Non-alcoholic Fatty Liver Disease: East Versus West

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Non-alcoholic fatty liver disease (NAFLD) is an important cause of liver disease worldwide with prevalence ranging from 10% to 30% in various countries. It has become an important cause of unexplained rise in transaminases, cryptogenic cirrhosis, and cryptogenic hepatocellular carcinoma. Pathogenesis is related to obesity, insulin resistance, oxidative stress, lipotoxicity, and resultant inflammation in the liver progressing to fibrosis. Pharmacological treatment in patients with NAFLD is still evolving and the treatment of these patients rests upon lifestyle modification with diet and exercise being the cornerstones of therapy. While there are many similarities between patients with NAFLD from Asia and the West, there are certain features which make the patients with NAFLD from Asia stand apart. This review highlights the data on NAFLD from Asia comparing it with the data from the West. (J Clin Exp Hepatol 2012;2:122–134)

Non-alcoholic fatty liver disease (NAFLD) is a recently coined entity and includes patients with simple steatosis and non-alcoholic steatohepatitis (NASH). Under the broad umbrella of NAFLD, NASH is an intermediate stage of liver damage and has a propensity of progressing to cirrhosis and hepatocellular carcinoma (HCC). Ludwig et al[1] introduced the term NASH in 1980 to describe histological changes indistinguishable from alcoholic hepatitis in patients with no or insignificant alcohol intake of <20 g/day. In the absence of alcohol intake, patients with insulin resistance (IR), with or without metabolic syndrome (MS), may develop hepatic steatosis due to increased lipolysis in adipocytes leading to excess fatty acid delivery and deposition in the liver. Some of these patients with hepatic steatosis develop hepatic oxidative stress and recruitment of various cytokines which lead on to hepatic inflammation and or fibrosis in addition to steatosis and progression from a stage of simple steatosis to NASH, setting the stage for future complications like cirrhosis and HCC. Non-alcoholic fatty liver disease is probably as common in Asia if not more than in the West. With emerging data from Asia on the prevalence, risk factors, pathogenesis, natural course, and treatment of patients with NAFLD, many similarities and certain differences have come up between the data from Asia and that from the West. This review compares the data on NAFLD as available from Asia and the West.

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Abbreviations: ALT: alanine aminotransferases; APO C3: apolipoprotein C3; CC: cryptogenic cirrhosis; CLD: chronic liver disease; DM: diabetes mellitus; FRAP: ferric-reducing ability of plasma; GSH: glutathione; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HDL: high-density lipoprotein; HTN: hypertension; IR: insulin resistance; ITT: insulin tolerance test; MS: metabolic syndrome; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; PCC: protein carbonyl; PNPLA3: patatin-like phospholipase domain-containing protein 3; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances; TG: triglyceride; TGF-β: transforming growth factor-beta; TNF-α: tumor necrosis factor-alfa; VLDL: very low density lipoproteins
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PREVALENCE OF NON-ALCOHOLIC FATTY LIVER DISEASE

General Population

Not all patients with metabolic risk factors develop NAFLD and only some patients with simple steatosis progress on to have progressive form of the disease, i.e., NASH. Further, the prevalence of NAFLD varies with different geographical locations but even in the same location, some races are more prone to develop NAFLD and NASH. The data on the prevalence of NAFLD are available from almost all the countries in Asia including China (5–24%), Japan (9–30%), South Korea (18%), Sri Lanka (33%), Malaysia (17%), and Indonesia (30%) to name a few.[2,3] Prevalence of NAFLD in India varies from 9% to 35%. The lower prevalence is from the most recent community-based epidemiological study where 1911 inhabitants of a rural administrative unit of West Bengal were analyzed for the prevalence of NAFLD. The diagnosis was based on dual radiological screening protocol consisting of ultrasonographic and computed tomographic examination of the liver. Transient elastographic examination and liver biopsy were performed in a subset to identify significant liver disease. The prevalence of NAFLD, NAFLD with elevated alanine aminotransferases (ALT), and cryptogenic cirrhosis (CC) was 8.7%, 2.3%, and 0.2%, respectively.[4] The figures for the prevalence of NAFLD are higher in the urban parts of India, ranging from 16.6% in Western India to 24.5% in Eastern India to 32% in South India.[5–7]

Thus, except for the lower prevalence in rural parts of India and China where the 'Eastern' lifestyle may be protective, the prevalence of NAFLD in urban India and other parts of Asia, which now have largely 'Western' lifestyles, is at least as high as that in the West where both population and hospital-based studies describe that around 10–24% have NAFLD and 3–4% have NASH.[8]

Racial Differences

Weston et al[9] looked at the prevalence of NAFLD in different ethnic groups of patients in the USA who were recently diagnosed as having chronic liver disease (CLD). Of 742 patients, 333 (47.7%) had definite or probable NAFLD. The proportion of Whites, Hispanics, Asians, and African Americans in the general population and NAFLD subgroup was 59% and 45%, 10% and 28%, 16% and 18%, and 9% and 3%, respectively. Hispanics had disproportionately higher representation in the NAFLD group compared with base population and whites and African Americans had lower representation, suggesting that Hispanics had the highest risk of developing NAFLD, followed by Asians and least in Whites and African Americans. In another study, Petersen et al[10] looked at the prevalence of various metabolic risk factors among different races in Italy and found that in spite of having lower body mass index (BMI), the Asian Indians had the highest levels of fasting serum insulin and IR as measured by homeostasis model assessment for IR (HOMA-IR) in comparison with other Eastern Asians, Caucasians, Blacks, and Hispanics. Asian Indians also had higher hepatic triglyceride (TG) content in comparison with Caucasians. This interethnic difference in the prevalence of NAFLD and NASH is believed to be related not only to different lifestyles but also to a strong genetic predisposition (discussed later).

High-risk Populations

Diabetes and obesity are the major risk factors for the occurrence and severity of NAFLD. Non-alcoholic fatty liver disease is very common in patients with type-2 diabetes mellitus (DM) with majority of patients with DM having evidence of fatty liver on imaging. Prevalence of NAFLD in diabetics was 74% in a North American study and 70% in an Italian study.[11,12] A study from Mexico found that the prevalence of NASH in diabetics was 18.5% compared with 7.1% in controls.[13] We found that 35 of 40 (88%) non-alcoholic patients with DM had evidence of fatty liver on ultrasound.[14] In another study from India, 49 of 100 patients with type-2 DM had evidence of fatty liver on ultrasound. Thirty-two of these patients underwent liver biopsy. Mild, moderate, and severe diseases were present in 21/32 (65.5%), 4/32 (12.5%), and 3/32 (9.35%) patients, respectively, and fibrosis was present in 7/32 (21.8%) patients with one having grade 4 and three having grade 3 fibrosis.[15] In other parts of Asia, the prevalence of NAFLD in DM was 56% in Iran, 35% in Korea, 40–50% in Japan, and 35% in China.[2,16,17]

In the West, 57–74% of obese individuals have NAFLD and 15–20% have NASH.[8] Prevalence of NASH in morbidly obese patients undergoing bariatric surgery was very high in USA with 48–60% of men and 20–31% of women having histological NASH.[18]

Similar high prevalence of NAFLD in obese population has been shown in Asia, ranging from 50% to 80% in Japan, 70% to 80% in China, 10% to 50% in Korea, and 47% in Indonesia.[2,19–21] One study from Taiwan in morbidly obese patients undergoing bariatric surgery found the prevalence of NASH to be as high as 80%.[22]

NON-ALCOHOLIC FATTY LIVER DISEASE AS A CAUSE OF SIGNIFICANT LIVER DISEASE

Enough evidence exists in the literature that NAFLD is responsible for a large number of patients presenting with unexplained raised transaminases and is an important cause of CC and cryptogenic HCC (CHCC).

Non-alcoholic Fatty Liver Disease and Unexplained Raised Transaminases

Non-alcoholic fatty liver disease/NASH is one of the most common causes of unexplained raised transaminases. In a study from Maryland, USA, 79% of cases of raised transaminases could not be explained by common etiologies like alcohol, viral hepatitis, or iron overload. These patients could have had NAFLD because of higher BMI, waist circumference, TG, fasting insulin, and lower high-density lipoprotein (HDL) compared with other etiologies of raised transaminases.[23] In a study from France including 272 patients of unexplained rise in transaminases, liver biopsy showed minimal lesions in 18.7%, steatosis in 26.8%, and NASH in 32.7%. Thus, NAFLD accounted for about 60% of these unexplained patients with raised transaminases.[24] A study from India found that NASH and chronic viral hepatitis were the most common causes of asymptomatic rise in hepatic transaminases, each accounting for 36% of patients.[25] In our experience, NASH is the third most common cause of raised transaminases (20%) after chronic viral hepatitis (38%) and alcoholic liver disease (40%).[26] In a study from Iran, 80% of patients with raised transaminases could not be explained by common etiologies like alcohol and viral hepatitis and of these about 80% had fatty liver. Thus, NAFLD probably accounted for about 60–65% of all patients with raised transaminases in Iran.[27] In Taiwan, 34% of all patients with raised transaminases are due to NAFLD, with the figures increasing to 60% in patients without alcohol or viral hepatitis.[28] Non-alcoholic fatty liver disease accounts for relatively lower proportion of patients with raised transaminases in India and Taiwan as hepatitis B and C together account for about 40% of cases in these populations.[25,26,28]

Overall, serum transaminases have poor correlation with histological severity in patients with NAFLD. Patients with raised ALT may not have histological NASH and severe disease cannot be excluded in those with normal ALT.[29] Mofrad et al[29] demonstrated in 51 patients with NAFLD and normal ALT and 50 with elevated ALT that there was no difference between the groups for fibrosis stage (1.5 vs 1.4, respectively), or prevalence of MS constituents like obesity, DM, hypertension (HTN), and dyslipidemia or demographic factors like age, gender, and ethnicity. Only steatosis grade was higher in the raised ALT group (2.16) vs normal ALT group (1.6). Amarapurkar et al[30] from India showed in 25 patients with NASH and normal ALT that 60% of these patients had histological evidence of cirrhosis in spite of persistently normal ALT. In fact, the clinical and histological spectrum of NAFLD was not different among 25 patients with normal ALT and 56 patients with abnormal ALT.

Non-alcoholic Fatty Liver Disease and Cryptogenic Cirrhosis

Non-alcoholic fatty liver disease/NASH is the most common cause of CC as shown by many studies from the West. Like any CLD, NAFLD/NASH may be asymptomatic in the beginning and may present later as CC or even HCC. It may be difficult in these patients to recognize NAFLD/NASH even on histology because liver fat decreases with increasing fibrosis and the characteristic changes of NAFLD/NASH may not be evident once it goes into the stage of cirrhosis. Large series from the West indicate that 50–75% of patients with CC have obesity and diabetes[31,32] and 33% of patients transplanted for CC show evidence of NAFLD on histology of their explanted livers.[33]

In a study from India, NASH with or without cirrhosis, and CC occurred more commonly in patients with diabetes than those without it. The incidence of diabetes in CC was 57% vs 30% in non-CC, suggesting the role of DM and NAFLD in causing cirrhosis.[34] The most convincing data on high prevalence of NAFLD in CC come from a recent study from a large volume liver transplant center in India. Clinicopathological features of NAFLD were explored by the clinical data and by examining the explant livers in living donor liver transplant recipients. Among 103 adult liver transplant recipients with different types of CLD, 30 had a pre-liver transplant diagnosis of CC. Of the 30 CC cases, 19 (63.3%) were finally labeled as NAFLD-related cirrhosis and showed histological features in several respects different from those reported for the early and established phases of NAFLD.[35] We recently published results of surrogate markers of NAFLD in 65 patients with CC and compared the results with 50 patients with virus-related cirrhosis of comparable age, gender, and severity of liver disease. All possible etiologies for cirrhosis including viral, autoimmune, Wilson's disease, and iron overload were excluded. Screening for occult HBV infection [by total antibodies against core antigen (anti-HBc total)] and celiac disease (by anti-tissue transglutaminase antibodies, anti-endomysial antibodies, and duodenal biopsies) was also done in 16 and 10 patients, respectively. Mean BMI was higher in patients with CC ($26.06 \pm 5.96\,Kg/m^2$) in comparison with virus-related cirrhosis ($22.12 \pm 1.71\,Kg/m^2$). Higher number of patients with CC had abnormal waist [38 (58.5%) vs 15 (30%)], type-2 DM [26 (40%) vs 5 (10%)], and lower serum HDL [35 (53.8%) vs 3 (6%)] in comparison with virus-related cirrhosis, again suggesting NAFLD as the possible etiology in these patients of CC.[36] Data on NASH as a cause of CC from other countries in Asia are sparse. A study from Japan found that 5% of cirrhotics did not have a definite viral, alcohol, autoimmune, or primary cholestatic disease as etiology. Two percent had features of NASH either histologically or metabolically and 3% were genuinely cryptogenic. So, NASH accounted for at least 40% of cases without an obvious etiology and figures could be higher as some of the cryptogenic cirrhotics may have had burnt out NASH with disappearance of steatosis with worsening histology.[37]

Non-alcoholic Fatty Liver Disease and Cryptogenic Hepatocellular Carcinoma

In a study from the US, CC accounted for 29% of all HCC, and was the second most important cause after HCV infection. Half of these patients had clinical or histological features of NAFLD.[38] Non-alcoholic fatty liver disease accounts for 40% of cases of HCC in UK and is the most common etiology.[39] A study from Italy showed that patients with CHCC had higher prevalence of obesity and DM and higher TG and cholesterol levels suggesting NAFLD as the underlying etiology.[40]

On the Eastern side, a study from Japan found that 17 of 320 (5.3%) HCC cases were either associated with NASH or had a cryptogenic etiology.[41] Cryptogenic hepatocellular carcinoma accounted for 5.4% of all HCC in Korea, and this group had higher BMI, TG levels, DM, and HTN compared with HCC with well-defined etiologies.[42] In another study from Korea, 20 of 36 (56%) patients with CHCC had at least two risk factors for NAFLD.[43] The lower proportion of NAFLD/CC as etiology for HCC in Asia could be due to higher burden of chronic viral hepatitis, especially hepatitis B compared with the West as discussed in later sections.

We analyzed surrogate markers of NAFLD in 39 patients with CHCC with all possible etiologies for HCC excluded and compared the results with 39 patients with virus-related HCC (VHCC). Patients with CHCC had a higher BMI ($24.35 \pm 4\,Kg/m^2$ vs $22.5 \pm 3.4\,Kg/m^2$) and higher prevalence of type-2 DM [15 (38.5%) vs 7 (17.9%)] in comparison with VHCC. There was no difference in abnormal HDL, serum TGs, and HTN among patients

with CHCC and VHCC. Higher prevalence of metabolic risk factors if taken as surrogate markers of NAFLD suggests that NAFLD is an important cause of both CC and CHCC, thus contributing to significant liver disease in India.[36]

Type-2 DM and obesity may predispose to the formation of HCC beyond that expected with advanced fibrosis as seen in a Japanese series of 11 patients of NASH-related HCC where 7 patients did not have cirrhosis and 91% were obese or had type-2 diabetes, HTN, or dyslipidemia.[44] Another study from Japan found that majority of patients with NASH-related HCC were either noncirrhotic or Child A cirrhotic compared with Child B and C cirrhosis in other etiologies like hepatitis B, C, and alcohol. Also, the prevalence of obesity and diabetes was significantly higher in this cohort.[41] Formation of HCC in noncirrhotic livers in NASH is a cause of concern, and appropriate screening protocols for HCC in NASH need to be formulated like those for hepatitis B infection.

RISK FACTORS AND PATHOGENESIS

Risk factors for NAFLD include increasing age, male gender (in Asians), obesity, and IR. Though the pathogenesis of the NASH is not completely understood, it involves the deposition of fat in the liver; increased fatty acid oxidation, oxidative stress, and cytokines may then lead on to steatohepatitis and fibrosis in some of these patients. Various pathogenetic mechanisms responsible for these insults include interplay of cytokines (tumor necrosis factor-alfa [TNF-α], adiponectin, resistin, leptin, interleukins, transforming growth factor-beta [TGF-β], etc.) causing IR, serum and liver iron overload and oxidative stress leading to necro-inflammation and fibrosis. Finally, genetics play an important role in determining the occurrence and progression in patients with NAFLD. Differences among various ethnicities have been recently explained by various gene polymorphisms including the emerging data on apolipoprotein C3 (APO C3) and patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene variations.

Age

The prevalence of NAFLD increases with age. Patients >60 years of age were more likely to have NAFLD than those <50 years or between 50 years and 60 years of age. This was correlated with a higher prevalence of MS constituents in the older population. Also, older patients are more likely to have advanced fibrosis, cirrhosis, and HCC.[45,46] Whether this is due to longer duration of disease or actually more virulent disease in elderly is not clear. A study from China did not find a worse outcome for older patients.[47] Also, the mean age at which NAFLD is detected differs between the West and the East. In most of the Western studies, the mean age of patients with NAFLD is around 50–55 years.[1,48,49] which is somewhat higher than seen in most Asian studies with a mean age of 45 years in Pakistan,[50] 42 years in the Phillipines,[51] and 45 years in Japan[19] and China.[52] Our own data show the mean age of patients with NAFLD to be 38 years.[53] Similarly, data from the Western part of India found the mean age of patients with NAFLD to be around 39 years.[5] Data from the USA also suggest that the Asians have a lower age at presentations (46 years) compared with Caucasians (52 years) and African Americans (61 years).[9]

Gender

Based on the Western data, it was previously believed that NAFLD is more common in females.[48,49] However, it has now been shown from a multiethnic study in the USA that in Asians, 79% of patients are male, compared with only 44% in Caucasians.[9] Our data also show that 70% of our patients are male,[53] which is similar to Japan where 61% of NAFLD patients are males.[19] Data from other Asian countries show that the prevalence of fatty liver was higher in males being 13.3% and 2.7%, respectively, in males and females in China,[52] and 21.6% and 11.2%, respectively, in Korea.[17] The reason for this difference in gender distribution could be linked in part to the difference in lifestyles of women in Western and Eastern settings, but the role of a genetic predisposition in the Asian men seems likely given the recent discovery of the role of APO C3 polymorphisms in the Asian Indian men with NAFLD.

Overweight/obesity

Mean BMI in Western studies of patients with NAFLD has been around 30–35 Kg/m^2.[48,49] This is in sharp contrast to Asian studies where the mean BMI is about 29 Kg/m^2 in India[53,54]; 24 in Korea,[17] 27 in Sri Lanka,[55] 23 in China,[52] and 23 in Japan.[19] Initial studies from India that used the international criteria[56] for defining overweight and obesity found obesity in only 12–30% of patients[57,58] compared with 30–100% in Western studies depending on the population studied.[8] We found overweight and obesity in 64% and 12% of patients with NAFLD[59] using the international criteria but our figures got reversed to 20% (overweight) and 68% (obesity) when we used the Asia-Pacific criteria[60] for overweight and obesity in our later studies, involving more than 100 patients.[53,54] Even at lower BMI, the Asians have been found to have a high percentage of body fat compared with white Caucasians and Blacks. At a given percentage of body fat, the BMI values of the Asians including Asian Indians was 3 Kg/m^2 lower than that in white Caucasians.[61] This is partly explained by the body build (trunk to leg-length ratio), low muscularity, adaptation to chronic calorie deprivation, and ethnicity.[62] More importantly, the morbidity and mortality associated with higher body fat occur more frequently at lower BMI

in Asians than in white Caucasians. In a study from Korea, the optimum BMI cut-off to predict diabetes, HTN, and dyslipidemia was lower than the internationally recommended cut-off.[63] A study from Delhi showed that about 66% of men and 88% of women classified as non-obese based on the international cut-off of BMI had one or more cardiovascular risk factors.[62] Based on these data, it has been suggested that the BMI limits for overweight and obesity should be lower for Asians.[60] Recommendations for BMI are normal 18–22.9 Kg/m^2, overweight 23–24.9 Kg/m^2, and obesity as BMI ≥ 25 Kg/m^2. A high prevalence of abdominal obesity is seen in Asians, including Asian Indians even when the BMI is < 25 Kg/m^2.[64] Similarly, Asians have been found to have more intra-abdominal adipose tissue than Caucasians, in spite of having smaller waists.[64] Lower cut-offs (waist circumference ≥ 90 cm in males and ≥ 80 cm in females) are also recommended for identifying abdominal obesity in Asians.[65]

Insulin Resistance

Insulin resistance is thought to be the key factor, which leads to increase in lipolysis and increased uptake of fatty acids by hepatocytes. Hyperinsulinemia occurring as a result of IR also increases the intrahepatocytic fatty acids by increasing the glycolysis and decreasing the APO B-100 and resulting in decreased export as very low density lipoproteins (VLDL). The end result is the increase in fatty acids and TGs in the hepatocytes leading to steatosis. Insulin resistance is almost a universal phenomenon in patients with NAFLD and is related to the imbalance between proinsulin (adiponectin) and anti-insulin cytokines (TNF-α) particularly those secreted from the adipose tissue (adipokines).[66] Though glucose clamp studies are ideal method of studying IR, most studies in patients with NAFLD have used HOMA-IR. Studies from Italy have demonstrated that NAFLD is associated with higher IR compared with controls, even after excluding overweight and obese subjects,[67] and that IR increases with increasing degree of steatosis.[68] Similar data are available from Australia where it has been shown that IR independently predicts fibrosis[69] and that IR is seen in both obese and lean cases of NAFLD.[70] The prevalence of IR in NASH was 75% compared with 8% of matched hepatitis C virus (HCV) cases.[70] Insulin resistance is seen in about 63% of NAFLD cases in the USA[71]; 83–98% of our patients had evidence of IR tested by both insulin tolerance test (ITT) and HOMA-IR.[53,54,59] Data from Delhi are similar and show the prevalence of IR to be 80% in patients with NAFLD.[72]

It was shown in a multiethnic trial from Italy that non-obese Indian men have a higher prevalence of IR compared with Eastern Asians, Caucasians, and Hispanics, and that this higher IR was associated with higher intrahepatic TG and serum IL-6 levels.[10] This may account for the development of NAFLD at lower BMI in Asian Indians compared with other ethnicities.

Diabetes Mellitus

Non-alcoholic fatty liver disease has been associated very closely with the presence of type-2 DM. Diabetes mellitus is an important determinant of both the presence and severity of NAFLD.[15] Even though NAFLD is very common in patients with DM, the presence of diabetes in patients of NAFLD presenting with raised transaminases is not very common. However, over the years, many patients with NAFLD do develop frank DM.

We observed the presence of DM in only 12–14% of our patients of NAFLD despite IR being present in > 80%.[53,54] Other centers from India have also reported low prevalence of DM at presentation in patients with NAFLD ranging from 4% to 10%.[4,72–74] Similarly, only 15% of Chinese patients with NAFLD have diabetes at presentation.[20] Diabetes was seen in only 4.2% and 4.8% of males and 5.8% and11.6% of females with steatosis and NASH, respectively, in Korea.[75] This is in contrast to the Western studies where the prevalence of diabetes was 30–60% in patients with NAFLD.[48,49] The reasons for this difference are not clear but could be related to lower BMI and lower prevalence of obesity in Asians compared with the West. Development of diabetes on long-term follow-up in patients with NAFLD has been shown from both Western and Asian countries. A study from Japan showed that patients with nondiabetic NAFLD have a 5.5 times higher risk of developing diabetes even after adjusting for age and BMI compared with controls with 8% developing diabetes over 4 years follow-up compared with 2% in controls.[76] Similarly, a study from China found that 20% NAFLD developed diabetes over 6 years follow-up compared with just 5% in matched controls.[77] In the USA, 22% of nondiabetic NAFLD developed diabetes over a 7.6 year follow-up.[48] It has been shown that DM is an independent predictor of mortality in patients with NAFLD[48,78]

Metabolic Syndrome

Currently, NAFLD is considered an integral part of the MS with IR as a central pathogenic factor. Metabolic syndrome is characterized by the presence of IR in association with other metabolic abnormalities like obesity, diabetes, dyslipidemia, and HTN. According to adult treatment panel III (ATP III) criteria, MS is defined by the presence of at least three of five conditions namely obesity, DM, HTN, low HDL, and high TG.

In Italy, MS was present in 36% of patients with NAFLD and increasing prevalence of NAFLD was seen with increasing BMI (18% of normal weight subjects, 29% of overweight, and 67% of obese). Eighty-eight percent of patients with NASH had MS compared with 53% with simple steatosis. Metabolic syndrome was associated with a high-risk of severe disease with OR of 3.2 and 3.5 for NASH and severe fibrosis, respectively, even after correcting for age, BMI, and gender.[79] Data from the Framingham Heart

Study from the US show that 63% of patients having NAFLD have MS compared with 25% of controls (OR 5.22, 95% CI, 4.15–6.57).[71] Metabolic syndrome as defined by the ATP III criteria was present in 50% of our patients.[53,54] Other centers from India have also reported the MS in 21–68% of patients with one metabolic risk factor being present in almost all patients.[72,73] Metabolic syndrome was detected in 36.1%, 46.6%, and 55.4% of patients with NAFLD in the normal weight, overweight, and obese groups in China.[80] Metabolic syndrome was seen in 8.5% and 22% of males and 8.6% and 31% of females with steatosis and NASH, respectively, in Korea.[75] The overall prevalence of MS in NAFLD is lower in Asia compared with the West, mainly due to lower prevalence of individual constituents like diabetes, HTN, and obesity (by international criteria) in the Asian patients.

Oxidative Stress and Mitochondrial Dysfunction

The increased load of fatty acids in the hepatocytes increases the mitochondrial β-oxidation and also the cytochrome P-450 4A and cytochrome P-450 2E1 levels, leading to increase in reactive oxygen species. The increased mitochondrial oxidative stress leads to the second hit from steatosis to steatohepatitis and fibrosis by three main mechanisms, namely lipid peroxidation, cytokine induction, and Fas ligand induction. A high ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) protects individuals against oxidative stress.

A British study found reduced glutathione content, superoxide dismutase (SOD) activity and the ferric-reducing ability of plasma (FRAP) in patients with steatosis and even more so in those with NASH. CYP2E1 activity was significantly increased in NASH patients.[81] Another study from Australia showed increased CYP2E1 by immunohistochemistry on biopsy specimens of NASH patients which was of a similar pattern to that seen in alcoholic liver disease. Also, this increased activity was localized to areas of steatosis.[82] An Italian study looked at several cytokines and inflammatory markers like TNF-α, adiponectin, resistin, leptin, interleukin-6, and markers of nitrosative stress and found that nitrotyrosine and adiponectin levels were associated with raised transaminases and poorer liver histology.[83]

Decreased hepatic GSH levels have been demonstrated and an association has been shown between the depleted hepatic GSH levels and lipid peroxidation in Indian patients with NAFLD.[84,85] In one of the studies, oxidative stress and antioxidant status were studied in 29 prospectively enrolled patients with NAFLD, 25 diseased controls with chronic viral hepatitis, and 23 healthy controls. Apart from standard biochemical parameters, lipid peroxidation products were measured as thiobarbituric acid reactive substances (TBARS). As measures of antioxidant capacity, SOD, vitamin C levels, and FRAP were measured. Products of lipid peroxidation were significantly increased among patients with NAFLD in comparison with chronic viral hepatitis or healthy controls.[84] In an attempt to explore the inter-relationship of oxidative stress and IR in NAFLD subjects with and without type-2 diabetes, 200 subjects were recruited from the Chennai Urban Rural Epidemiology Study Group and tested for TBARS, protein carbonyl (PCC), and glutathione levels. Thiobarbituric acid reactive substances and PCC levels were significantly elevated and GSH/GSSG ratio was significantly decreased in diabetic subjects with NAFLD compared with all other groups, suggesting that oxidative stress markers are significantly associated with NAFLD even after adjusting for age, gender, BMI, and glycemic status.[85]

Mitochondrial dysfunction may play a key role in the oxidative stress pathogenesis of NAFLD. Megamitochondria with linear crystalline inclusions have been observed in patients with NASH as described from USA.[86] Impaired activity of mitochondrial respiratory chain complexes that may promote oxidative stress has been observed in patients with NAFLD.[87] We have also found similar changes in mitochondria on electron microscopy in patients with NAFLD (unpublished data).

Iron/hemochromatosis Gene Mutations

The relationship between iron overload and NAFLD is complex and unresolved. There are studies both favoring and refuting the role of iron in the pathogenesis of NAFLD. Saturation of mitochondrial β-oxidation leads to peroxisomal oxidation and generation of hydrogen peroxide, which in the presence of increased iron is converted to hydroxyl radicles, thus adding to the oxidative stress and further injury. It has recently been reported in a study from USA that elevated serum ferritin is independently associated with a diagnosis of NASH, higher NAS score, and more severe fibrosis.[88] It is postulated that increased hepatic iron stores, which may be due to mutations in the hemochromatosis gene (HFE), may lead to NASH and advanced fibrosis in NAFLD via increased oxidative stress[89] as murine models have shown that iron overload combined with high fatty diet upregulates lipid synthesis genes.[90]

Many studies from the West have shown increased hepatic iron levels with some studies also showing high prevalence of HFE gene mutations.[91,92] In a study from USA, 8.4% patients of NASH were homozygous and 61% were heterozygous for HFE gene mutations compared with 3% and 38%, respectively, in controls.[92] In a study from Italy, 7.4% of patients had signs of peripheral iron overload, 9% had signs of hepatic iron overload, and 21.1% had hyperferritinemia. Serum ferritin was the independent predictor of fibrosis.[91] However, in our own data, patients with NAFLD did not have significant serum iron abnormalities (abnormal serum iron, ferritin, and transferrin saturation in 3%, 7%, and 8%, respectively, of a total of 60 patients).

Majority [20 (67%)] of our 30 patients had negative Perls staining for iron on liver biopsy and only 6 (20%) and 4 (13%) patients had 1+ or 2+ iron staining, further proving the point that iron probably had little role to play in our patients with NAFLD. None of the patients had C282Y HFE gene mutation and only 4 (13%) of 30 patients were found to be heterozygotes for H63D gene mutation.[93,94] Our results suggest that iron abnormalities do not play much role in the pathogenesis of NAFLD in Indian population. Similarly, none of the patients with NAFLD/NASH in Japan was found to have HFE gene mutations.[95] In a multiethnic study from Australia, only Anglo-Celtic patients were found to have increased C282Y heterozygosity in NASH compared with controls, although it did not predict for disease severity or fibrosis. None were homozygous for the mutation.[96] Thus, the role of HFE mutations and iron overload seems to be an ethnic phenomenon, seen predominantly in the West.

Apolipoprotein C3 and Non-alcoholic Fatty Liver Disease

There are three types of apolipoproteins C, that is, C1, C2, and C3. Apolipoprotein C3 is the most abundant of the C apolipoproteins and inhibits lipoprotein lipase that hydrolyzes TGs to generate free fatty acids before their uptake by the muscle and adipose tissue. The proposed mechanism by which APO C3 variants increase the risk of NAFLD is that the increased plasma levels of APO C3 inhibit lipoprotein lipase and TG clearance, leading to fasting and postprandial hypertriglyceridemia due to an increase in chylomicron remnants. The chylomicron remnants are preferentially taken up by the liver, resulting in NAFLD and hepatic IR. Two single nucleotide polymorphisms (SNPs) in the promoter region of the APOC3 gene [rs2854117 (–482C>T) and rs2854116 (–455T>C)], which are in strong linkage disequilibrium with each other, have been reported to be associated with hypertriglyceridemia, MS, and coronary artery disease.[97] Thus, they are expected to be associated with NAFLD also. However, literature on the role of APO C3 polymorphism in patients with NAFLD is controversial. A study from the USA comparing the presence of APO C3 polymorphism in Asian Indian men and non-Asian Indian men found that in healthy non-obese subjects, 38% of the Asian Indian men with APO C3 variants (C-482T and T-455C) had NAFLD and marked IR, whereas no APO C3 wild-type homozygotes had NAFLD. In the non-Asian Indian cohort, the difference in NAFLD between those with and without variant alleles was 9% and 0%, respectively. The prevalence of variant APO C3 alleles was similar among the Asian Indian men and non-Asian Indian men, suggesting that other factors are responsible for the increased prevalence of NAFLD (38% vs 9%) among the Asian Indian men compared with other ethnicities.[98] In contrast, other studies in various ethnic groups like Americans, European Americans, African Americans, and Hispanics have shown that APO C3 variants probably have no effect in causing NAFLD, nor are associated with IR.[99,100] Even other Asian ethnicities like Chinese did not have this association.[101] This discrepancy could be due to racial differences which make Indians more susceptible to the effects of APO C3 polymorphisms. As this gene is located close to several other genes involved in lipid metabolism, linkage disequilibrium with another gene may be responsible for the increased NAFLD seen in Indians with APO C3 polymorphisms. Also, a major difference in the Peterson study was the selection of non-obese patients (mean BMI 24 Kg/m^2) compared with obese patients in other studies. Effect of APO C3 may be dependent upon adiposity status as shown in a study by Peter et al where gene expression and intrahepatic TG content were quantified in liver tissue samples from 50 Caucasians. Individuals carrying at least one of the minor alleles of the rs3854116 or the rs2854117 SNPs in the APO C3 were found to have higher liver fat content than those homozygous for the wild-type only in the subgroup with the lowest tertile of waist circumference.[102] The polymorphic forms of APO C3 gene are relatively resistant to the suppressive action of insulin, leading to high levels of APO C3 protein in the liver. In pre-existing IR states like obesity and MS, the effect of the polymorphism may become redundant. Asian Indians are less obese than their Western counterparts and this could be allowing a higher phenotypic expression of the APO C3 genetic polymorphism. However, this explanation is refuted by studies from other centers where association of APO C3 with NAFLD was not found despite low BMI of study subjects.[99,101] Further multiethnic studies with large sample sizes in each group are needed to further clarify the role of APO C3 in the pathogenesis of NAFLD.[103]

Patatin-like Phospholipase Domain-containing Protein 3 and Non-alcoholic Fatty Liver Disease

Patatin-like phospholipase domain-containing protein 3, also known as adiponutrin, is an enzyme that in humans is encoded by the PNPLA3 gene. Patatin-like phospholipase domain-containing protein 3 is a triacylglycerol lipase that mediates triacylglycerol hydrolysis in adipocytes. The encoded protein, which appears to be membrane-bound, may be involved in the balance of energy usage/storage in adipocytes. In animals and humans, PNPLA3 is primarily expressed in white adipose tissue and liver, its expression is nutritionally regulated and increases with obesity. Patatin-like phospholipase domain-containing protein 3 may also have a role in adipogenesis, being upregulated during the differentiation of white adipocytes. Patatin-like phospholipase domain-containing protein 3 expression is influenced by insulin; it is still unclear whether its expression is decreased in subject with IR.

Two SNPs have been described in relation to NAFLD: a G-to-C change leading to substitution of isoleucine with methionine at codon 148 (I148M; rs738409) and a G-to-T change leading to substitution of serine with isoleucine at codon 453 (S453I; rs6006460). The S453I variant is protective for NAFLD and is common among African Americans, which may explain the low prevalence of NAFLD in them.[104] The I148M variant has been shown to be associated with NAFLD in multiple ethnicities in the USA independent of BMI and diabetes status based on genome-wide association studies. This allele was most commonly found in Hispanics who are also the group with the highest prevalence of NAFLD.[105] G allele was also associated with increased steatosis and higher fibrosis in an Italian and British study.[106] Association of G allele with NAFLD is seen in children, adults, obese, non-obese, diabetics, and nondiabetics.[107] Similar association has been reported from Asian countries.[101,108] A recent meta-analysis has confirmed not only the role of this polymorphism in the development of NAFLD across all ethnicities, but also its association with more severe disease with 3.24 times higher risk of high necro-inflammatory scores and liver fibrosis in GG compared with CC genotype.[109]

SEVERITY, PROGRESSION, AND OUTCOME

Severity at Diagnosis

There are some data to suggest that NAFLD may be less severe in Asians, at least at initial presentation compared with Western counterparts. In a study done in France and Hong Kong, advanced fibrosis or cirrhosis was found in 28% of Caucasian patients but in only 17% of Chinese patients.[110] Even though data from some other Asian countries have found NASH to be present in about 60–80% of patients with NAFLD which is similar to the data from the West,[111,112] our data showed that histological NASH was seen in only 22 of our 43 patients and of these 22 patients, all had mild (10 patients) or moderate (12 patients) inflammation. Six patients did not have fibrosis, whereas stage 1, 2, and 3 fibrosis was seen in seven, five, and four patients, respectively. None of the patients had cirrhosis.[53,54] Similarly, data are available from another center in India where it was found that of 51 patients of NAFLD only 53% patients had NASH of whom 63% had grade 1 and 31% had grade 2 inflammation. Twenty-three (45%) and 19 (37%) patients had stage 0 and stage 1 fibrosis, 8 (16%) had stage 2 and 1 (2%) had stage 3 fibrosis while none had cirrhosis on index biopsy.[72] This is contrast to the data from USA where 80% had NASH, 18% has stage 3, and 13% had stage 4 fibrosis on index biopsy.[48]

Natural History

Histological Progression and Cirrhosis

Non-alcoholic fatty liver disease in general is a relatively slowly progressive disease. However, disease progression depends upon baseline histology. Non-alcoholic steatohepatitis progresses faster than simple steatosis as evidenced by population-based and biopsy-based studies and can lead on to cirrhosis and HCC. In a study of 132 patients with biopsy-proven NAFLD from the USA, after 8 years of follow-up, 11% of patients with NASH developed liver-related mortality, compared with only 2% of the patients with non-NASH. After 18.5 years of follow-up in the same NAFLD cohort, liver-related mortality had risen to 18% for NASH and 3% for patients with non-NASH.[49,78] In a study from Hong Kong, after 3 years of follow-up of 13 patients with simple steatosis at baseline, 2 (15%) had a normal liver at month 36, 3 (23%) continued to have simple steatosis, 5 (39%) developed borderline NASH, and 3 (23%) developed NASH. Of 22 patients with borderline NASH at baseline, four (18%) had simple steatosis and 13 (59%) had borderline NASH, whereas 5 (23%) developed NASH. Among 17 patients with NASH at baseline, 10 (59%) continued to have NASH and 6 (35%) had borderline NASH at month 36. Only 1 (6%) patient regressed to simple steatosis.[113] Similarly, a study from the USA based on paired liver biopsies on 22 patients with NASH over a mean of 5.7 years showed that 32% of these patients had progression with either increased inflammation or fibrosis score with one-third of these patients rapidly progressing to advanced fibrosis.[114] These data from the East and the West show that NASH is usually a progressive disorder, but can occasionally revert to simple steatosis. Overall, 10–15% of NASH develops progressive fibrosis and cirrhosis.[115] Once cirrhosis occurs, the clinical outcomes are similar between NASH and other etiologies. The 5-year survival rates are 75% and 74% for NASH- and HCV-related cirrhosis, respectively.[116]

Development of Hepatocellular Carcinoma

Incidence of HCC with NASH is quite high once cirrhosis has developed, but slightly lower than that for hepatitis C infection. Data from the USA found the incidence to be 2.6% and 4.0% per annum, respectively, for NASH cirrhosis and HCV cirrhosis, with 13% and 20% patients developing HCC, respectively, after a mean follow-up of 3.2 years.[45] A study from Japan found the 5-year HCC rate to be 11% in NASH cirrhosis vs 31% in HCV cirrhosis.[117] The incidence of HCC is much lower in patients with noncirrhotic livers at baseline with only 0–0.5% NAFLD and 0–2.8% of NASH subjects developing HCC over long-term follow-up, mostly after they had developed cirrhosis.[118] However, recent studies have demonstrated that HCC can develop without cirrhosis in patients with NASH.[44] Overall, 26% of NASH-related HCCs arise in noncirrhotic livers, are diagnosed later, and have poorer outcomes than of HCC with other etiologies as these patients are not on regular surveillance programs.[44] Risk factors for the development of HCC in NASH include older age, advanced fibrosis or cirrhosis, DM, obesity, and iron deposition

in the liver.[118] No studies are available to compare racial differences in the development of HCC in NAFLD.

Nonliver-related Outcomes

A population-based study from the USA found that the mortality in patients with NAFLD was 12.6% after a follow-up of 7.6 years with standardized mortality ratio of 1.34 compared with general population. Liver disease was the third leading cause of death in NAFLD compared with 13th in the population. However, liver-related causes accounted for only 7 of the 53 (13%) deaths in the NAFLD population. Extrahepatic malignancies (28%), ischemic heart disease (25%), and infections (11%) were the other leading causes of death.[48] Similar results were obtained in another community-based study from the USA with important causes of death being cardiovascular disease (25%), neoplasms (24%), cerebrovascular disease (6%), and liver disease (6%).[119] Asian studies have also confirmed that patients with NAFLD have higher prevalence of diabetes, HTN, dyslipidemia and CRP, and higher cardiovascular risk by Framingham scoring compared with the general population, with NASH having higher risk than patients with simple steatosis.[75] Hazard ratios for cardiovascular events/mortality compared with controls were 1.6% and 1.8% in males and 2.9% and 5.3% of females with steatosis and NASH, respectively, in Korea.[75] Studies from around the world uniformly show that NAFLD is strongly associated with increased carotid-artery intimal medial thickness and an increased prevalence of carotid atherosclerotic plaques.[120] This suggests that extrahepatic complications associated with the MS play a major part in mortality of NAFLD patients, especially those who do not have progressive liver disease. However, even after adjusting for the presence of MS constituents, mortality remains higher in NAFLD patients with a hazard ratio of 9.22 for liver-related mortality and 1.12 for nonliver-related mortality compared with general population.[119] Possible mechanisms include the presence of chronic inflammation, hypercoagulation, hypofibrinolysis, atherogenic dyslipidemia, IR, and oxidative stress associated with NAFLD.[121]

We found that patients with NAFLD have increased atherosclerosis (as studied by the carotid intima media thickness and flow-mediated vasodilatation) and increased risk for cardiovascular disease (as studied by PROCAM score), but increased atherosclerosis and cardiovascular risk in these patients was dependent on the presence of MS (unpublished data).

TREATMENT

Weight loss and exercise are the only established treatment of NAFLD. However, less than 50% of patients achieve target weight loss. Studies are available from Asia,[74,122,123] including one from India,[74] documenting the beneficial effects of this approach in children and adults. Pharmacological therapy including insulin sensitizers, antioxidants, and pentoxifylline has yielded some benefit but not without adverse effects.[124] There are no studies on thiazolidinediones from India, but uncontrolled trials have shown benefit with ursodeoxycholic acid (UDCA),[53] metformin,[59] vitamin E,[125] and pentoxifylline.[126,127] Lack of a control arm makes interpretation of these studies difficult. Though most of the randomized trials in NAFLD are available from the West, a few randomized controlled trials are also available from Asia. A study from Singapore demonstrated improvement in liver enzymes with pentoxifylline.[128] Studies from Turkey have demonstrated beneficial effects of metformin[129] and pioglitazone[130] in patients with NAFLD. A study from China demonstrated the beneficial effects of polyunsaturated fatty acids in hyperlipidemic patients of NAFLD.[131] However, most of these studies are limited by the small sample size and lack of histological assessment. A meta-analysis has shown that bariatric surgery improves histology in patients with NASH[132] and similar data are now available from Asia as well.[133]

CONCLUSION

Non-alcoholic fatty liver disease is ubiquitous disease affecting all parts of the globe with a prevalence of about 10–30% in most societies. Non-alcoholic fatty liver disease accounts for relatively lower proportion of patients with raised transaminases in the East as chronic viral hepatitis are responsible for a high proportion of cases in these populations. Rural populations from Asian countries have a lower prevalence of NAFLD, though in metropolitan areas the prevalence of NAFLD is rising rapidly. Whether residing in the East or the West, the Asians seem to be more at risk than their Caucasian and African American counterparts. While in the West patients are more likely to be older, female, and obese, Asians develop NAFLD at a younger age, are more likely to be male, and are less likely to be obese. Traditional risk factors for NAFLD including advanced age, obesity, diabetes, and MS are less common. The role of HFE mutations and iron overload also seems to be an ethnic phenomenon, seen predominantly in the West. Thus, there is a strong empirical reason to suspect additional risk factors such as a genetic predisposition for the development of NAFLD in Asians, and recent data on the APO C3 gene seem to bear this out. Patatin-like phospholipase domain-containing protein 3 SNPs seem to be strongly associated with NAFLD irrespective of ethnicity. However, further research is needed before a definite genetic basis can be established or genetic screening/diagnosis advocated. Asians have milder disease at diagnosis, which may be linked to younger age and shorter duration of illness. However, the disease is progressive and significant number of cases of HCC and

cirrhosis are now accounted for by NASH. This proportion is lower than that seen in the Western countries due to a high burden of chronic viral hepatitis in Asia, but with successful immunization programs and effective treatment of viral hepatitis, NAFLD is likely to soon become the largest contributor to liver-related morbidity and mortality like it has in the West. This is in addition to the significant nonliver-related mortality directly attributable to NAFLD over and above that of co-existent metabolic conditions. Pharmacological therapy for NAFLD is still evolving and large-scale primary prevention health education and lifestyle programs are required to stem this tide while further research is done to identify the missing links in the pathogenesis and treatment of NAFLD.

CONFLICTS OF INTEREST

All authors have none to declare.

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