Unexplained elevation of aminotransferases as an independent predictor of insulin resistance

Thesis
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Introduction

Mild elevation of ALT is a common clinical problem of various causes. Except for those that can be attributed to viral hepatitis, alcohol or other chemical toxin exposure, most of cases are nonspecific causes or fatty liver related (Ghen et al., 2007).

A growing number of studies have suggested that elevation of ALT in absence specific liver diseases was associated with insulin resistance (IR) (Hanly, 2012).

Vosarova et al., 2013 had reported that elevated ALT of non specific causes was associated with obesity and hepatic insulin resistance and might predict the development of T2DM (Vosarova et al., 2013).

Both ethnicity and gender seem to play roles in the prevalence of mild elevation of ALT. Compared to women, men were 1.5-2 times more prone to have unexplained elevation of ALT although they had approximately equal prevalence of T2DM (Clark, 2010).

Serum ALT and AST level is well recognized markers of liver injury and may represent a consequence of the second event of pathogenesis of NAFLD. On the other hand, it has been demonstrated that augmented ALT activities can be a predictor to the development of IR, DM, cardiovascular diseases and metabolic syndrome in NAFLD patients (Farrell, 2012).
NAFLD

Nonalcoholic fatty liver disease (NAFLD) refers to the presence of hepatic steatosis when no other causes for secondary hepatic fat accumulation (eg, heavy alcohol consumption) are present. NAFLD may progress to cirrhosis and is likely an important cause of cryptogenic cirrhosis (Poonawala A, et al. 2000).

Patients with nonalcoholic fatty liver disease (NAFLD) have hepatic steatosis, with or without inflammation and fibrosis. In addition, no secondary causes of hepatic steatosis are present. (Poonawala A, et al. 2000).

NAFLD is subdivided into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). In NAFL, hepatic steatosis is present without evidence of significant inflammation, whereas in NASH, hepatic steatosis is associated with hepatic inflammation that may be histologically indistinguishable from alcoholic steatohepatitis. Other terms that have been used to describe NASH include pseudoalcoholic hepatitis, alcohol-like hepatitis, fatty liver hepatitis, steatonecrosis, and diabetic hepatitis (Sheth SG, et al. 1997).

Pathogenesis

Nonalcoholic fatty liver disease (NAFLD) is a clinico-histopathological entity with histological features that resemble alcohol-induced liver injury, but by definition, it occurs in patients with little or no history of alcohol consumption. It encompasses a histological spectrum that ranges from fat accumulation in hepatocytes without concomitant inflammation or fibrosis (simple hepatic steatosis) to hepatic steatosis with a necro-inflammatory component (steatohepatitis) that may or may not have associated fibrosis. The latter condition, referred to as nonalcoholic steatohepatitis (NASH), may progress to cirrhosis in up to 20 percent of patients. NASH is now recognized to be a leading cause of cryptogenic cirrhosis (Matteoni CA, et al. 1999).
The pathogenesis of nonalcoholic fatty liver disease has not been fully elucidated. The most widely supported theory implicates insulin resistance as the key mechanism leading to hepatic steatosis, and perhaps also to steatohepatitis. Others have proposed that a "second hit," or additional oxidative injury, is required to manifest the necroinflammatory component of steatohepatitis. Hepatic iron, leptin, antioxidant deficiencies, and intestinal bacteria have all been suggested as potential oxidative stressors. (Matteoni CA, et al, 1999).

CAUSES OF TRIGLYCERIDE ACCUMULATION — Hepatic steatosis is a manifestation of excessive triglyceride accumulation in the liver. This can occur from the excessive importation of free fatty acids (FFA) from adipose tissue, from diminished hepatic export of FFA (secondary to reduced synthesis or secretion of very low-density lipoprotein [VLDL]), or from impaired beta-oxidation of FFA. The major sources of triglycerides are from fatty acids stored in adipose tissue and fatty acids newly made within the liver through de novo lipogenesis. (Donnelly KL, et al, 2005).

- Excessive importation of FFA can result from either increased delivery of triglycerides to the liver (as seen with obesity and rapid weight loss), or from excessive conversion of carbohydrates and proteins to triglycerides (eg, secondary to overfeeding or use of total parenteral nutrition) (Donnelly KL, et al, 2005).
- Impaired VLDL synthesis and secretion can result from abetalipoproteinemia, protein malnutrition, or choline deficiency. Patients with nonalcoholic steatohepatitis (NASH) may have a defect in postprandial Apo B secretion, leading to triglyceride accumulation. In addition, a defect in the lipidation of Apo B, caused by an inhibition of microsomal triglyceride transfer protein (MTP), may be a key mechanism in drug-induced nonalcoholic fatty liver disease (NAFLD), such as seen...
with amiodarone and tetracycline. Depletion of the orphan receptor small heterodimer partner (SHP) results in increased VLDL secretion, elevated MTP levels, and increased insulin sensitivity, whereas induction of SHP results in the rapid accumulation of hepatocyte lipids. Impaired VLDL synthesis and secretion were also more apparent in patients with NASH compared with patients with hepatic steatosis. This may have resulted in the induction of lipid oxidation and oxidative hepatocyte damage.

- Treatment of hypertriglyceridemia with eicosapentaenoic acid (EPA) reduces steatosis, oxidative stress, inflammation, and progression of fibrosis in a NASH animal model (Kajikawa S, et al, 2010).
- In the 96-week Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial, patients who had resolution of NASH seen on biopsy also had significant decreases in triglyceride levels (-21.1 mg/dL versus -2.3 mg/dL) (Corey KE, et al, 2015).
- Impaired beta-oxidation of FFA to adenosine triphosphate (ATP) may be seen with vitamin B5 (pantothenic acid) deficiency, excessive alcohol consumption, or coenzyme A deficiency (as can occur with valproic acid or chronic aspirin use). Activation of peroxisome proliferator-activated receptor alpha appears to have a central role in stimulating beta-oxidation and disposing hepatic fatty acids in NASH. The ability to recover from hepatic ATP depletion is severely impaired in patients with obesity-related NASH. Compromised hepatic ATP homeostasis may predispose to injury from other insults. Adiponectin, a fat derived hormone, appears to have a pivotal role in improving fatty acid oxidation and decreasing fatty acid synthesis. Administration of adiponectin improved hepatomegaly, steatosis, and alanine aminotransferase levels in obese, leptin deficient mice. Also implicated in the steatosis pathway is the cannabinoid receptor type 1 (CB1). Administration of a CB1 receptor antagonist rapidly
abolished hepatic steatosis, improved aminotransferase levels, reduced the levels of proinflammatory cytokines, and increased adiponectin levels in leptin-deficient mice (Gary-Bobo M, et al, 2007).

- Triglyceride synthesis and oxidation appear to be regulated, at least in part, by the enzyme acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1). DGAT1 deficient hepatocytes were protected from hepatic steatosis by reducing synthesis and increasing oxidation of fatty acids in an animal knockout model. DGAT1 activity was necessary for the manifestation of hepatic steatosis (Villanueva CJ, et al, 2009).

- Micro RNA (MiRNA) also has a role in hepatocellular lipid metabolism and immunity. These are small non-coding RNAs that post-translationally modulate gene function and are involved in cellular processes, including cellular proliferation, inflammation, and apoptosis. Alterations in MiRNA activity result in hepatocellular injury, apoptosis, and portal fibrosis. In NAFLD, specific MiRNA appear to regulate genes involved in fatty acid biosynthesis. Antagonism of specific MiRNA leads to decreased fatty acid synthesis and increased hepatic fatty acid oxidation (Jin X, et al, 2009).

**INSULIN RESISTANCE** — Insulin resistance has a key role in the development of hepatic steatosis and, potentially, steatohepatitis. Obesity and type 2 diabetes, conditions associated with peripheral insulin resistance, are frequently observed in patients with nonalcoholic fatty liver disease (NAFLD). Insulin resistance has also been observed in patients with nonalcoholic steatohepatitis (NASH) who are not obese and those who have normal glucose tolerance. Despite the strong association, not all patients with NASH exhibit insulin resistance. This suggests that NASH may be a heterogeneous syndrome with more than one cause (Kim HJ, et al, 2004).
The genetic basis for insulin resistance associated with NASH remains unclear. One report found an association with certain polymorphisms in the gene encoding for apoliprotein C3, while another study demonstrated that IL-6 polymorphisms are associated with NAFLD and markers of insulin resistance and inflammation. A third report found polymorphisms in a gene encoding for protein expressed in adipose tissue (adiponutrin) and involved in triglyceride metabolism. (Rotman Y, et al, 2010).

Certain variants of the gene were strongly associated with the histologic severity of NAFLD. In addition, alterations in the transcriptional activity of the peroxisome proliferator-activated receptor γ coactivator 1α (PPARGC1A) promoter correlated with the insulin resistance phenotype and the presence of NAFLD. In both adults and children, a single nucleotide polymorphism in the peroxisome proliferator-activated receptor-gamma coactivator 1-alpha gene (PPARGC1A) has been associated with an increased risk for developing NAFLD. A clinical trial supports the receptor's pathophysiological role. Patients who took elafibranor (an agonist of peroxisome proliferator-activated receptors alpha and delta) 120 mg daily for 52 weeks had resolution of NASH with improvements in fibrosis, liver enzymes, glucose and lipid profiles, and systemic inflammatory markers more often than those who received placebo (Ratziu V, et al, 2016).

Increases in visceral adipose tissue and intrahepatic fat correlate with increased gluconeogenesis, increased free fatty acid (FFA) levels, and insulin resistance. Visceral fat has also been associated with liver inflammation and fibrosis in patients with NASH independently of insulin resistance, an effect possibly mediated by interleukin-6 (a proinflammatory cytokine). Increased expression of hepatic interleukin-6 correlated with insulin resistance in another report. Several other cytokines and adipokines involved in insulin receptor signaling appear to be altered in omental adipose tissue of NASH patients. The activation
of tumor necrosis factor alpha-converting enzyme correlated with levels of insulin resistance, macrovesicular steatosis, and balloon degeneration in an animal model (Fiorentino L, et al, 2010).

Further supporting the role of insulin resistance are the observations from pilot studies, which have demonstrated beneficial effects of glucose-sensitizing medications in patients with NAFLD. Treatment with rosiglitazone reduced the expression of hepatic acute phase reactants (C-reactive protein and serum amyloid A), suggesting that improvements in insulin sensitivity correspond with a reduction in inflammation. Similarly, pioglitazone therapy led to significant improvements in steatosis and necroinflammation in patients with NASH and impaired glucose tolerance, correlating with decreases in adipose tissue insulin resistance. In other reports, patients with NAFLD and glucose intolerance were significantly more insulin resistant than glucose intolerant patients without fatty liver (Gastaldelli A, et al, 2009).

Resistance to the action of insulin results in important changes in lipid metabolism. These include enhanced peripheral lipolysis, increased triglyceride synthesis, and increased hepatic uptake of fatty acids. Each of these may contribute to the accumulation of hepatocellular triglyceride, which in turn results in a preferential shift from carbohydrate to FFA beta-oxidation, an occurrence that has been demonstrated in patients with insulin resistance. Significantly increased FFA levels have been observed in patients with NAFLD and type 2 diabetes mellitus, compared with type 2 diabetics without NAFLD. The molecular pathways leading to insulin resistance are complex and have not been completely elucidated. Several molecules appear to be involved in interfering with the actions of insulin on a cellular level (Kelley DE, et al, 2003).
Lipophilic bile acids have been demonstrated to promote insulin sensitivity and decrease hepatic gluconeogenesis and triglyceridemia via binding to the farnesoid X nuclear receptor. A randomized trial with 141 patients demonstrated that 45 percent of the patients who received obeticholic acid (a potent farnesoid X nuclear receptor activator) had a two point or greater improvement in NAFLD activity score on liver biopsy after 72 weeks of treatment compared with 21 percent of patients in the placebo group, supporting the receptor's role in NAFLD (Neuschwander-Tetri BA, et al, 2015).

HEPATOCELLULAR INJURY — FFAs are inducers of several cytochrome p-450 microsomal lipoxygenases, capable of producing hepatotoxic free oxygen radical species. Furthermore, the shift to FFA beta-oxidation in the setting of preexisting defects in mitochondrial oxidative phosphorylation may result in increased free radical formation, hepatocellular injury, and fibrosis. Electron microscopy of hepatocytes from patients with NAFLD demonstrated that significant mitochondrial structural abnormalities were present in patients with NASH, but not those with simple hepatic steatosis (Angulo P, 2002).

These investigators hypothesized that, in the absence of these mitochondrial defects, peripheral insulin resistance will lead only to the development of simple fatty liver. Consistent with this theory is the observation that several genes important for mitochondrial function were significantly underexpressed in NASH patients, suggesting that there is a transcriptional basis for impaired mitochondrial function. On the other hand, it is possible that mitochondrial structural abnormalities may simply be a consequence of increased lipid peroxidation since lipid peroxidation products alter both mitochondrial DNA and mitochondrial respiration (Sreekumar R, et al, 2003).

Others have suggested that the development of hepatocellular injury requires the presence of both insulin resistance and a second defect that results in the
accumulation of damaging free oxygen radical species. Several potential oxidative stressors have been proposed to result in necroinflammation. (Sreekumar R, et al, 2003).

The activation of nuclear factor kappa-beta and increased cytokine production appear to mediate the hepatocyte inflammatory process. Numerous proinflammatory cytokines and inflammatory mediators have been identified as having a role in hepatocyte inflammation and injury, including the activation of TNF-alpha, the complement system, plasma myeloperoxidase, natural killer cells, among others. Estrogens may protect against fibrogenesis in NAFLD patients as men and postmenopausal women have been found to have a high risk for more severe fibrosis compared with premenopausal women (Rensen SS, et al, 2009).

Furthermore, the induction of the Hedgehog ligand pathway appears to correlate with the severity of portal inflammation in both adult and pediatric patients with NAFLD. Evidence also points to a pathogenic role of caspase-2, a protease involved in cellular apoptosis, whose expression strongly correlates with liver disease severity in patients with NAFLD. In animal models, caspace-2 deficiency protected the liver from steatohepatitis (Machado MV, et al, 2016).

ANTIOXIDANTS — Lipid peroxidation and free oxygen radical species can deplete antioxidant enzymes such as glutathione, vitamin E, beta-carotene, and vitamin C, thus rendering the liver susceptible to oxidative injury. Serum levels of xanthine oxidase, a generator of reactive oxygen species, are higher in patients with non-alcoholic steato-hepatitis (NASH) compared with controls, whereas levels of multiple antioxidant enzymes are lower. In addition, the induction of heme oxygenase-1, an antioxidant defense enzyme, interrupted the progression of steato-hepatitis by inducing an antioxidant pathway and
suppressing proinflammatory cytokines. A correlation between disease severity and increased expression of oxidative scavenger receptors has been described. Serotonin has been implicated as a source of reactive oxygen species in NASH. Increased catabolism of serotonin resulted in increased levels of reactive oxidative species and necroinflammation in an animal NASH model (Yu J, et al, 2010).

There is indirect evidence supporting the role of antioxidants in preventing oxidative liver injury. Vitamin E therapy normalized serum aminotransferase elevations in children with fatty liver disease. In another report, a six-month course of combination therapy with vitamin E and vitamin C resulted in significant histological improvement, both with respect to inflammation and fibrosis scores. In a third report, dietary intake of antioxidant vitamins was significantly lower in NASH patients, compared with age and body mass index-matched controls (Harrison SA, et al, 2003).

The beneficial effect of antioxidant therapy may be mediated by regulatory T cells, which are depleted in steatotic mice. Antioxidant therapy resulted in reduced regulatory T-cell apoptosis and decreased hepatic inflammation (Ma X, et al, 2007).

IRON — Increased hepatic iron may also have a role in the development of nonalcoholic steatohepatitis (NASH).

- Insulin resistance is associated with increased hepatic iron levels, and improved glycemic control is associated with improvements in serum ferritin and hepatic iron concentrations (Viganò M, et al, 2000).
- The prevalence of heterozygosity for the hemochromatosis gene mutation (HFE) may be increased in patients with NASH associated with
increased hepatic iron concentrations and alanine aminotransferase (ALT) levels. In one report, there was a significantly increased prevalence of HFE mutations and iron overload in patients with primary hypertriglyceridemia, a condition that is associated with nonalcoholic fatty liver disease (NAFLD). However, another report found no association between the presence of an HFE mutation or a specific HFE genotype with the severity of hepatic fibrosis in patients with NAFLD (Valenti L, et al, 2010).

- Increased parenchymal hepatic iron concentration in NASH appears to correlate with the severity of fibrosis. A study of 840 patients with NAFLD demonstrated that the pattern of iron staining correlated with the severity of histological injury. Among the 35 percent of patients with NAFLD who had stainable hepatic iron, those with a reticuloendothelial system (RES) cell pattern of iron staining were more likely to have portal inflammation, hepatocellular ballooning, definite steatohepatitis, and fibrosis. Patients with RES iron were also significantly more likely to have advanced fibrosis (Nelson JE, et al, 2011).

The specific mechanism by which hepatic iron may contribute to necroinflammation is unknown, but may be related to the generation of free oxygen radical species that occurs in the process of reduction of Fe 3+ to Fe 2+.

In one study, iron, even at normal levels, was an important factor in determining sensitivity to insulin. In addition, compared with glucose intolerant patients without fatty liver disease, glucose intolerant patients with fatty liver disease were 2.5 times more hyperinsulinemic at baseline. In those with fatty liver disease, both hyperinsulinemia and aminotransferase elevations were exceptionally responsive to iron depletion, even though all of the patients had normal iron indices. Interestingly, the glucose intolerant patients without fatty liver disease did
not demonstrate significant improvements in fasting insulin levels after phlebotomy. (Facchini FS, et al, 2002).

Still unexplained is the observation that homozygosity for the hemochromatosis gene mutation does not appear to confer an increased risk for the development of NAFLD. Furthermore, the clinical significance of iron overload in NASH with respect to clinically relevant endpoints is unclear. In an unselected cohort of 65 patients with NASH, iron accumulation was not associated with increased overall mortality, liver-related mortality, or development of cirrhosis. Additionally, a prospective study did not demonstrate an improvement in hepatic steatosis (based on magnetic resonance imaging), ALT levels, or insulin sensitivity indices among patients who underwent six months of phlebotomy (Adams LA, et al, 2015).

**LEPTIN** — Leptin is a peptide produced primarily in adipose tissue. An absence of leptin is associated with massive obesity in mice (ob/ob) and in humans. Leptin may contribute to the development of fibrosis in NASH. Leptin induces dephosphorylation of insulin-receptor substrate 1, rendering hepatocytes more insulin-resistant. Blood leptin levels correlate with the degree of fibrosis in patients with chronic hepatitis C, and leptin-deficient obese mice that are exposed to a methionine-choline-deficient diet, a necroinflammatory insult, do not develop hepatic fibrosis. Administration of leptin into the central nervous system (CNS) of mice with fatty liver corrected insulin resistance and fatty liver, while peripheral administration did not. This suggests resistance to leptin in the central nervous system, rather than the liver, may be important in the pathogenesis of NASH. On the other hand, no relationship between leptin levels and fibrosis stage (after adjusting for potential confounders) was found in a study of 88 patients with NAFLD (Asilmaz E, et al, 2004).
ADIPONECTIN — Adiponectin is a hormone secreted exclusively by adipose tissue that produces beneficial effects on lipid metabolism, enhancing both lipid clearance from plasma and beta-oxidation of fatty acids in muscle [12]. It also has direct anti-inflammatory effects, suppressing tumor necrosis factor-alpha production in the liver. In one report, low serum adiponectin levels correlated with the presence of NAFLD, hepatic fibrosis, and the severity of the metabolic syndrome. Furthermore, adiponectin expression was markedly reduced in adipose tissue from ob/ob (leptin-deficient) mice (Xu A, et al, 2003).

A study in nonalcoholic obese ob/ob mice demonstrated significant improvements in hepatic steatosis, hepatomegaly, and aminotransferase elevations following administration of adiponectin. Reduced circulating levels of adiponectin correlate with the severity of liver histology in NASH. Adiponectin appears to have a role in modulating insulin sensitivity. In one report, plasma adiponectin levels were significantly associated with hepatic insulin sensitivity. Furthermore, administration of pioglitazone increased adiponectin levels, which correlated with improvements in hepatic steatosis, necroinflammation, and fibrosis (Gastaldelli A, et al, 2010).

RESISTIN — Resistin is an adipose-derived protein that may have an important physiological role in the development of insulin resistance. Overexpression of resistin in a mouse model led to glucose intolerance, hyperinsulinemia, and impaired suppression of free fatty acid levels. In addition, administering antisense oligonucleotides against resistin mRNA completely reversed the marked increase in resistin levels and severe insulin resistance that developed in mice fed high-fat diets (Muse ED, et al, 2004).

INTESTINAL MICROBES — Intestinal microbes have been implicated as a potential source of hepatotoxic oxidative injury. In one report, small intestinal bacterial overgrowth was observed significantly more often in patients with
nonalcoholic steatohepatitis (NASH) compared with controls. Another report found increased intestinal permeability in patients with nonalcoholic fatty liver disease (NAFLD), possibly related to small intestinal bacterial overgrowth (Miele L, et al, 2009).

Studies suggest that the specific composition of gut microbiota may play a role in both the inflammatory and fibrosis responses in patients with NAFLD. Among 57 patients with biopsy-proven NAFLD, those with Bacteroides genus counts in the second and third tertile had a twofold increase in NASH compared with those with lower Bacteroides counts who were found to also have an abundance of Prevotella bacteria. With respect to fibrosis stage, those with Ruminococcus counts in the third tertile were found to have a twofold increase in stage 2 or greater fibrosis compared with those with lower levels of Ruminococcus (Boursier J, et al, 2016).

One proposed mechanism pertains to the production of endogenous alcohol and acetaldehyde. Colonic bacteria and yeast possess an enormous metabolic capacity for generating both ethanol and acetaldehyde, and can oxidize ethanol to high levels of acetaldehyde, even at low ethanol concentrations. Acetaldehyde is easily absorbed into the portal blood stream and can initiate histological changes similar to those seen in NAFLD. High concentrations of endogenous alcohol production have been found in humans and animals with intestinal blind-loops. Increased breath alcohol levels have been described in patients with Candida albicans overgrowth given a carbohydrate load and in obese females (Nair S, et al, 2001).

Intestinal bacteria may also contribute to hepatic injury by means of endotoxin production. Rats injected with lipopolysaccharide develop steato-hepatitis, while anti-tumor necrosis factor antibodies can improve steatosis. Increased intestinal permeability and increased portal endotoxemia have been
demonstrated in genetically obese mice, which are consistent with this theory. Significant increases in markers of intestinal permeability and a higher prevalence of small intestinal bacterial overgrowth have been demonstrated in humans with NAFLD [Jin X, et al., 2007]. These changes correlated with the severity of hepatic steatosis.

Other possible mechanisms by which intestinal bacteria may contribute to hepatocellular injury include deconjugation of bile salts and inactivation of hepatic lipotropes, such as choline. Further supporting a pathogenic role for intestinal bacteria is the observation that the administration of antibiotics, such as polymyxin B, improved steatosis grades in both rats and humans on total parenteral nutrition, and in alcohol-exposed rats. In addition, metronidazole administration improved hepatic steatosis following intestinal bypass surgery. Finally, the administration of probiotics to mice with NAFLD led to improvements in steatosis, hepatomegaly, and nuclear factor kappa-beta activity after four weeks of therapy [Li Z, et al., 2003].

**OTHER** — Obstructive sleep apnea has been proposed to have a role in inducing inflammation in NAFLD. Compared with controls, mice on a high-fat, high-cholesterol diet exposed to six months of chronic intermittent hypoxia exhibited histological signs of liver injury, including lobular inflammation and fibrosis. Also noted were significant increases in hepatic lipid peroxidation and levels of pro-inflammatory cytokines. An observational study in obese patients undergoing gastric bypass surgery found that patients with higher oxygen desaturation index scores (number of drops in oxygen saturation of 3 percent per hour) had more severe histopathologic changes on liver biopsy than patients with lower oxygen desaturation index scores. This observation was confirmed in a study of 362 obese patients who underwent bariatric surgery. In this cohort, the severity of obstructive sleep apnea, as determined by the apnea-hypopnea
index, correlated with the histological severity of NAFLD (Benotti P, et al, 2016).

Dietary cholesterol may also be an independent factor in the development of hepatic inflammation. Hyperlipidemic mice fed high-cholesterol diets developed steatosis with severe inflammation, compared with normolipidemic control animals that developed only steatosis. Hepatic inflammation was linked to increased plasma very low-density lipoprotein (VLDL) cholesterol levels. Omitting cholesterol and lowering VLDL levels prevented hepatic inflammation. On the cellular level, activation of the nuclear receptor constitutive androstane receptor (CAR) is important in the development of lipid peroxidation and steatohepatitis. In a dietary model of NASH, activation of the CAR receptor resulted in hepatic inflammation and fibrosis, in contrast to increased steatosis alone in CAR negative animals (Wouters K, et al, 2008).

FIBROSIS — Peri-sinusoidal (zone 3) fibrosis in patients with NASH is primarily a consequence of the chronic inflammatory process with activation of lobular stellate cells. Portal fibrosis is commonly a feature of progressive disease. It stems from the activation of a secondary replicative pathway involving hepatic progenitor cells. Hepatic progenitor cells appear to proliferate in the setting of primary replicative senescence from chronic hepatocyte injury. A ductular reaction ensues, leading to periportal fibrogenesis. Increases in the ductular reaction correlated with the grade of NASH activity, the degree of fibrosis, and the extent of primary hepatocyte replicative arrest, which in turn correlated with insulin resistance (Richardson MM, et al, 2007).

CLINICAL MANIFESTATIONS — Most patients with nonalcoholic fatty liver disease (NAFLD) are asymptomatic, although some patients with nonalcoholic steatohepatitis (NASH) may complain of fatigue, malaise, and
vague right upper abdominal discomfort. Patients are more likely to come to attention because laboratory testing revealed elevated liver aminotransferases or hepatic steatosis was detected incidentally on abdominal imaging (Bacon BR, et al, 1994).

**Physical findings** — Patients with NAFLD may have hepatomegaly on physical examination due to fatty infiltration of the liver. In some patients, hepatomegaly is the presenting sign of NAFLD (Amarapurkar D, et al, 2007).

The reported prevalence of hepatomegaly in patients with NAFLD is highly variable:

- In a population-based study of 1168 participants from Mumbai, NAFLD was detected in 9 percent (19 percent of those older than 20 years of age). Among those with NAFLD, 5 percent had hepatomegaly (Amarapurkar D, et al, 2007).
- In a study of 12 patients with NASH who underwent computed tomography (CT) scanning, 11 had hepatomegaly (defined as a liver span of >18 cm), with a mean liver span for all 12 of 21 cm (Oliva MR, et al, 2006).
- In a study of 144 patients with NASH, 18 percent were noted to have hepatomegaly on examination and/or ultrasound, and there was a trend toward an increased rate of hepatomegaly among those with more advanced fibrosis (28 percent) (Angulo P, et al, 1999).

The population-based study likely provides a better estimate of the prevalence of hepatomegaly in patients with NAFLD since it does not subject to referral bias. However, the study did not differentiate between patients with nonalcoholic fatty liver and those with NASH, and as suggested by the third
study, it is possible that hepatomegaly is more prevalent in patients with more advanced disease (Angulo P, et al, 1999).

**Laboratory findings** — Patients with NAFLD may have mild or moderate elevations in the aspartate aminotransferase (AST) and alanine aminotransferase (ALT), although normal aminotransferase levels do not exclude NAFLD. The true prevalence of abnormal transaminases among patients with NAFLD is unclear, since many patients with NAFLD are diagnosed because they are noted to have abnormal aminotransferases. When elevated, the AST and ALT are typically two to five times the upper limit of normal, with an AST to ALT ratio of less than one (unlike alcoholic fatty liver disease, which typically has a ratio greater than two). The degree of aminotransferase elevation does not predict the degree of hepatic inflammation or fibrosis, and a normal alanine aminotransferase does not exclude clinically important histologic injury (Charatcharoenwitthaya P, et al, 2012).

The alkaline phosphatase may be elevated to two to three times the upper limit of normal. Serum albumin and bilirubin levels are typically within the normal range, but may be abnormal in patients who have developed cirrhosis. Other laboratory abnormalities that may be seen in patients who have developed cirrhosis include a prolonged prothrombin time, thrombocytopenia, and neutropenia (Charatcharoenwitthaya P, et al, 2012).

Patients with NAFLD may have an elevated serum ferritin concentration or transferrin saturation. There is evidence that a serum ferritin greater than 1.5 times the upper limit of normal in patients with NAFLD is associated with a higher nonalcoholic fatty liver disease activity score (and thus, NASH) and with advanced hepatic fibrosis. Patients with NAFLD may also have positive serum autoantibodies (antinuclear antigen, antismooth muscle antibody), though the significance of these findings is unclear (Kowdley KV, et al, 2010).
Radiographic findings — Radiographic findings in patients with NAFLD include increased echogenicity on ultrasound, decreased hepatic attenuation on CT, and an increased fat signal on magnetic resonance imaging (MRI). (Schwenzer NF, et al, 2009).

Associated disorders — In addition to the findings related to NAFLD, patients often have findings associated with the metabolic syndrome (table 1).

DIAGNOSIS — The diagnosis of nonalcoholic fatty liver disease (NAFLD) requires all of the following:

- Demonstration of hepatic steatosis by imaging or biopsy
- Exclusion of significant alcohol consumption
- Exclusion of other causes of hepatic steatosis
- Absence of coexisting chronic liver disease

In those undergoing a radiologic evaluation, radiologic findings are often sufficient to make the diagnosis if other causes of hepatic steatosis have been excluded. While not indicated for the majority of patients, a liver biopsy may be indicated if the diagnosis is not clear or to assess the degree of hepatic injury. In addition, liver biopsy is the only method currently available to differentiate nonalcoholic fatty liver (NAFL) from nonalcoholic steatohepatitis (NASH) (Schwenzer NF, et al, 2009).

Laboratory tests — Laboratory tests, such as the serum aminotransferase and ferritin levels, are often abnormal in NAFLD. However, these abnormalities are neither required nor sufficient for making the diagnosis, as laboratory tests may be normal in patients with NAFLD and may be abnormal in patients with numerous other conditions. However, laboratory testing is required to evaluate for other conditions in the differential diagnosis of hepatic steatosis (Schwenzer NF, et al, 2009).
**Rule out other disorders** — Differentiating NAFLD from the other items in the differential diagnosis begins with a thorough history to identify potential causes such as significant alcohol use, starvation, medication use, and pregnancy-related hepatic steatosis.

We test all patients with hepatic steatosis for hepatitis C virus infection. We also test for hepatitis A and B. We do this to both to rule out these infections in patients with elevated aminotransferases and to determine immunity to guide future immunizations. We also rule out other chronic liver diseases such as autoimmune hepatitis and hemochromatosis.

We obtain the following tests in all patients:

- Anti-hepatitis C virus antibody.
- Hepatitis A IgG.
- Hepatitis B surface antigen, surface antibody, and core antibody.
- Plasma iron, ferritin, and total iron binding capacity.
- Serum gammaglobulin level, antinuclear antibody, antismooth muscle antibody, and anti-liver/kidney microsomal antibody-1

Other disorders that should be considered based upon the patient's history, associated symptoms, and family history include Wilson disease, thyroid disorders, celiac disease, alpha-1 antitrypsin deficiency, HELLP, and Budd-Chiari syndrome.

**Radiographic examinations** — Various radiologic methods can detect NAFLD, but no imaging modality is routinely used to differentiate between the histologic subtypes of nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). Our approach in patients who have not already undergone imaging is to obtain an ultrasound. However, computed tomography...
(CT) and magnetic resonance imaging (MRI) can also detect hepatic steatosis. (Schwenzer NF, et al, 2009).

We consider a radiographic diagnosis to be sufficient for diagnosing NAFLD if all of the following conditions are met:

- Radiographic imaging is consistent with fatty infiltration
- Other causes for the patient's liver disease have been excluded.
- The patient does not have signs or symptoms of cirrhosis
- The patient is not at high risk for advanced fibrosis or cirrhosis (e.g., a younger patient who does not have diabetes and has a normal serum ferritin is at lower risk for having fibrosis or cirrhosis) (Schwenzer NF, et al, 2009).

If these criteria are not met, patients will typically require a liver biopsy to make the diagnosis or to assess the degree of liver injury.

**Ultrasound** — Ultrasonography often reveals a hyperechoic texture or a bright liver because of diffuse fatty infiltration. A meta-analysis of 49 studies with 4720 patients found that the sensitivity and specificity for ultrasound were 85 and 94 percent, respectively, when using liver biopsy as the gold standard. However, the sensitivity appears to be decreased in patients who are morbidly obese. In a study of 187 morbidly obese patients undergoing bariatric surgery, hepatic steatosis was present histologically in 95 percent but was only detected by ultrasound in 49 percent (Schwenzer NF, et al, 2009).

**Vibration controlled transient elastography** — Vibration controlled transient elastography, which is routinely used to grade fibrosis based on liver stiffness, is also being developed to grade hepatic steatosis. However, additional data are needed to show how transient elastography measurements are reproducible, valid, and associated with clinical outcomes. In a meta-analysis of 19 biopsy-
controlled studies including over 2700 patients, the optimal cutoff value for steatosis grade >S0 was 248 dB/m (95% CI 237-261) and for steatosis grade >S1 was 268 dB/m (95% CI 257-284) (Wong GL, Wong VW.2015).

**CT, MRI, and magnetic resonance spectroscopy** — Both CT and MRI can identify steatosis but are not sufficiently sensitive to detect inflammation or fibrosis. Magnetic resonance spectroscopy (MRS) has the advantage of being quantitative rather than qualitative or semiquantitative, but it is not widely available (Szczepaniak LS, et al, 2005).

One of the difficulties in determining the sensitivity and specificity of CT and MRI for diagnosis of hepatic steatosis is that not all patients undergo confirmation by liver biopsy. In a study that did use histology as the gold standard, the sensitivity of CT scan for detecting hepatic steatosis was poor, whereas MRI had low specificity. It included a total of 131 patients who had a radiologic evaluation with noncontrast CT, contrast-enhanced CT, or MRI before undergoing a partial hepatectomy, usually for malignancy. The sensitivities of noncontrast CT, contrast-enhanced CT, and MRI for detecting hepatic steatosis were 33, 50, and 88 percent, respectively. The specificities were 100, 83, and 63 percent, respectively. In addition, the accuracy of noncontrast CT fell with increasing body mass index. However, in a study of 33 patients with diabetes at risk for NAFLD, the sensitivity and specificity of in-phase and out-of-phase MRI for hepatic steatosis were 95 and 98 percent, respectively (Borra RJ, et al, 2009).

Unlike CT and MRI, MRS allows for quantification of hepatic fat, and may be particularly helpful in patients with small amounts of hepatic steatosis. A study that compared MRS with liver biopsy in 12 patients found a close correlation between the measurement of intrahepatocellular lipid by MRS and the histologic assessment of cirrhosis (r = 0.94). However, not all scanners have the
capability of obtaining spectroscopic sequences, and it is not routinely used (Springer F, et al, 2010).

**Role of liver biopsy** — While liver biopsy is the gold standard for diagnosing NAFLD, in many cases a presumptive diagnosis can be made based upon the patient's history, laboratory tests, and imaging findings, provided other disorders have been excluded. However, some patients will continue to have an unclear diagnosis following a non-invasive evaluation. In such cases, a liver biopsy is indicated (Neuschwander-Tetri BA, et al, 2010).

In addition, imaging studies and laboratory tests do not reliably differentiate patients with NAFL from those with NASH, or predict the severity of liver disease. The only way to definitively confirm or exclude the diagnosis of NASH and to determine disease severity is with a liver biopsy. This information can be used to guide patient care and may motivate patients to enact lifestyle modifications. As examples, patients found to have cirrhosis will require screening for esophageal varices and hepatocellular carcinoma, whereas patients with early fibrosis may be motivated to lose weight to decrease the risk of progressing to cirrhosis (Neuschwander-Tetri BA, et al, 2010).

A potentially useful non-invasive method for excluding advanced fibrosis is measurement of liver stiffness with transient elastography. However, the approach is not widely available and has not been extensively studied in NASH. Other indirect markers of cirrhosis such as the aspartate aminotransferase to platelet ratio index are also being studied to identify patients with fibrosis. (Neuschwander-Tetri BA, et al, 2010).

**Which patients to biopsy** — There is no clear consensus about which patients require a liver biopsy. We obtain a liver biopsy in patients with suspected NAFLD if the diagnosis is unclear after obtaining standard laboratory tests and
hepatic imaging, if there is evidence of cirrhosis, if the patient wants to know if inflammation or fibrosis is present, or if the patient is at increased risk for advanced fibrosis or cirrhosis (Neuschwander-Tetri BA, Caldwell SH.2003).

Specifically, we obtain a biopsy if the patient

- Has peripheral stigmata of chronic liver disease (suggestive of cirrhosis)
- Has splenomegaly (suggestive of cirrhosis)
- Has cytopenias (suggestive of cirrhosis)
- Has a serum ferritin >1.5 times the upper limit of normal (suggestive of NASH and advanced fibrosis)
- Is >45 years of age with associated obesity or diabetes (increased risk of advanced fibrosis)

**Histologic findings** — NAFLD is subdivided into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) although these are considered to be part of a spectrum. The minimum criterion for a histologic diagnosis of NAFLD is >5 percent steatotic hepatocytes in a liver tissue section. In addition to steatosis, patients with NAFLD may also have hepatic iron deposition (EASL-EASD-EASO2016).

Nonalcoholic fatty liver can be distinguished from NASH based on histologic findings. NAFL is present when the liver biopsy shows any of the following:

- Steatosis alone.
- Steatosis with lobular or portal inflammation, without hepatocyte ballooning.
- Steatosis with hepatocyte ballooning but without inflammation (Kleiner DE, Brunt EM.2012).
The histologic diagnosis of NASH requires the presence of hepatic steatosis in association with hepatocyte ballooning degeneration and hepatic lobular inflammation (typically in acinar zone 3. Fibrosis is not a required diagnostic feature, but may be seen. The appearance of NASH may be histologically indistinguishable from alcoholic steatohepatitis (Kleiner DE, Brunt EM.2012).

Additional histologic findings of NASH include:

- Apoptotic (acidophil) bodies
- Mild chronic portal inflammation (inflammation that is severe or is disproportionate to the acinar lesions is suggestive of concurrent hepatitis C)
- Perisinusoidal collagen deposition that may result in zone 3 accentuation in a "chicken wire" pattern (related to the deposition of collagen and other extracellular matrix fibers along the sinusoids of zone 3 and around hepatocytes)
- Portal fibrosis without perisinusoidal or pericellular fibrosis
- Cirrhosis, which is typically macronodular or mixed
- Mallory-Denk bodies (previously called Mallory bodies or Mallory's hyaline)
- Megamitochondria
- Glycogenated (vacuolated) nuclei in periportal hepatocytes (rarely seen in alcoholic steatohepatitis)
- Lobular lipogranulomas
- PAS-diastase-resistant Kupffer cells
- Hepatic siderosis (typically mild) involving periportal hepatocytes or panacinar reticuloendothelial cells (Brunt EM, Tiniakos DG.2010).
As fibrosis progresses to cirrhosis, steatosis and inflammation may not be reliably identified, resulting in a diagnosis of "cryptogenic" cirrhosis. It is possible that portal fibrosis alone may represent a variant of NASH. In biopsy specimens from children, portal inflammation may be more prominent than in adults (Brunt EM, Tiniakos DG.2010).

NASH may exist concurrently with other liver diseases, though diagnosing NASH in that setting can be difficult. As an example, patients with NASH may also have alcoholic liver disease, but there is no way to differentiate the relative contributions of the two processes from a liver biopsy. In a series of 3581 liver biopsies from patients with various chronic liver diseases, concurrent steatohepatitis was found in 5.5 percent of patients with hepatitis C (some with significant alcohol use). Among patients with other chronic liver diseases of nonalcoholic etiology, the prevalence ranged from 1.6 percent (autoimmune hepatitis) to 7.9 percent (alpha-1 antitrypsin deficiency). None of the patients with steatohepatitis with chronic liver disease from a cause other than hepatitis C had significant alcohol consumption (Brunt EM, Tiniakos DG.2010).

**NAFLD activity score** — The NAFLD activity score (NAS) is a validated score that is used to grade disease activity in patients with NAFLD. The NAS is the sum of the biopsy's individual scores for steatosis (0 to 3), lobular inflammation (0 to 3), hepatocellular ballooning (0 to 2). Fibrosis is not included in the NAS. In the original study that derived the NAS, scores of 0 to 2 occurred in cases largely considered not diagnostic of NASH; scores of 3 to 4 were evenly divided among those considered not diagnostic, borderline, or positive for NASH; and scores of 5 to 8 occurred in cases that were largely considered diagnostic of NASH (Kleiner DE, et al.2005).

**Noninvasive assessment of hepatic fibrosis** — There are now several noninvasive methods to detect fibrosis in patients with liver disease. One of the
scores, the NAFLD fibrosis score, is specific to NAFLD. The score takes into account the patient's age, body mass index, hyperglycemia, aminotransferase levels, platelet count, and albumin. Studies suggest that higher NAFLD fibrosis scores may be associated with increased mortality from cardiovascular disease (Brunt EM, Tiniakos DG. 2010).

**DIFFERENTIAL DIAGNOSIS**

**Alternative causes of hepatic steatosis** — There are multiple causes of hepatic steatosis that should be considered in a patient with suspected nonalcoholic fatty liver disease (NAFLD). Causes of hepatic steatosis in addition to NAFLD include:

- Alcoholic liver disease
- Hepatitis C (particularly genotype 3)
- Wilson disease
- Lipodystrophy
- Starvation
- Parenteral nutrition
- Abetalipoproteinemia
- Medications (amiodarone, methotrexate, tamoxifen, glucocorticoids, valproate, anti-retroviral agents for HIV)
- Reye syndrome
- Acute fatty liver of pregnancy
- HELLP (hemolytic anemia, elevated liver enzymes, low platelet count) syndrome
- Inborn errors of metabolism (LCAT deficiency, cholesterol ester storage disease, Wolman disease)
**Significant alcohol consumption** — Several definitions have been proposed for what constitutes significant alcohol consumption. We define significant alcohol consumption as an average consumption of >21 standard drinks per week in men or >14 standard drinks per week in women over at least a two-year period, a definition that is consistent with a guideline from the American Association for the Study of Liver Diseases (Chalasani N, et al, 2018).

A standard alcoholic drink is any drink that contains about 14 grams of pure alcohol, according to the National Institute on Alcohol Abuse and Alcoholism. One finding that suggests alcoholic fatty liver disease rather than NAFLD is an aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio >2 (it is typically <1 in patients with NAFLD). The alcoholic liver disease to NAFLD index (ANI) is a model that has been developed to predict the probability that steatohepatitis is due to alcoholic liver disease (Dunn W, et al, 2006).

The model is based upon aminotransferase levels, mean corpuscular volume (MCV), body mass index (BMI), and sex:

\[ \text{ANI} = -58.5 + 0.637 \times \text{MCV} + 3.91 \times \frac{\text{AST}}{\text{ALT}} - 0.406 \times \text{BMI} + 6.35 \]

for men

An ANI greater than zero favors a diagnosis of alcoholic liver disease, whereas an ANI less than zero favors a diagnosis of NAFLD. The probability of the patient having alcoholic liver disease rather than NAFLD is then calculated using the value obtained for the ANI:

\[ \text{Probability} = \frac{e^{\text{ANI}}}{1+e^{\text{ANI}}} \]

The ability of the model to accurately categorize patients ranged from good to excellent in validation cohorts (Dunn W, et al, 2006).

**SCREENING** — One issue that arises is whether to screen patients for nonalcoholic fatty liver disease if they are at increased risk because of an
associated condition such as diabetes or obesity. The American Association for the Study of Liver Diseases guidelines do not recommend screening because there are uncertainties around which diagnostic test to use (since liver enzyme levels may be normal in patients with NAFLD), how to treat NAFLD if discovered, and whether screening is cost-effective (Chalasani N, et al., 2018).
Summary

Mild elevation of ALT is a common clinical problem of various causes. Except for those that can be attributed to viral hepatitis, alcohol or other chemical toxin exposure, most of these cases are of non-specific causes or fatty liver related.

Also, many causes of mildly elevated serum ALT levels should be excluded before the decision of an explained elevation is taken.

A growing number of studies have suggested that elevation of ALT in absence of specific liver disease or DM was associated with insulin resistance.

Both ethnicity and gender seem to play roles in the prevalence of mild elevation of ALT. Compared to women, men were more prone to have unexplained elevation of ALT although they had approximately equal prevalence of T2DM.

In this study, obesity (BMI ≥ 30) and hypercholesterolemia (total cholesterol ≥ 200 mg/dl) were identified to be significantly associated with abnormal ALT especially in younger males (age, 35 years). It has been demonstrated that augmented ALT activities can be predictor of development of IR, MD, Cardiovascular disease and metabolic syndrome.

The longitudinal increment of individuals’ ALT activity over time increased the incidence risk of metabolic syndrome and the effects generated by longitudinal increments of ALT on MetS was higher than that generated by baseline ALT.

Studies are needed to better define the risks, particularly better diagnostic criteria allowing differentiation of NAFLD, and most importantly NASH. This serious and potentially preventable public health issue has major implications for health and wellbeing in this health disparity population. Failing to intervene
with preventive measures will have considerable impact on the community and the economy and will impact costs and capacity within the already stretched health care system.

Recommendations

- Study the prevalence of liver disease and IR among wide scale population through employees and governmental institutes for better analysis and good statistical informations
- Survey for NAFLD prevalence through out population with pressure on young adolescents and paediatrics.
- Increase the time of studies for better assessment of relative risk factor and follow up the treatment.
- Education of population about NAFLD and its development and how to early diagnose and manage.
Abstract

Background

Although alanine aminotransferase (ALT) is well known to be associated with metabolic syndrome (MetS), prospective data on longitudinal increments in ALT activities and incident cases of MetS are limited. We analyzed the impact of longitudinal increments of ALT on MetS based on a health check-up population in China.

Methods

A total of 4491 subjects free of MetS who completed at least two annual health examinations during March 2010 to April 2016 were enrolled in this cohort study. The MetS was defined according to the Joint Interim Statement criteria 2009. The RRs of incident MetS were estimated by using the Cox model and the Joint model in R software.

Results

The cumulative incidence of MetS was 18.55% during the 7 years of follow-up. In the Cox model, the estimated RR of developing MetS was 1.751 (95% CI =1.532–2.000) for 1 unit augmented in LNALT-0 level. In the Joint model, the estimated RR of developing MetS was 3.626 (95% CI =2.721–4.831) for 1 unit augmented in LNALT activity longitudinally.

Conclusions

The longitudinal increment of individuals' ALT activity over time increased the incidence risk of MetS and the effects generated by longitudinal increments of ALT on MetS was higher than that generated by baseline ALT.