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Non- Invasive Biomarkers and Their Diagnostic Utility in Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH)

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is characterized by an abnormal accumulation of fat in the liver, due to causes other than excessive alcohol use. NAFLD is a continuum of liver abnormalities, from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH). These diseases begin with fatty accumulation in the liver (hepatic steatosis). A liver can remain fatty without disturbing liver function (NAFL), but by various mechanisms and possible insults to the liver, it may also progress into a non-alcoholic steatohepatitis (NASH), a state in which steatosis is combined with inflammation and sometimes fibrosis (steatohepatitis). The development of emerging biomarkers of disease has become a major focus of interest in non-alcoholic fatty liver disease (NAFLD). The large prevalence of the disease and the invasive nature of the investigation mean that screening with liver biopsy is impractical. In addition to screening, the differentiation of those with simple steatosis *vs.* steatohepatitis and fibrosis is clinically important as the prognosis of each differs.

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a spectrum of liver pathologies associated with liver injury and inflammation (hepatitis) caused by excessive accumulation of fat in the liver (steatosis). By definition, it is not etiologically related to excess alcohol consumption. NASH is defined histologically and diagnosed by liver biopsy findings (i.e., steatosis plus hepatocyte damage and liver inflammation). It occurs in a subset of individuals with non-alcoholic fatty liver disease (NAFLD), a clinically defined disease entity that broadly encompasses simple hepatic steatosis, NASH, NASH with fibrosis, and NASH related cirrhosis. NAFLD is strongly associated with obesity and, like obesity; NAFLD is a rapidly emerging health concern in many industrialized countries. Most individuals with NAFLD have simple steatosis, which is considered a generally benign condition. However, it is estimated that ~30% of the patients who have fatty liver may have NASH[1,2]. Unlike simple steatosis, NASH is considered a potentially progressive disorder because hepatocyte damage and liver inflammation may prompt collagen synthesis and deposition (i.e., fibrosis). Liver biopsy series indicate that progressive fibrosis develops in 32–53% of NASH cases, and thus, advanced fibrosis/cirrhosis ultimately ensues in ~10% of the NAFLD population overall. Liver fibrosis is the only histologic variable that independently predicts liver-related morbidity, liver-related mortality, and all-cause mortality in NAFLD. Individuals with NAFLD and mild to moderate liver fibrosis (stage 1–2) are twice as likely to die of any disease than are NAFLD patients without any fibrosis, and the risk of developing life-threatening consequences of liver disease is >80-fold higher in NAFLD patients with advanced hepatic fibrosis (stage 3–4) than in NAFLD patients with no hepatic fibrosis. Differences in the propensity for liver fibrosis explain why the cumulative incidence of liver-related death was reported to be as high as 18% among patients with NASH versus 3% among those with simple steatosis in an 18-year observational study. In general, NAFLD risk increases with the degree of obesity. However, NAFLD risk is also modified by other bio physiological attributes, such as the extent of peripheral versus visceral adiposity, and the degree of insulin resistance in adipose depots and other insulin-sensitive tissues, such as muscle and liver. Thus, body size [e.g., body mass index (BMI)] is an imperfect predictor of NAFLD risk, and NAFLD can occur in the context of a normal or low BMI.

The criterion standard for diagnosis and assessing progression of disease is liver histology, though this fatty liver disease (NAFLD) develops when the liver has difficulty in breaking

down fats, which causes a build-up in the liver tissue. The cause isn't related to alcohol. NAFLD is diagnosed when more than 5 percent of the liver is fat.

There has been much focus on the development and validation of emerging biomarkers of NAFLD in recent years. There is an urgent need for a less invasive method than biopsy of screening the population, stratifying disease severity and following disease progression. This is particularly relevant in the paediatric population. Many markers of inflammation, hepatocyte apoptosis, fibrosis and oxidative stress are under investigation. In common with all biomarkers which are "biological markers of disease presence and progression", important characteristics include; sufficient sensitivity to identify those with disease, specificity to exclude those without disease, cost-effectiveness, ease of use and reproducibility. There are several different approaches to the identification of biomarkers: the first is the use of clinical or biochemical markers that have been derived from large association studies. The second is the use of algorithms including markers of extracellular matrix turnover in the case of fibrosis and inflammation/cell death in the case of inflammatory change. The third is the non-hypothesis driven new-technology based approach such as microarray techniques, proteomics and glycolic.

Biomarkers of fibrosis

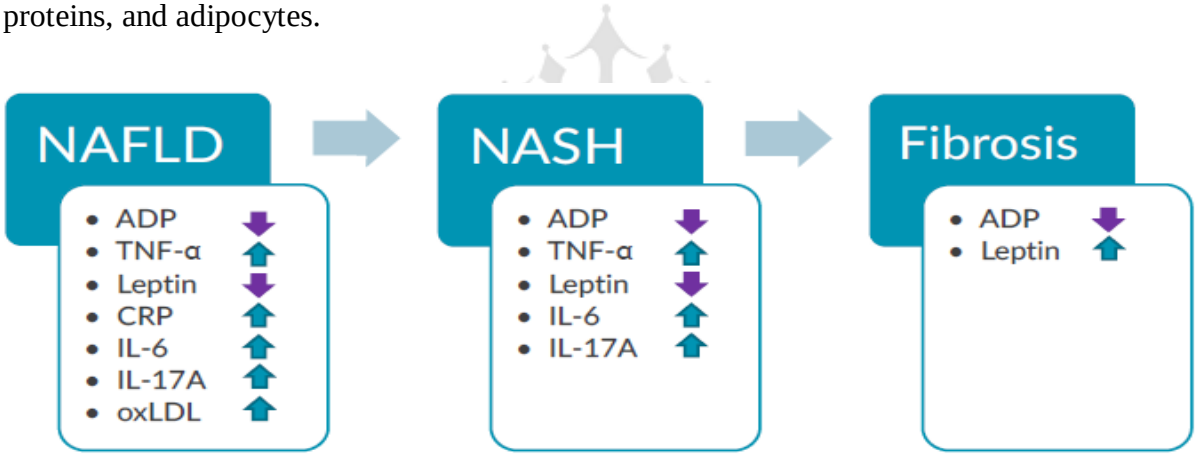
A strong association has been found between NASH, diabetes mellitus and fibrosis in obese patients [3]. Several non-invasive biomarkers of fibrosis were utilized in NAFLD patients, such as AST/platelet ratio index, NAFLD fibrosis score, BARD score, FIB-4 score. These biomarkers were suggested to be contributed in predicting high risk of developing liver complications [4,5,6]. Liver-related adverse events and shorter survival rate has been associated with higher scores of these assays in patients with advanced fibrosis. However, Liver-related complications have not been correlated with the degree of steatosis or the presence of NASH, suggesting the need to accurate assessment of liver fibrosis, using noninvasive biomarkers to predict clinical outcomes of NAFLD patients. Moreover, all four assays were accurately differentiated low risk patients and higher risk in both liver-related complications[5]. These markers have also been used to characterize fibrosis [7]. Non-invasive fibrosis scoring systems has high negative predictive value but low positive predictive value indicating that these markers can be used in exclusion of fibrosis in NAFLD patients [8]. A novel physical parameter had been proposed by De Lédinghen et al [9] based on the properties of ultrasonic

signals properties resulted by the FibroScan which can be used with a higher sensitivity to assess fibrosis and steatosis simultaneously.

Irvine et al [10] has been identified novel serum biomarkers for advanced liver fibrosis and thought to improve the diagnostic accuracy. Their findings have suggested that MMP7 might be a valuable marker of advanced fibrosis and may play a crucial role in liver fibrogenesis. In another study conducted by Ajmera et al [11] reported that serum biomarkers such as soluble interleukin-1 receptor I, monocyte chemoattractant protein-1, Interleukin-8, resistin, tumor necrosis factor alpha and soluble interleukin-2 receptor alpha were significantly elevated whereas insulin-like growth factor 2 levels were observed to be down regulated in NAFLD patients.

Emerging Biomarkers of NAFLD and NASH

More recently, researchers have been studying various types of biomarkers to detect, classify and monitor NAFLD and NASH. These include hormones, pro-inflammatory cytokines, proteins, and adipocytes.



Cytokines and Inflammatory Markers

Inflammatory cytokines and chemokines such as TNF-alpha, IL-6, chemokine CC-chemokine ligand-2, C-reactive protein are widely used to diagnose NASH [12]. Inactivation of cytokine signalling-3 resulted in decrease in hepatocellular insulin receptors expression and elevates hepatic insulin resistance [13].

Previous studies have been quantified inflammatory markers in NAFLD patients [14, 15, 16-19]. TNF-alpha was found to be elevated in NAFLD patients compared to non-NAFLD individuals [16,18]. It was observed that TNF-alpha levels have also been increased in NASH

compared to non-NASH individuals [19]. However, IL-6 levels were not significantly differed between NASH and non-NASH (14,19). Also, hs-CRP is another important inflammatory marker which found in higher levels in NAFLD patients compared with non-NAFLD controls [16, 18]. CRP levels have also been higher in NASH patients compared to non-NASH individuals [14] TNF-alpha, TNF-beta hs-CRP, IL-8, IL-6, and IL-10 levels were more or less similar in both NASH and non-NASH group.

Circulating MicroRNAs as Biomarkers in NAFLD and NASH

Previously several studies have been evaluated the role of miRNAs and their association with liver diseases [20]. A recent study was evaluated the circulating miRNAs in NASH patients and observed that out of 84 circulating miRNAs, miR-125b, miR-122, mir-19a, miR-19b miR-192 and miR-375 levels were significantly elevated [21]. In another study with comparison of simple steatosis with steatohepatitis, the levels of miR-192, miR- 122 were observed to be increased along with TGFb whereas miR-375 levels were associated with disease severity.

The elevated levels of serum mir-122 in NAFLD patients were observed in other studies [22, 23] and positive association was demonstrated between serum miR-122 levels and hepatic steatosis [23]. Moreover, increased levels of serum miR-34a, miR- 21, and miR-451 were observed in NAFLD patients [23].

Tan et al. Proposed a panel of miRNAs (miR-1290, miR-37 3p miR-122 5p, miR- 192 5p) which showed adequate diagnostic accuracy for NAFLD and suggested to be good predictive markers than ALT or FIB-4 [24]. Further, 23 miRNAs were observed to be down regulated or up regulated in patients with NASH compared to normal livers [25]. The biological functions of these miRNAs are inflammation, cell proliferation, oxidative stress, apoptosis, and metabolism.

CONCLUSION

In view of the high prevalence of NAFLD in the population, in both adults and children, and the fact that up to a one third will develop end stage liver disease and/or hepatocellular carcinoma, it is important that we develop non-invasive methods to diagnose and monitor this liver condition. A differentiation needs to be made between those with advance disease/or are at risk of developing advanced disease from those who have simple steatosis and are unlikely to progress. Liver biopsy is not a practical tool for this mass screening though the disease is

still defined histologically. Emerging biomarkers either in blood or imaging techniques show promise in this context and in many centres are used routinely. It is possible that a combination of blood biomarkers with methods such as transient elastography or acoustic radiation force impulse may yield the highest diagnostic discrimination. Noninvasive biomarkers are may help to diagnose patients with NAFLD or NASH by replacing liver biopsy. These biomarkers may provide accurate and reproducible data. Further research on biomarker discovery may lead to personalized therapeutic options and monitoring of treatment outcome in individuals with fatty liver disease.

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