).

Histological Changes: Histopathological reports of 182 patients were available but 5 of them did not have fatty change on microscopy. We have included 177 patients for further analysis. There was no significant difference between biopsied and non- biopsied patient regarding clinical, anthropometric and biochemical variables. Steatosis of < 33% was 73(41.2%), 33 – 66 % was 82 (46.4 %) and > 66 % was 22 (12.4%). Lobular inflammation was absent in 10 (5.6 %), mild in 93 (52.5 %), moderate in 70 (39.5 %) and severe in 4 (2.3 %). Ballooning was absent in 5 (2.8 %), few ballooning in 138 (78.0 %) and prominent ballooning in 34 (19.2%) (Figure I). No fibrosis was seen in 28 (15.8%), stage I in 94 (53.3%), stage II in 40 (22.5 %) and stage III in 15 (8.3%). None had stage IV fibrosis(Table I).

According to NAS scoring system NASH was absent in 10 (5.6%) cases, borderline NASH was 92 (52.6%) and diagnostic of NASH was 75 (42.4 %). So NNFL was 102 (57.6%) and NASH was 75 (42.4%).

Predictors of NASH:

Prevalence of NASH in NAFLD was 75 (42.4%). There were no significant difference of age, BMI, waist circumference, Serum HDL and triglyceride level, insulin resistance index,sex, hypertension, metabolic syndrome did not differed in NASH and Non NASH. Mean age, BMI and waist circumference was similar in NNFL and NASH patients. Mean triglyceride was higher in NASH and mean HDL was lower in NASH but could not establish statistically significant value. Presence of diabetes could significantly ($p = 0.001$) differentiate NASH from NNFL. Serum ALT and AST level could not detect NASH in NAFLD. But serum GGT level was significantly ($p = 0.05$) higher in NASH than that of NNFL (Table II). GGT level for NASH was (51.7 ± 32.8) U/L and for NNFL was (40.4 ± 22.6) U/L. Multivariate regression analysis also explore that presence of diabetes could influence the development of NASH ($p= 0.04$) and GGT could differentiate NASH from NNFL ($p= 0.01$) (table III). But area under the curve is 59.3 % for GGT to differentiate NASH, with a sensitivity of 45 % and specificity of 68 % only for 44.5 U/L (Figure II).

Table-I

Histopathological features of biopsied patients

Variable	Number	Percent
Lobular inflammation		
Absent	10	5.6
Mild	93	52.5
Moderate	70	39.5
Severe	4	2.3
Ballooning		
Absent	5	2.8
Few	138	78.0
Prominent	34	19.2
Fibrosis		
Absent	28	15.8
Stage I	94	53.3
Stage II	40	22.5
Stage III	15	8.3
NASH	75	42.4

Table-II

Clinical, anthropometric and biochemical differences of NNFL and NASH

Variable	NNFL N=102	NASH N=75	*P*value
Age (yr)Mean ± SD	39.3 ± 9.4	41.0 ± 9.7	0.24
Sex: Male/ female	42/60	31/44	1.00
Body Mass Index (Kg/m^2)	27.8 ± 3.9	27.8 ± 4.6	0.998
Waistcircumference in cm Male	93.0 ± 5.5	93.0 ± 9.8	0.081
Female	95.8 ± 9.9	95.6 ± 11.0	0.927
HDL in mg/dl Male	36.3 ± 8.9	34.2 ± 6.5	0.337
Female	39.8 ± 10.3	39.2 ± 10.3	0.801
Serum Triglyceride mg/dl	225.2 ± 165.8	239.8 ± 111.6	0.509
Insulin Resistance Index	1.8 ± 1.3	1.5 ± 0.7	0.337
Diabetes Present / Absent	13/86	25/48	0.001
Hypertension Present / Absent	17/65	17/48	0.555
Metabolic SyndromePresent/ Absent	41/41	39/32	0.328
ALT U/L	56.9 ± 38.8	56.3 ± 31.8	0.603
AST U/L	46.9 ± 63.7	46.1 ± 22.2	0.916
GGT U/L	40.4 ± 22.6	51.7 ± 32.8	0.05

NASH; Non alcoholic steatohepatitis, NNFL; Non nash fatty liver

Table-III

Multivariate regression analysis for variable detecting NASH

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig
	B	Std. Error	Beta		
(Constant)	1.247	.517		2.411	.018
BMI	.014	.018	.124	.780	.438
Diabetes	.260	.125	.227	2.084	.040
Serum Triglyceride	.000	.000	-.105	-.919	.361
GGT	.005	.002	.289	2.473	.015
Waist Circumference	-.004	.008	-.077	-.491	.624

a. Dependent Variable: nash and nnfl
NASH; Non alcoholic steatohepatitis, NNFL; Non nash fatty liver

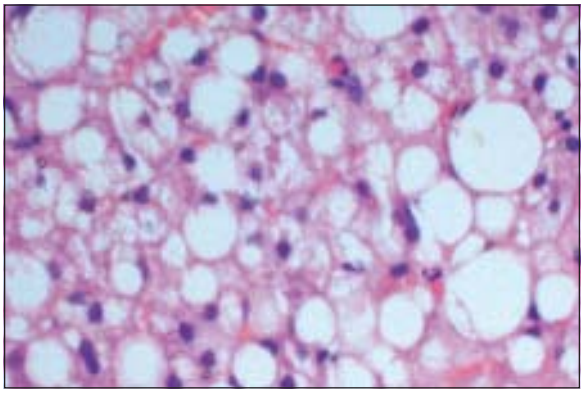

Fig.-1: *Microscopic feature of Nonalcoholic steatohepatitis:steatosis and ballooning degeneration.*

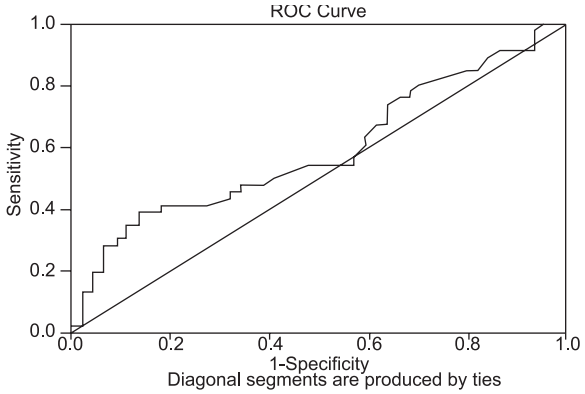

Fig.-2: *Receiver Operating Characteristic curve for GGT to differentiate NASH from NNFL.*

Discussion:

This study is the largest series from Bangladesh on NAFLD. Report of biopsy proven NASH and NNFL is also rare. University hospital is a tertiary care "center of excellence" hospital only and patients are referred from whole over the country. So this study may be the representative of prevalence of NASH in NAFLD of the country. Population based prevalence of NAFLD was not yet done in Bangladesh. Most of our NAFLD patients are of 30 to 50 years; this is similar to several reports from Asia[6,19-20]. But age could not influence the development of NASH. Female preponderance in NAFLD is dissimilar from reports from developed counties. Many recent studies have reported that male gender is a risk factor for fatty liver disease[21]. For example, in a study of 26,527 subjects undergoing medical checkups; the prevalence of NAFLD was 31% in men and 16% in women[22]. This female preponderance 250 (57.0 %) in our study may be the social conservative attitude which bounded most of our ladies to stay home for house hold activities without job leading to sedentary life style. Similar female preponderance was observed in one population studies from India[23]. But in accordance with previous studies sex did not influenced the development of NASH in NAFLD[22].

Centrally obese was 422 (96.2 %) outnumbered the overall obesity 330 (75.1%). The prevalence of NAFLD was increased according to the increase of BMI or abdominal circumference reported from Japan[24]. But other report concluded that waist circumference is an independent predictor of advance histological changes in NAFLD than BMI[25, 26]. But waist circumference was similar in NASH and NNFL in our series. It could be explained by that waist circumference indicate visceral obesity but no influence on pathogenesis of NASH at the stage of 2nd hit. Hypertriglyceridemia was very common 320 (72.8 %) in this study with no difference between NASH and NNFL. TG was long been considered as major factor in the development of NAFLD,[5-8] but there is mounting evidence that such non-TG lipid molecules are implicated in the pathogenesis of NASH by the process of lipotoxicity. Conversely, formation of TG may actually be a cytoprotective mechanism in liver[27, 28]. Our study revealed similar role of TG in NAFLD.

Our study explored that prevalence of NASH was 75 (42.4%) in NAFLD which is much higher. It is alarming for the country like Bangladesh. It was neither addressed previously nor considered anyway. In previous review, NAFLD wasfoundhighly prevalent (15% to 45%) in modern societies, only 10% to 25% of cases develop NASH, hepatic fibrosis leading to cirrhosis, end-stage liver disease or hepatocellular carcinoma[29].In other studies prevalence of NASH was 10 to 30 % in NAFLD [30] and it is less in Asian than that of European[31.32] . We were unbiased in selecting patient for liver biopsy and it was irrespective of clinical, biochemical and anthropometric status of the study population. So it is the representative of prevailing situation in the society. This finding warrants further extensive study on prevalence of NASH in Bangladesh and awareness of clinician is essential to diagnose NASH and to advice possible intervention as early as possible.

Presence of diabetes signified the presence of NASH in our study population (p= .001). Metabolic syndrome was prevailing in 188 (42.9%) population. NAFLD is strongly associated with insulin resistance (IR) and other components of the metabolic syndrome, like T2DM, central obesity, hyperlipidemia, and hypertension[33]. The pathogenesis of NASH appears to be a multiple hit process. The initial insult is the development of macrovesicular steatosis with the accumulation of hepatic fat from decreased hepatic free fatty acid oxidation and D or increased hepatic de novo lipogenesis, and D or decreased lipid export from the liver. Although IR can contribute to this dysregulation of lipid metabolism, once fatty liver develops, it can worsen hepatic IR and diabetes, contributing to a vicious cycle[34].

Serum ALT and AST levels were similar in NASH and NNFL in this study. But GGT were significantly (P= 0.05) higher in NASH than that of NNFL. NASH has been associated with slight elevation of liver enzymes mostly ALT[35]. In other reports NAFLD patient typically present with asymptomatic serum aminotransferase elevations of 2-3 times the normal[36] · This difference was due to different selection criteria. GGT is a sensitive indicator of liver damage[37]. Excess deposition of fat in the liver is associated with an elevated serum GGT[38]. Recent reports suggest that an increased GGT level is a risk factor for advanced fibrosis in NAFLD and, with weight loss, a decrease in GGT activity is predictive of improved lobular inflammation and fibrosis of liver[39].

The limitation of the study was that we had not done it at the community level rather at a tertiary level hospital of the country.

In conclusion, Females were predominant sufferer of NAFLD in Bangladesh. Prevalence of NASH was much higher in NAFLD. Diabetes was the main culprit in developing NASH in NAFLD. GGT was the only biochemical predictor of NASH but with low sensitivity and specificity. We recommend liver biopsy in NAFLD with diabetes and raised GGT.

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