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Original Article

Frequency of Nonalcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis in patients with Metabolic Syndrome

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Abstract

Background: Obesity and metabolic syndrome is an epidemic seen in the developed, as well as developing countries. Early recognition of this disorder may prevent major non-communicable diseases such as type 2 diabetes mellitus, hypertension and dyslipidemia. Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis are the hepatic manifestations of metabolic syndrome.
Study type, settings and duration: Prospective, observational, cross-sectional study was conducted in Endocrine Clinic of Jinnah Postgraduate Medical Centre, Karachi from June 2008 to September 2010.
Patients and Methods: This study was conducted in patients suspected to have metabolic syndrome, as defined by International Diabetes Federation. Patients fitting the clinical consensus definition, having either palpable liver or ultrasound evidence of fatty infiltration were enrolled. Detailed history, physical examination, anthropometrics and biochemical measurements were recorded. Liver biopsies were performed where possible and were assessed according to Brunt et al's classification.
Results: A total of 101 patients who met the inclusion criteria were enrolled in the study. Liver biopsy was done in 31 patients. On biopsy, non-alcoholic fatty liver disease was confirmed in 28 (90%), non-alcoholic steatohepatitis in 18 (58%) and fibrosis in 8 (25%) patients. Of the biopsied cases, fatty infiltration on ultrasound was seen in 14 (87.5%) cases. Alanine aminotransferase was higher in patients having fibrosis. There was a direct correlation of histopathological changes with rising waist circumference, total cholesterol, triglyceride, low density lipoprotein & alanine aminotransferase.
Conclusion: There was high prevalence of non-alcoholic fatty liver disease / non-alcoholic steatohepatitis in patients with metabolic syndrome and liver biopsy confirmed this in 90.3% patients who consented to this procedure.
Key words: Obesity; Metabolic syndrome; NAFLD.

Introduction

Metabolic syndrome (MetS) is characterized by an aggregation of disorders including obesity, dyslipidemia (DYS), hypertension (HTN), impaired glucose tolerance (IGT), chronic inflammation, pro-coagulation and impaired fibrinolysis. The coexistence of these conditions in the same patient is associated with increased risk of cardiovascular diseases and their dramatic consequences[1].

Insulin resistance (IR) and raised fasting glucose are common in most individual components of MetS and its risk factors. A portion of the population with increased fasting blood glucose (FBG) eventually develops type 2 diabetes mellitus (T2DM)[2].

Non-alcoholic fatty liver disease (NAFLD) is the hepatic component of MetS; obesity and IGT/T2DM are two important risk factors for the development of MetS. The true incidence and prevalence of NAFLD is difficult to ascertain, as there is no single reliable screening test that can be applied to the entire population. However, as it is closely linked with obesity and MetS, high prevalence of this disease is seen in selected subgroups[3].

To observe the frequency of NAFLD in patients with MetS, the definition of International Diabetes Federation (IDF) is used[3]. The IDF consensus definition of MetS in children and adolescents is divided in different age groups: age 6 to 10; 10 to 16; and 16 or older[4]. For children age 10 or older, MetS is diagnosed in cases having abdominal obesity (using waist

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circumference percentiles) along with two or more features of MetS. The only exception is that a single cut-off for HDL is used, rather than the sex-specific cut-offs as in adults. For children older than 16 years, MetS is defined according to the IDF adult criteria.

Diagnosis of NAFLD is based on clinical hepatomegaly, abnormal liver function tests (LFTs), or fatty liver on ultrasound; Non-alcoholic Steatohepatitis (NASH) is diagnosed by the presence of steatosis/ steatohepatitis on liver biopsy[5].

The prevalence of NAFLD/NASH is increasing worldwide due to the increasing prevalence of MetS. In a study from Pakistan, NAFLD was found in 15.3% of visitors of the Hepatitis Awareness Programme, who were screened for liver ailments[6]. Hepatic steatosis has also been found as a reliable marker for carotid intima-media thickness, reflecting increased cardiovascular risk in these patients[7]. NAFLD/NASH is an increasingly recognized cause for cirrhosis, found in 1.4% of 74 cases of liver cirrhosis evaluated in one study[8].

Patients and Methods

This prospective, observational, cross-sectional study was done in obese patients aged 12 years or more, who came to the Endocrine Clinic of Jinnah Postgraduate Medical Centre. Diagnosis of MetS was made as defined by IDF[3]. Patient sampling was done by non-probability, convenient sampling between June 2008 and September 2010.

The study was conducted after getting approval from the Institutional Review Board. Written informed consent was taken from all patients and each patient underwent detailed history, physical examination, complete blood count (CBC), LFTs, fasting lipids, glucose and ultrasound of abdomen, to grade fatty infiltration of liver. Data was recorded on a pre-designed proforma. NAFLD was diagnosed on having enlarged liver either on clinical examination or on ultrasound showing fatty liver. Coagulation profile, Hepatitis B surface Antigen, Anti HCV were done prior to liver biopsy .The patients having chronic liver disease due to hepatitis B, hepatitis C, alcohol; bleeding or clotting disorders; severe (stage 3-5) chronic kidney disease or class III/IV heart failure; women who were either pregnant or lactating; people on hepatotoxic drugs; patients with rapid or profound weight loss or on parenteral nutrition; those who had jejuno-ileal bypass surgery or who were hypothyroid, were excluded from the study.

Liver biopsy was done in patients who consented. All biopsies were analyzed by an independent histopathologist, who graded it according to the Brunt system in three categories i.e. macro-vesicular steatosis, necro-inflammatory activity and fibrosis[9]. All patients were advised lifestyle modifications including diet and physical activity.

A database was developed on SPSS version 10.0 on the basis of filled in proformas. Qualitative variables of complaints were presented by their frequencies along with percentages. A 95% Confidence interval was also computed for the frequencies. Quantity variables of laboratory investigations were presented by their mean ± S.D. The Chi-Square test or Fisher's Exact test (for less than 5 expected frequencies) was employed to know the association of NAFLD with other categorical variables. The result was considered significant at $p<0.05$.

Results

A total of 150 patients having obesity and related disorders were screened for MetS. Thirty-seven patients did not meet the criteria and 12 patients were lost to follow up, leaving 101 patients for final analysis. Majority of patients (86.1%) were females. Mean values of baseline characteristics are shown in Table-1. Only 31 patients consented for liver biopsy (3 males and 28 females). Their mean age was 30.26 years. Frequency of NAFLD in biopsied cases which showed macro-vesicular steatosis was 28 (90.3%), NASH 18 (58.1%) and fibrosis 8 (26%) patients. Moreover, 90% cases who had steatosis on biopsy also had enlarged fatty liver on ultrasound. However, 3 patients with normal ultrasound had macro-vesicular steatosis on histopathology, showing relative imprecision of ultrasound to diagnose NAFLD.

Low-density lipoprotein (LDL) levels were higher in those having histological evidence of NAFLD as compared to those with normal biopsy (120.3 mg/dl NAFLD vs. 86.67 mg/dl non-NAFLD group; $p = 0.05$, as shown in Table-2. Body mass index (BMI), waist circumference, and cholesterol were increased in macro-vesicular steatosis group, showing a positive trend (not significant). According to histopathological grading, majority of patients with macro-vesicular steatosis had Grade 1 changes (58.1%); Grade 2 and 3 changes were seen in 29% and 3.2% respectively.

Fasting blood glucose was significantly higher, although still normal, in patients with NASH (91 mg/dl NASH vs. 83.46 mg/dl non-NASH group; $p=0.05$, as shown in Table-3). BMI and liver size also showed a positive trend. According to histopathological grading, grade 1 NASH was seen in 32.3% while grade 2 NASH was seen in 25.8% patients.

Alanine aminotransferase (ALT) levels were significantly higher in patients with fibrosis (52.38 IU fibrosis vs. 30.87 IU non-fibrosis group; $p=0.02$, shown in Table-4). BMI, FBG, Triglycerides (TG) and liver size were higher in people with fibrosis, but the difference was not significant. Stage 1 and 2 fibrosis was seen in 19.4% and 6.5% patients, respectively.

Table 1: Patients' baseline characteristics.

Variables	*Biopsy Group (n=31)*			*Non-Biopsy Group (n=70)*		
	Male		*Female*	*Male*		*Female*
Sex (F:M)	3		28	11		59
	Mean	*Min-Max*	*SD*	*Mean*	*Min-Max*	*SD*
Age (years)	30.26	12-50	9.16	33.47	12-50	10.06
BMI (kg/m^2)	37.72	22-70	9.75	36.12	22-63	8.96
W. Circum (cm)	110.11	87-150	14.98	107.24	83-157	15.91
SBP (mmHg)	128.26	90-170	19.99	125.60	80-170	18.28
DBP (mmHg)	85.48	60-110	13.68	82.89	60-120	13.24
ALT (IU)	36.42	12-112	23.71	29.04	10-118	17.43
FBG (mg/dl)	87.84	72-115	11.06	98.73	62-264	41.35
Cholesterol (mg/dl)	192.29	110-282	39.61	197.53	110-290	40.47
HDL (mg/dl)	31.32	22-43	4.43	30.76	22-42	3.27
LDL (mg/dl)	117.13	65-180	30.54	125.66	66-198	34.33
TG (mg/dl)	193.58	69-440	103.55	147.61	65-360	70.93
Liver size (cm)	15.80	12-21	2.18	15.55	12-21	1.96

Table 2: Correlation of variables and NAFLD (Macrovesicular Steatosis).

	NAFLD		*Non-NAFLD*		
No. of Patients	28		3		
	Mean	*SD*	*Mean*	*SD*	*p Value*
Age (years)	29.86	9.40	34.67	8.73	0.40
BMI (kg/m^2)	38.09	10.13	34.28	4.78	0.52
W. Circum (cm)	110.6	15.56	105.0	7.21	0.54
SBP (mmHg)	124.5	30.19	127.0	14.73	0.89
DBP (mmHg)	85.18	13.97	88.33	12.58	0.71
FBS (mg/dl)	88.36	11.41	83.00	6.24	0.43
ALT (IU)	37.29	24.77	28.33	6.35	0.54
Cholesterol (mg/dl)	194.46	38.30	172.0	55.24	0.35
HDL (mg/dl)	31.18	4.51	32.67	4.04	0.58
LDL (mg/dl)	120.3	29.31	86.67	29.29	0.05
TG (mg/dl)	190.7	94.94	219.6	195.0	0.65
Liver size (cm)	15.86	2.26	15.19	1.40	0.61

Table 3: Correlation of variables with NASH.

	NASH		*Non-NASH*		
No. of Patients	18		13		
	Mean	*SD*	*Mean*	*SD*	*p Value*
Age (years)	31.72	9.33	28.38	9.29	0.33
BMI (kg/m^2)	39.92	11.42	34.68	5.98	0.14
W. Circum (cm)	111.22	14.14	108.57	15.22	0.63
SBP (mmHg)	127.22	21.09	121.38	37.90	0.58
DBP (mmHg)	85.52	15.42	85.38	11.44	0.97
FBS (mg/dl)	91.00	11.89	83.46	8.35	0.05
ALT (IU)	38.06	27.47	34.15	18.07	0.65
Cholesterol (mg/dl)	189.83	48.89	195.69	22.69	0.69
HDL (mg/dl)	31.28	5.13	31.38	3.42	0.94
LDL (mg/dl)	116.72	33.96	117.69	26.42	0.93
TG (mg/dl)	192.94	110.8	194.46	96.94	0.96
Liver size (cm)	16.14	2.10	15.33	2.29	0.31

Among the biopsied patients, all patients with raised ALT levels had proven NASH on biopsy with 50% having stage 1 fibrosis and beyond. In the group that refused biopsy, 13 patients had an elevated ALT (>40 IU). It may be extrapolated that these patients also had NASH. Therefore, it seems possible that 21 (20%) of all the enrolled patients had NASH.

Table 4: Patient characteristics with fibrosis.

No. of Patients	*Fibrosis* 8		*No Fibrosis* 23		
	Mean	*SD*	*Mean*	*SD*	*p Value*
Age (years)	31.63	9.18	29.87	9.52	0.65
BMI (kg/m^2)	40.41	13.67	36.79	8.17	0.37
W. Circum (cm)	108.5	13.36	110.67	15.74	0.73
SBP (mmHg)	125.0	23.29	124.7	31.08	0.98
DBP (mmHg)	83.75	16.75	86.09	12.78	0.68
FBS (mg/dl)	92.63	15.26	86.17	9.02	0.15
ALT (IU)	52.38	36.14	30.87	15.04	0.02
Cholesterol (mg/dl)	190.5	45.24	192.9	38.5	0.88
HDL (mg/dl)	31.13	6.46	31.39	3.66	0.88
LDL (mg/dl)	110.25	37.21	119.5	28.43	0.46
TG (mg/dl)	218.7	109.9	184.8	102.3	0.43
Liver size (cm)	16.08	2.51	15.7	2.11	0.67

Discussion

In the present study of MetS, the prevalence of NAFLD was 90.3%. The incidence of MetS has reached epidemic proportions. The prevalence of MetS in U.S adults in 1999-2006 was 34.1±0.8%, which showed a significant increase from 1988-1994 and more so in women (28.4%) than in men (16.8%) applying the revised AHA/NCEP ATPIII definition[10]. Early diagnosis, treatment goals, therapeutic approaches and comprehensive management are now the focus of both clinical care and research.

IDF has defined various cut-offs for abdominal obesity for different ethnic populations, using waist circumference measurements. An important limitation of BMI as a measure of obesity is that it tends to ignore the distinction between fat and fat free mass. A more accurate definition of overweight and obesity should be based on the total amount of body fat[11].

NAFLD is described as a spectrum of disorders characterized by macro-vesicular steatosis, that occurs in the absence of consumption of alcohol in amounts considered to be harmful to the liver[12]. A strong association between NAFLD and each component of MetS, including central obesity, hypertriglyceridemia, T2DM and HTN has been demonstrated independently in several studies and is now considered as the hepatic manifestation of MetS[13]. In a study of 4,401 Japanese subjects, MetS was a strong predictor of new-onset NAFLD and also had an impact on the clinical course[14]. Prevalence of NAFLD in obese people varies between 57.5 – 74%, but our study showed it to be 90.3% in cases with MetS who underwent biopsy[10]. This high prevalence in our population might be a reflection of racial differences in genetic susceptibility to visceral obesity including liver fat.

Individuals with NAFLD have significantly higher levels of glucose, insulin, TG, serum ALT, and a higher BMI and waist circumference than controls[15]. The strongest independent predictors of NAFLD are waist circumference, BMI, and TG level. Workers have reported NAFLD to be a strong and independent predictor of MetS, after adjusting for insulin and BMI[15]. In addition, obesity and diabetes mellitus are two important risk factors for the development of NAFLD[16]. A study from Karachi, Pakistan reported NAFLD in 60.8% diabetic cases[17]. Diabetes is not only associated with the presence of NAFLD but is also associated with the risk of having advanced hepatic fibrosis[18].

Non-invasive imaging such as ultrasound, computerized tomography (CT scan) and magnetic resonance imaging (MRI) may be used to diagnose NAFLD. Of these, MRI is the most expensive, while trans-abdominal ultrasound remains the most commonly used noninvasive method for detecting NAFLD, with remarkable sensitivity and specificity, but without accurate quantification of the degree of steatosis. Moreover, the diagnostic criteria for hepatic fat are highly operator-dependent and non-standardized. This study shows that among all the patients included, only 5 had normal liver on ultrasound, while 96 patients (95%) had some degree of fatty infiltration. Patients may also have a normal ultrasound but steatosis on biopsy, as has been shown in a small number of this cohort. Non-invasive tests include transient elastography, using which, excellent results have been reported in diagnosing liver fibrosis and cirrhosis in patients with NAFLD/NASH[19].

Serum ALT level is used as a screening test to diagnose NAFLD on population basis[20]. However, there are problems associated with its specificity, sensitivity, and predictive value. Serum ALT levels may be normal in patients with advanced grade of steatohepatitis or even cirrhosis[21]. The need to revise the normal limits for ALT values has also been considered. Decreasing the upper limit of normal for ALT level from 40 U/L to 30 U/L in men, and from 30 U/L to 19 U/L in women increases the sensitivity for detection of patients with liver injury from 55% to 76%, but decreases the specificity from 97% to 88% [22].

Liver biopsy is the gold standard for the evaluation of liver histology and determination of NASH. Unfortunately, it is invasive, requires skill, and is associated with discomfort and some risk. It is therefore not suitable for evaluation of all individuals with this condition. Liver biopsy helps to determine the stage of fibrosis and disease severity. Currently there is no consensus on indications for liver biopsy in NAFLD. However, biopsy is essential when the diagnosis is uncertain, and decision has to be individualized in clinical practice.

There are 2 recognized histological patterns of NAFLD i.e fatty liver alone and NASH. The latter represents a shift from simple steatosis to an inflammatory component. NASH is described by grading inflammatory lesion and staging degree of fibrosis. There are several methods of grading and staging NAFLD. The system proposed by Brunt remains one of the best known and most frequently used method[23].

In children and adolescents (5-19 years), the prevalence of obesity is rising rapidly in the developing countries[24]. Obesity and MetS are important modifiable risk factors for atherosclerotic cardiovascular disease (ASCVD)[25]. In an African study, current or past BMI strongly predicted cardio-metabolic risk factors[26]. As ASCVD begins within the first two decades of life, it is critical to recognize and intervene early in cases of childhood obesity.

Serum immunoglobulins have also been studied to predict fibrosis in patients with NAFLD. Serum IgA is elevated in patients with NASH as compared to those with simple steatosis and IgA level has been used as an independent predictor of advanced fibrosis[27,28].

In view of the heavy worldwide disease burden of NAFLD and NASH, especially in obese people with MetS, it is imperative that research into identification of some non-invasive surrogate marker be identified to detect individuals with NAFLD progressing to NASH. This will lead to identification of people with serious liver disease and risk modification in as far as is possible at this stage of knowledge.

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