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Specific

Non-alcoholic fatty liver disease: a metabolic syndrome on the increase

G N Somasundaram*, N P Somasundaram, S D Jayaratne*****

Journal of the Ceylon College of Physicians, 2004, **37**, 30-38

Introduction

Clinical scenario

A 41-year old Sri Lankan male presented to the clinic after a routine medical examination for the purpose of medical insurance. He had been informed that he has elevated liver enzymes and to seek specialised care. He has an aspartate aminotransferase (AST) of 118 units/l, and alanine aminotransferase (ALT) of l00 units/l. His fasting plasma glucose is 109 mg/dl. He is over weight with a BMI of 24.9 and a waist hip ratio of 1.2, his blood pressure was 150/95 and 150/100 on two separate occasions. The lipid profile revealed his total cholesterol is 208, fasting triglycerides 180, LDL cholesterol 130 and HDL cholesterol 35 mg/dl. An abdominal ultrasound suggests that he has fatty liver. A standard liver screen, including hepatitis serology, is negative.

- What is the aetiology of fatty liver in this patient?
- Should this patient have a liver biopsy?
- Does he need any medical treatment?

Finding mild to moderate elevation of the liver enzymes that cannot be explained by the occurrence of common liver diseases is an increasingly recognised scenario. It has been shown, after more than two decades of extensive research, that in most instances this is due to non-alcoholic fatty liver disease. At one time it was thought that fatty liver associated with diabetes or otherwise, is essentially a benign condition. For this reason active intervention was not considered necessary. However, data emerging in the past two decades demonstrate that fatty liver, irrespective of its aetiology, has the potential to progress to more advanced forms of liver diseases such as steatohepatitis[1-4], fibrosis, cirrhosis[5] and even to hepatocellular carcinoma[6-7-8].

* *Senior Registrar,*

*** *Senior Lecturer and Consultant Physician, University Medical Unit, Colombo South Teaching Hospital.*

** *Consultant Endocrinologist, National Hospital of Sri Lanka.*

Non Alcoholic Fatty Liver Disease (NAFLD) encompasses spectrum of diseases such as steatosis, steatohepatitis, and cirrhosis. The popular term "Non Alcoholic Steatohepatitis" (NASH) in fact refers to only a stage within the spectrum of Non Alcoholic Fatty Liver Diseases (NAFLD). Many retrospective cohort studies, paired liver biopsy analysis and clinical follow up data show that patients with simple steatosis generally have a benign course as opposed to patients with steatohepatitis (NASH) who have a higher propensity to progress to cirrhosis and its complications[4,9]. However progression has been observed even in mild NAFLD and any amount of fat in the hepatocytes may sensitise the liver to injury from other causes. A high proportion of patients previously thought to have cryptogenic cirrhosis share many of the clinical and demographic features of patients with NAFLD, suggesting that the aetiology of their cryptogenic cirrhosis is often unrecognised fatty liver disease[3,5,7,10].

Estimates based on imaging and autopsy studies suggest that about 20-30% of adults in the western countries have excess fat accumulation in the liver[11,12]. Among these adults about 10% meet current criteria for NASH. Sustained liver injury leads to progressive fibrosis and cirrhosis and in possibly a one third of those with NASH, progress to develop cryptogenic cirrhosis[13,14,15]. Among diabetic patients, it is estimated that 75% have some form of fatty liver[16-19]. Five and ten year survival in NASH has been estimated at 67% and 59% respectively, although death may often be due to co morbid conditions[102].

As more data of epidemiology and pathogenesis of the condition have emerged, we are faced with new questions about the aggressiveness of approach and modes of management.

Diagnosis of NAFLD

NAFLD is suspected when a patient presents with a liver disease that is not due to the common causes of liver damage such as alcohol, viruses, drugs and toxins and after excluding autoimmune, genetic or metabolic disorders. This necessitates careful

exclusion of alcohol intake that has the potential to induce liver injury. A daily intake of 20g of alcohol in females and 30g in males may induce liver injury in some. (8g = 1unit)[21,22]. Many centres accept a cut off level of 14-28 units/week to differentiate between alcohol related and non-alcoholic fatty liver disease[23]. Evidence suggest that NAFLD is by far the most likely histological diagnosis in the increasing number of patients presenting with persistently elevated transaminases or other liver enzymes[24,25].

NAFLD is often detected during routine evaluation of liver enzymes and imaging for unrelated conditions. Liver transaminases, (if elevated), are usually only marginally elevated[19].

The gold standard of diagnosis of NAFLD is liver biopsy. Non invasive tests such as liver biochemistry and imaging studies, though very useful in developing and supporting the clinical suspicion, cannot be used to confirm the diagnosis, and assessing the severity or progression of the disease. In fact there is poor correlation between the three tests.

The entire histologic spectrum of NAFLD can be seen in individuals with normal ALT values and NAFLD, similar to those with elevated ALT levels and NAFLD[26]. Normal liver enzyme values do not guarantee freedom from underlying steatohepatitis with advanced cirrhosis.

Imaging

Ultrasonography (US), computerised tomography (CT), and magnetic resonance imaging (MRI) though able to show the presence of steatosis, are all insensitive to degrees of steatosis less than 25-30%. The presence of more than 33% fat on liver biopsy is optimal for detecting steatosis on radiological imaging. Focal hepatic steatosis and focal fatty sparing are conditions that can create potential diagnostic difficulties as these can be confused with other fat containing tumours. In the presence of diffuse steatosis, hepatic mass lesions can be difficult to discern on both CT and US with reported decreased sensitivity and specificity of the lesion detected. Although the presence of fat can be demonstrated with CT or US, MRI is the most specific imaging technique for demonstration of both microscopic and macroscopic fat and characterisation of both hepatic steatosis and coexistent hepatic masses[27]. Magnetic resonance spectroscopy (MRS) is a sensitive and quantitative measure of the hepatic triglyceride content[11,28,29].

However none of the radiological features are specific for fibrosis and therefore do not distinguish between NASH and other types of NAFLD[30].

Therefore, physicians face the dilemma of whether or not to perform an invasive procedure that carries some risk for morbidity and mortality to diagnose and stage a disease in apparently asymptomatic patients.

Predictors of advanced disease

A number of studies have addressed clinical predictors of more advanced histology on the initial diagnostic biopsy. Age more than 45 years, obesity of BMI>28, Type 2 diabetes and hypertriglyceridaemia of are the significant independent predictors of more advanced form of disease[7,13-15,31]. The other factors identified as predictors of advanced disease are female gender, impaired glucose tolerance, insulin resistance index or fasting C peptide, hypertension, elevation of AST and ALT and an AST: ALT ratio of more than one[4,13-15,31,32]. These factors are generally used to identify patients in whom liver biopsy may provide the most prognostic information. Firm recommendations of when to perform a liver biopsy in routine clinical setting have not yet been developed. Selecting patients with possible advanced disease to perform liver biopsy is adopted by many units.

It is important to identify patient with possible NAFLD in an early stage to protect them from further damage to their liver and to treat them for other possible metabolic disorders.

Since currently available non-invasive markers of liver damage are unreliable, there is an urgent need for a reliable mode of diagnosis.

Predictors of NASH and advanced fibrosis on index liver biopsy

Age >45
Obesity- BMI >28
Type 2 diabetes
Hypertriglyceridaemia
Female gender
High AST and ALT
AST: ALT ratio >1
Hypertension
Insulin resistance index >5

Aetiology

A feature of metabolic syndrome

The main associations of NAFLD are obesity, Type 2 diabetes, Hyperlipidaemia and insulin resistance, which are also the main features of the metabolic

syndrome. The working definition of the metabolic syndrome is based on a combination of 5 risk factors (central obesity, hyperglycaemia, hypertension, hypertriglyceridaemia, and low HDL cholesterol levels.), which are commonly shared by patients with NAFLD[33]. Many recent studies suggest that NAFLD can be considered as the hepatic consequence of the metabolic syndrome. Among 282 patients with fatty liver on ultrasonography who had no history of alcohol abuse and negative tests for the presence of hepatitis B and C virus, more than half the subjects had insulin resistance metabolic syndrome. Obesity, diabetes and hypertriglyceridaemia were more common by 5.3-fold, 4.0-fold, and 6.7-fold, respectively in subjects with severe steatosis on histology as compared to controls[34]. Presence of metabolic syndrome carried a high risk of NASH among NAFLD subjects[33]. These studies suggest a correlation between the severity of metabolic syndrome and more severe form of NAFLD.

NAFLD in patients with metabolic disorders

The clinical significance of liver disease is frequently under estimated in patients with metabolic disorders such as Type 2 diabetes, obesity and hyperlipidaemia. Prevalence of NAFLD in obese patients and Type 2 diabetics can be as high as 80-90%[16-18,34]. A study which compared patients who had chronically elevated transaminase levels in the metabolic disorders clinic and patients attending liver unit for investigation and management of NAFLD showed that the liver disease in patients routinely cared for in metabolic units is similar to that observed in patients cared for in liver units[35]. Liver biopsy was performed in 65 of the 147 patients attending the metabolic clinics and 82% (53) of these biopsies fulfilled histological criteria for NASH. Patients with NAFLD and diabetes are at risk for the development of an aggressive outcome, such as cirrhosis and mortality, probably because of the potential role of insulin resistance in the development of poor clinical outcomes in patients with NAFLD[36].

In Italy, a population based study in Type 2 diabetics showed that the standardised mortality rates for cirrhosis were 2.5 times higher than expected[37]. Other studies which looked at the outcome of patients with NAFLD and diabetes also report more aggressive form of disease, higher overall mortality and mortality related to liver disease[19,38]. All the available data on NAFLD in diabetic patients suggest that these patients must be carefully selected, investigated and followed up, in view of prognostication and management. This should prompt us to include liver surveillance in the comprehensive management plan of patients with Type 2 diabetes and other metabolic disorders, if it is not already done routinely.

Decades of possible mechanisms involved revealed one common link, namely induction of cytochrome P4502E1 (CYP2E1)[50]. Its substrates, fatty acids, ketones and ethanol, when present chronically in large amounts, induce the activity of the enzyme. This reaction is associated with the release of free radicals, which can cause lipid peroxidation, mitochondrial damage and liver injury. CYP2E1 mediated generation of oxidative stress-related parameters and diminished anti-oxidant capacity were shown in the presence of fatty liver[51,52].

Other possible aetiological factors

Genetic studies aimed at various possible aetiological factors have shown association of a number of genes with NAFLD. Genes responsible for insulin resistance, pro-fibrotic polymorphisms promoting fibrosis in the presence of steatosis[53], polymorphism of cytochrome P450IIE1 (CYPIIE1) are among these. Association of insulin resistance, hepatic steatosis, NASH and cirrhosis in patients with partial and total lipodystrophy has given further scope for molecular and genetic investigations. Mutations in the genes encoding PPAR-γ, PPARG and other regulators of insulin signalling pathway have been identified in partial lipodystrophy[54-58].

Variably distributed genes creating the background for a disease, which in most cases, become clinically apparent because of life style changes could be a possible scenario for the pathogenesis of NAFLD (Similar to Type 2 diabetes, which is a polygenic-disease).

Elevated serum ferritin levels were found in half the patients with NASH in one series and increased transferrin saturation was found in 6-11 percent of patients. Hepatic iron index and hepatic iron levels are usually in the normal range[1,31].

But studies which looked at the possibility of hepatic iron overload as a possible aetiological factor proved that hepatic iron deposition and the HFE gene of Familial haemochromatosis are not necessary for the development of NAFLD[44,45,58-60].

Non-organ specific auto-antibodies were found to be more prevalent in patients with NAFLD. Anti Nuclear Antibodies (ANA) was more prevalent than other antibodies like Smooth Muscle Antibodies (SMA), Anti Mitochondrial Antibodies (AMA) and higher titre of ANA was associated with more severe insulin resistence[1,7,61]. Whether this has any aetiological significance is not understood.

Racial and ethnic distribution

Racial and gender variation among patients with NAFLD has been shown in a study in the USA; Hispanic and Asians being the more affected ethnic groups. In this study NAFLD was 3.5 times more common among Asian males than females, and the BMI of Asians is significantly lower than the other ethnic groups[62]. Racial and gender variations may reflect differences in genetic susceptibility to visceral adiposity, including hepatic involvement.

Treatment

Currently there are no proven effective therapies available for the treatment of NAFLD. Although there are number of studies that have explored various treatments for NAFLD, these generally consist of small number of patients and sub optimal endpoints.

Treatment strategies of NAFLD can be broadly divided into two categories.

1. **Treatment or control of underlying risk factors:**

This is aimed at obesity, diabetes, and hyperlipidaemia. In patients with diabetes, good metabolic control is always recommended, but it is not always effective in reversing NAFLD. Weight reduction results in improvement in liver biochemistry, and the degree of steatosis[63-66]. Degree of necroinflammation and fibrosis was found to worsen with drastic weight reduction[67-69]. Gradual weight reduction continues to be the cornerstone of management including in the less obese and centrally obese patients and exercise is beneficial even in the absence of weight reduction.

Patients with NAFLD often have elevated triglycerides levels. Lipid lowering agents such as gemfibrosil, bezafibrate, and atorvastatin have been tried in patients with some promising results in a few small trials[70-73]. The effects of various anti lipid agents need to be established in larger studies in this group of patients.

Whether alcohol use should be prohibited or diminished to levels less than 20g/day is unclear. Dixon et al have suggested that limited alcohol intake is protective against NASH[13]. In the absence of data; the recommendation is to tailor this to the histology, with abstinence if significant fibrosis is present[23].

2. **Specific pharmacotherapy directed at the possible aetiological factors**

   - **Insulin sensitizers** – Metformin has been studied in the treatment of NAFLD in mice and in limited number of patients with promising resuts[74-76]. Metformin down regulates hepatic gluconeogenesis as well as may have beneficial effects through inhibition of hepatic expression of TNFα and TNF inducible factors that promote lipid accumulation and among patients with NAFLD who are not known to have diabetes the incidence of impaired or diabetic glucose tolerance is prominent and therefore performing OGTT in cases with NAFLD may be useful for early screening of diabetes[39].

Insulin resistance as an aetiological factor

NAFLD has been clearly shown to be associated with insulin resistance on laboratory testing and on clinical grounds[19,40,41]. Hyperinsulinaemia, hyperleptinaemia and insulin resistance has been repeatedly demonstrated not only in patients with Type 2 diabetes and obesity but also in non-diabetic patients with normal glucose tolerance, and patients with normal BMI[42-46]. Reduced insulin sensitivity rather than reduced insulin secretion has been found in patients with NAFLD[44-47].

Adiponectin levels are reduced in insulin resistance states and adiponectin has been shown to maintain liver integrity through the regulation of insulin sensitivity and or inflammatory response. Plasma adiponectin level is decreased in NAFLD and is related to insulin resistance and hepatic fat content[48,49]. General obesity, which is another feature of insulin resistance, was found to be in high prevalence in patients with NAFLD especially in the subgroup with normal Body Mass Index (BMI)[42-44,45].

Oxidative stress as an aetiological factor

In some individuals, hepatic steatosis of whatever aetiology, never progresses to steatohepatitis, and individuals show variable incidence, severity and progress of steatohepatitis and fibrosis. Multiple possible sources of oxidant stress in fatty liver have been identified. One known mechanism that links steatosis to necro inflammation and fibrosis is lipid peroxidation. Studies carried over the last three ATP depletion in the liver[77,78]. Metformin was shown to reduce the incidence of diabetes in non-diabetics or pre-diabetics who had elevated fasting and post-load plasma glucose concentrations[79]. With relatively safe side effect profile, metformin may prove useful in patients with NAFLD, insulin resistance and metabolic syndrome.

The insulin sensitising agents of the thiazolidinedione class is currently being tested for the treatment of NAFLD. These agents led to improvement in liver enzymes as well as histologic findings in previously reported smaller trials[80-84]. Thiazolidinediones are

synthetic ligands of the PPAR-γ and their actions are to improve insulin action, improve circulating adipocytokine levels[49,85], reduce central adiposity, decrease the plasma concentrations of TNF-α, and other cytokines, and other anti inflammatory functions all of which may help to reduce the necroinflammation seen in NASH[86-88].

- **Antioxidants** – Vitamin E at a dose of 400-1200 IU/day showed improvement of liver enzymes in a paediatric population with NASH but the liver enzyme abnormality recurred when treatment was discontinued. Histological improvement was not assessed in this study[89]. Betaine, a metabolite of choline showed decrease in aminotransferase levels and a trend towards histological improvement in a small number of adult patients[90]. N-acetylcystine was used in one trial which showed improvement in liver enzymes[91].

- **Cytoprotective agents** – Ursodeoxycholic acid was thought to have some beneficial effect in some preliminary studies[92-95]. But it failed to show any histological improvement after 2 years of treatment in the recently completed multi center randomized trial[96].

Reported treatment modalities for NAFLD

Life style changes
- Exercise
- Diet
- Gradual weight reduction

Cytoprotective agents
- Ursodeoxycholic acid

Antioxidants
- Vitamin E
- Betaine
- N-Acetyl cysteine

Lipid lowering drugs
- Gemfibrosil
- Bezafibrate
- Atorvastatin

Insulin sensitizers
- Metformin
- Thiazolidinediones

Resolution of histologic abnormalities as determined by liver biopsy remains the gold standard for treatment outcomes. However, normalisation of aminotransferases, loss of fat detected by imaging studies, indices of insulin resistance, body mass index, waist circumference and lipid profiles are some of the endpoints that can be routinely assessed in clinical practice until a non-invasive predictor of disease activity is established.

NASH may recur in the transplanted liver in patients with previously established NASH[97-100] and in patients with cryptogenic cirrhosis[101-102] who undergo transplantation for end-stage liver disease.

Obesity and Type 2 diabetes are increasing among children and adults in epidemic propotions[103-104]. Life style changes are seen worldwide exposing a large number of people at risk of liver disease due to NAFLD. Many issues remain unresolved regarding the prevalence of the disease among various populations, including ethnic groups, natural history of the disease, diagnosis and treatment of NAFLD.

Prevention and treatment of metabolic syndrome is one major step that will reduce the burden of the disease in the future.

Diabetes and metabolic disorder clinics are ideal places to screen for patients with NAFLD for more aggressive life style changes and to explore possible treatment modalities.

Outcome of clinical scenario:

The case scenario presented in the introduction of this article describes a patient with metabolic syndrome at relatively high risk of NASH as a result of being over weight with a waist hip ratio of >1, hypertension, hypertriglyceridaemia and having an AST/ALT ratio >1. On oral glucose tolerance test he had impaired glucose tolerance. He under went liver biopsy, which showed grade 1 steatohepatitis. He was treated with atorvastatin, metformin and antihypertensives. He was advised to have an active life style, exercise regularly and to consume low fat diabetic diet. His LFTs improved as did his glycaemia, blood pressure and dyslipidaemia in six months' time.

Practice Points:

- NAFLD is an increasingly recognised condition and is common among Asians.
- Type 2 diabetes, Hyperlipidaemia and obesity which are increasing in epidemic proportions world wide, are risk factors of NAFLD
- NAFLD has the potential to progress to cirrhosis and end stage liver disease.
- Prevalence of NAFLD as a cause of advanced liver disease is expected to increase world wide in parallel to the rapidly increasing prevalence of Type 2 diabetes and obesity.
- Prevention and treatment of metabolic syndrome may reduce the burden of the disease in future.
- More research on NAFLD is called for among various sub groups of Sri Lankans.

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