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Keratin 18, Apoptosis, and Liver Disease in Children

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Abstract

Keratins, a major component of epithelial cell intermediate filaments, provide structural support to the cell and are important for the maintenance of structural integrity. Beyond its role of structural integrity in hepatocytes, keratin 18 (K18) is a known marker of apoptosis and has been proposed as an indicator of progression in chronic liver diseases such as nonalcoholic fatty liver disease (NAFLD). NAFLD is the most common cause of chronic liver disease in children and adolescents in the United States and throughout the world and comprises a wide spectrum of disease ranging from simple steatosis (fatty liver) to nonalcoholic steatohepatitis (NASH) and cirrhosis. While simple steatosis is typically benign in nature, NASH is a more serious condition that may progress to end-stage liver disease and liver failure. Currently, liver biopsy is considered the most reliable method of assessing the histological severity of disease and differentiating between simple steatosis and NASH. Because biopsy is invasive in nature, expensive, and subject to sampling error and/or variability in interpretation, it is not suitable as a screening test. Therefore, it is necessary to examine known mechanisms associated with the progression of liver disease, such as hepatocellular apoptosis, and identify potential biomarkers that could be used as a diagnostic tool in NASH. This review will focus on the role of apoptosis in pediatric liver disease and how K18, an early marker of apoptosis, can be utilized as a noninvasive biomarker to diagnose NASH.

Keywords

apoptosis; biomarker; children; keratin 18; liver disease; nonalcoholic liver disease

INTRODUCTION

BIOMARKERS OF LIVER DISEASE

Biomarkers are of increasing importance in the diagnosis, treatment, and prevention of pediatric liver diseases. Ideally, biomarkers of disease should be simple, reproducible, inexpensive, and readily available. In addition to the aforementioned characteristics, in some disease, biomarkers should be able to differentiate stages of disease and progression. This is particularly important in liver diseases that typically are slowly progressive over many years. While serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are widely used as markers of liver disease, they lack the specificity and sensitivity needed (1). Moreover, liver biopsy, (while providing more detail) is expensive, invasive, and associated with risks of clinical complications. Further, liver biopsies have potential problems of sample size issues and regionality of disease in the liver. Thus, there is an urgent need for investigators to use current knowledge of liver disease pathogenesis to identify novel noninvasive markers of disease.

ROLE OF KERATINS IN HEALTH AND LIVER DISEASE

Keratins are the largest subfamily of cytoplasmic intermediate filaments. Together with actin microfilaments and microtubules, intermediate filaments play a major functional role in the maintenance of mechanical stability and integrity of epithelial cells (2), protection from mechanical stress (3), and locomotion (4). Keratins, which are found specifically in epithelial cells, are subdivided into type I (acidic) and type II (basic) keratins (5). Keratins K9–K28 comprise type I keratins while keratins K1–K8 and K71–K80 comprise type II keratins. All epithelial cells contain at least one type I keratin and one type II keratin which form noncovalent heterodimers, the building units of the keratin filaments, in a 1:1 ratio (6). Formed heterodimers polymerize to generate intermediate filaments which normally extend from the nucleus to the plasma membrane (7). Keratin expression is tissue specific with different pairs being predominantly expressed in different cell populations (4). While specific keratins (K7, K8, K18, K19, K20, and K23) typically predominate in simple, single layered epithelial cells, there is variation in expression (8). For example, while K8 and K19 are typically found in simple epithelial cells, they can also be expressed in low levels in non-epithelial tissues such as muscle (9). Adult hepatocytes are unique, however, in that they express keratin 8 (K8; type II) and keratin 18 (K18; type I) exclusively (2).

Demonstrating the importance of keratins to cell function, specific keratin mutations have been shown to cause different tissue-specific human diseases (10). Ku et al. provided the first evidence that linked keratin mutations and predisposition to liver disease (11). They demonstrated that over-expression of a human mutant K18 caused K8/K18 filament disruption, mild chronic hepatitis, and increased hepatocyte fragility (11). In addition, expression of the K18 mutant in mice increased susceptibility to hepatocyte apoptosis (3). Similar findings have also been observed in K8 transgenic mice and both K8 and K18 null mice (9). These findings from rodent studies have encouraged the investigation of K8/K18 mutations in patients with liver disease. Initial studies identified K8 and K18 heterozygous point mutations in only 6 of 153 patients with liver disease (12). Subsequent studies also confirmed the presence of keratin point mutations in a relatively small percentage of patients

with liver diseases although significantly higher than typically found in healthy control subjects (3, 13). And, although K8/K18 variants are associated with liver disease, it is more likely that they predispose hepatocytes to injury rather than directly cause disease. Thus, K8 and K18 gene mutations are risk factors for the development of end stage liver disease (4, 14).

Degradation of keratins in hepatocyte death proceeds differently in apoptosis versus necrosis. Early studies demonstrated that apoptosis results in the caspase-mediated cleavage of K18 but not K8 (7, 15). The caspase-mediated cleavage of K18 at Asp₃₉₆ results in the release of a CK 18 fragment with exposure of a neoepitope that has been termed M30-antigen (16). Rylander et al. developed a monoclonal antibody, M30, that specifically recognizes K18 fragments and allows quantification (using enzyme-linked immunosorbent assay (ELISA) of caspase cleaved K18 (M-30 antigen) in the serum (17). Because exposure of the M30-neoepitope occurs early in apoptosis and is not detectable in vital or necrotic cells, K18 has substantial potential as a biomarker of apoptosis in liver disease. A second monoclonal antibody, M65, was developed to recognize K18 full-length protein (M65-antigen) released during secondary necrosis (18). Thus, measurements from serum or plasma samples of the M65 antigen would reflect total epithelial cell death by any cause (19). Multiple studies have utilized the combination of the M30 and M65 ELISA to estimate the relationship of cell death mode (necrosis versus apoptosis) which can then be used to correlate with stages of liver disease and progression (20–21).

NONALCOHOLIC LIVER DISEASE

Nonalcoholic fatty liver disease (NAFLD) is a clinico-pathologically defined liver disease that typically occurs in the setting of increased visceral adiposity and the other features of the metabolic syndrome. The diagnosis requires exclusion of other known causes of fatty liver, including alcohol consumption, medications, hepatitis C and anorexia nervosa. The hallmark of NAFLD is abnormal accumulation of fat in the hepatocytes, ie steatosis. Pathologically, this intracytoplasmic lipid deposition is typically visible as both large and small droplet fat (22). NAFLD occurs in a range of severity from steatosis to a more severe form that has inflammation, hepatocyte injury and often fibrosis along with the steatosis and is identified as nonalcoholic steatohepatitis (NASH). The minimum criteria for NASH includes 1) steatosis, 2) hepatocellular injury, and 3) lobular inflammation (23). Similar to some other forms of chronic liver disease, fibrosis is not required for the diagnosis of NASH however, is commonly present. Histologically, the hepatocellular injury is usually visible as hepatocyte ballooning, but it may also present as apoptotic (acidophilic) bodies and lytic necrosis (22). Hepatocellular ballooning is the presence of swollen, rounded hepatocytes, with rarefied cytoplasm, that may have a reticulated appearance or contain Mallory--Denk bodies. Keratin alteration seems to be important in NAFLD because immunostaining studies of these ballooned hepatocytes in NAFLD show loss of cytoplasmic keratin 8 and 18 (K8/18) while the Mallory-Denk bodies were positive for K8/18 (24), and this was not found in other chronic liver diseases such as autoimmune hepatitis and hepatitis B. However, the role of hepatocyte ballooning may not be uniform across NAFLD. In children, the pathologic pattern varies substantially from the more typical “adult” pattern and has been labeled “pediatric type” or type II. The primary difference is an alteration of the location of

the disease features to a predominance of the inflammation and steatosis as peri-portal rather than zone 3 and the uncommon appearance of hepatocyte ballooning compared to adult type (22). Because this pediatric type tends to occur in younger adolescents, is less common in older adolescents and virtually non-existent after age 18 years, it may be related to pubertal or growth effects. For clinical purposes, pathologic assessment of NAFLD typically consists of 1) grading steatosis, 2) staging fibrosis and 3) assessing inflammation and ballooning (23). A more precise quantification system based on these histologic features (the NAFLD activity score) has been developed for research purposes (25) and is often used in comparison studies of new biomarkers.

PREVALENCE OF NAFLD IN CHILDREN

NAFLD is the most common chronic liver disease in children in the US, likely because of its close association with childhood obesity (26). Although screening for NAFLD is problematic, thus decreasing the accuracy of prevalence estimates, it is likely that NAFLD affects over 10% of children in the US. The best prevalence estimates are from a San Diego County autopsy study which found that 38% of obese (>95th percentile for age and gender) children had NAFLD (26). Other estimates using ALT/AST have shown lower estimates (27–28). Similar to other features of the metabolic syndrome, prevalence of NAFLD varies substantially by race/ethnicity. The autopsy study estimated that population prevalence of NAFLD for Hispanic children (regardless of weight status) was 12%, compared to Asians (10%), white (8.6%) and African American children (1.3%) (29). The relative protection of overweight African American children from NAFLD has been confirmed in other studies (30). Generally, NAFLD does not appear in very young children, more often affecting those age 8 years and greater (26) although the age of onset in Hispanic children may be younger (31). Previous studies have also shown that adolescent boys are more likely affected than girls (28–29, 32).

NAFLD DIAGNOSIS

One reason that prevalence estimates for NAFLD vary greatly is because there is not yet an accurate non-invasive diagnostic test for NAFLD. Modalities used for screening and diagnosis include serological tests, imaging and liver biopsy. In the clinical practice, reference ranges of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are used to diagnose NAFLD. While plasma levels of AST and ALT are typically two to five times the upper limit of normal in NAFLD, it is well documented that AST and ALT levels can be normal despite the presence of steatosis and NASH. Similar to hepatitis C and other chronic liver diseases, AST and ALT levels appear to fluctuate in NAFLD and thus are not good discriminators of histologic severity (33).

Liver biopsy is considered the gold standard for assessment of histological severity of NAFLD, diagnosis of NASH, and determination of prognosis (34). The most important limitation of liver biopsy is its invasive nature, which can be associated with serious complications and mortality in a small percentage of cases. Studies in pediatric populations suggest an increased risk of bleeding in these younger subjects, especially in children with abnormal coagulation tests (35–36). Given the prevalence of NAFLD in the population, the use of liver biopsies as a diagnostic tool would require resources and impose risks that

would likely exceed benefit. Other limitations associated with liver biopsy include high cost of the procedure and variability in interpretation of liver damage. Therefore, other tests are particularly needed that both identify the presence of NAFLD and also help with distinguishing NAFLD from NASH to potentially focus the utilization of liver biopsy to the high risk group.

Ultrasound is the most commonly ordered non-invasive imaging technique used in screening for NAFLD. Because it visualizes steatosis as increased liver echogenicity, ultrasound is useful for establishing normal gross structure of the liver. This technology however is less accurate for steatosis identification when liver fat content is less than 30% (1, 37). Moreover, ultrasound is unable to differentiate NAFLD from other chronic liver diseases. Previous studies have shown that magnetic resonance imaging (MRI) is more precise for steatosis detection and can accurately measure lipid deposition to within $\pm 2\%$ or lower (37). Although MRI is capable of detecting changes in fat more accurately than its noninvasive counterparts, it is expensive and not easily accessible. Transient elastography (TE) is a non-invasive medical device that assesses liver stiffness as a function of the extent of hepatic fibrosis. In contrast to studies conducted in adults (38), TE reliably predicted the presence of any fibrosis, significant fibrosis, and advanced fibrosis in children (39). This difference in accuracy/efficacy of TE for the detection of fibrosis in children is likely attributed to differences in histopathological features versus adults. Nobili et al. also determined that TE was associated with a high level of interobserver agreement and was accurate and reproducible in children (39). However, it is not known whether TE is capable of differentiating fibrosis from significant steatosis or if its precision is sufficient to track changes in fibrosis over time and following treatment (40). While many of the aforementioned technologies are promising, it is necessary to examine and identify novel, non-invasive biomarkers to accurately differentiate between simple steatosis and NASH with or without fibrosis.

APOPTOSIS AND NASH

Emerging evidence suggests that dysregulation of hepatocyte apoptosis plays a central role in the pathogenesis of NAFLD (41), liver fibrogenesis (42), and development of NASH and cirrhosis (42). Previous work by Feldstein et al showed that hepatocyte apoptosis was significantly increased in patients with NASH but was absent in patients with simple steatosis (41).

Moreover, in these patients, increased apoptosis correlated with disease severity and hepatic fibrosis (41). Apoptosis is a highly organized form of cell death characterized by membrane blebbing, cell shrinkage, and nuclear fragmentation (43). Irrespective of the triggering stimuli, apoptosis is mediated by activation of cysteine-dependent aspartate specific proteases or caspases, which require proteolytic cleavage for activation (44). In NASH, cleavage of initiator caspase 9 elicits downstream activation of effector caspase 3. Caspase 3 then cleaves cellular substrates whose processing ensures the orderly dismantlement of the cell (45). K18, a major intermediate filament protein in the liver, is associated with apoptotic cell death of hepatocytes and is released into the bloodstream following cleavage by caspase 3 (45). Because K18 fragments can be measured by ELISA as an index of early hepatocyte

apoptosis, researchers have examined whether measurement of K18 fragments can be used clinically to differentiate steatosis from more severe histology associated with NAFLD.

Wieckowska et al. demonstrated that plasma K18 fragment levels were significantly higher in adult patients with NASH than controls (46). They further showed that risk of definitive NASH on liver biopsy increased with increasing plasma levels of caspase-3-generated K18 fragments suggesting that serum and liver K18 fragments are independent predictors of NASH (46–47). Papatheodoridis et al. extended the findings of Wieckowska by using a receiver operating characteristic (ROC) curve to determine if K18 fragments had diagnostic accuracy for differentiating adult patients with NASH from those with simple steatosis (48). The data showed that K18 fragments were found helpful for the diagnosis of NASH among patients with NAFLD (48). Moreover, K18 fragments were not only found superior in accuracy for the diagnosis of NASH but the only parameter that differed significantly between NASH patients and patients with fatty liver (48).

Data from studies in children show that significant differences exist between adult and pediatric presentation of NAFLD and NASH (49). Several groups have examined K18 fragments in liver biopsies and plasma of children to determine if clinical and histologic characteristics in pediatric NASH correlate with their presence. We have previously demonstrated that plasma K18 levels are elevated in children with a clinical diagnosis of NAFLD as compared to normal weight children and overweight children without NAFLD (50). Fitzpatrick et al showed that K18 fragments were significantly higher in children with NASH as compared to children with simple steatosis or borderline disease (51). They confirmed the association between the level of K18 fragments and NASH reported in adults and support the role of K18 fragments as an individual predictor for both inflammation and fibrosis (51). In our study, CK18 had a much clearer differentiation between children with NAFLD and normal, healthy children compared to CK18 fragments (50). This has not been completely explored in larger studies and could be related to the pediatric NAFLD pattern with less ballooning.

KERATIN 18 IN OTHER CHRONIC LIVER DISEASES

The use of K18 as a diagnostic biomarker holds promise for assessment of degree of injury in NAFLD. However, because apoptosis has been implicated in the pathogenesis of nearly all acute and chronic liver diseases, an important caveat is that K18 levels are likely elevated in a variety of liver pathologies. Previous studies have shown that K18 levels were significantly higher in hepatitis C infected patients as compared to healthy controls (52). Eren et al. demonstrated that serum levels of K18 are significantly higher in hepatitis B infected patients (53). Additional studies expand the aforementioned observations to include chronic liver disease patients with varying underlying disease etiology including but not limited to hepatitis B and C virus, autoimmune hepatitis, chronic cholestasis, and hepatocellular carcinoma. Gonzalez-Quintela et al. determined that serum levels of K18 were elevated in patients with chronic liver disease (54). Moreover, the data showed that the majority of patients with liver disease had serum levels of K18 at least 10 times the normal upper reference limit (54). Yagmur et al. showed similar results and therefore concluded that

hepatic apoptosis appears to be a general mechanism in liver disease independent of the underlying cause of damage (55).

CONCLUSION

K18 is an important keratin in the liver that is released during hepatocyte cell death. Because it is measurable in the serum or plasma, it, along with the K18 fragment generated in apoptosis, has substantial potential as a biomarker for disease presence and severity in NAFLD. The relative importance of K18 versus K18 fragment levels in pediatric NAFLD compared to adult NAFLD is still not completely understood. Larger studies with both histology and levels of K18/K18 fragments are needed for correlation with liver disease histopathology, treatment response and the progression of the disease over time, particularly in children and adolescents.

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LITERATURE CITED

1. Lewis JR, Mohanty SR. Nonalcoholic fatty liver disease: a review and update. *Dig Dis Sci.* Mar; 2010 55(3):560–78. [PubMed: 20101463]
2. Moll R, Divo M, Langbein L. The human keratins: biology and pathology. *Histochem Cell Biol.* Jun; 2008 129(6):705–33. [PubMed: 18461349]
3. Ku NO, Darling JM, Krams SM, Esquivel CO, Keeffe EB, Sibley RK, et al. Keratin 8 and 18 mutations are risk factors for developing liver disease of multiple etiologies. *Proc Natl Acad Sci U S A.* May 13; 2003 100(10):6063–8. [PubMed: 12724528]
4. Strnad P, Stumtpner C, Zatloukal K, Denk H. Intermediate filament cytoskeleton of the liver in health and disease. *Histochem Cell Biol.* Jun; 2008 129(6):735–49. [PubMed: 18443813]
5. Ndozangue-Touriguine O, Hamelin J, Breard J. Cytoskeleton and apoptosis. *Biochem Pharmacol.* Jul 1; 2008 76(1):11–8. [PubMed: 18462707]
6. Omary MB, Ku NO, Toivola DM. Keratins: guardians of the liver. *Hepatology.* Feb; 2002 35(2):251–7. [PubMed: 11826396]
7. Caulin C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. *J Cell Biol.* Sep 22; 1997 138(6):1379–94. [PubMed: 9298992]
8. Oriolo AS, Wald FA, Ramsauer VP, Salas PJ. Intermediate filaments: a role in epithelial polarity. *Exp Cell Res.* Jun 10; 2007 313(10):2255–64. [PubMed: 17425955]
9. Omary MB, Ku NO, Strnad P, Hanada S. Toward unraveling the complexity of simple epithelial keratins in human disease. *J Clin Invest.* Jul; 2009 119(7):1794–805. [PubMed: 19587454]
10. Omary MB, Coulombe PA, McLean WH. Intermediate filament proteins and their associated diseases. *N Engl J Med.* Nov 11; 2004 351(20):2087–100. [PubMed: 15537907]
11. Ku NO, Michie S, Oshima RG, Omary MB. Chronic hepatitis, hepatocyte fragility, and increased soluble phosphoglycokeratins in transgenic mice expressing a keratin 18 conserved arginine mutant. *J Cell Biol.* Dec; 1995 131(5):1303–14. [PubMed: 8522591]
12. Ku NO, Gish R, Wright TL, Omary MB. Keratin 8 mutations in patients with cryptogenic liver disease. *N Engl J Med.* May 24; 2001 344(21):1580–7. [PubMed: 11372009]
13. Schoniger-Hekele M, Petermann D, Muller C. Mutation of keratin 8 in patients with liver disease. *J Gastroenterol Hepatol.* Sep; 2006 21(9):1466–9. [PubMed: 16911694]

14. Ku NO, Lim JK, Krams SM, Esquivel CO, Keeffe EB, Wright TL, et al. Keratins as susceptibility genes for end-stage liver disease. *Gastroenterology*. Sep; 2005 129(3):885–93. [PubMed: 16143128]
15. Ku NO, Liao J, Omary MB. Apoptosis generates stable fragments of human type I keratins. *J Biol Chem*. Dec 26; 1997 272(52):33197–203. [PubMed: 9407108]
16. Leers MP, Kolgen W, Bjorklund V, Bergman T, Tribbick G, Persson B, et al. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. *J Pathol*. Apr; 1999 187(5):567–72. [PubMed: 10398123]
17. Rydlander L, Ziegler E, Bergman T, Schoberl E, Steiner G, Bergman AC, et al. Molecular characterization of a tissue-polypeptide-specific-antigen epitope and its relationship to human cytokeratin 18. *Eur J Biochem*. Oct 15; 1996 241(2):309–14. [PubMed: 8917424]
18. de Haas EC, di Pietro A, Simpson KL, Meijer C, Suurmeijer AJ, Lancashire LJ, et al. Clinical evaluation of M30 and M65 ELISA cell death assays as circulating biomarkers in a drug-sensitive tumor, testicular cancer. *Neoplasia*. Oct; 2008 10(10):1041–8. [PubMed: 18813353]
19. Kramer G, Erdal H, Mertens HJ, Nap M, Mauermann J, Steiner G, et al. Differentiation between cell death modes using measurements of different soluble forms of extracellular cytokeratin 18. *Cancer Res*. Mar 1; 2004 64(5):1751–6. [PubMed: 14996736]
20. Yilmaz Y, Dolar E, Ulukaya E, Akgoz S, Keskin M, Kiyici M, et al. Soluble forms of extracellular cytokeratin 18 may differentiate simple steatosis from nonalcoholic steatohepatitis. *World J Gastroenterol*. Feb 14; 2007 13(6):837–44. [PubMed: 17352011]
21. Younossi ZM, Jarrar M, Nugent C, Randhawa M, Afendy M, Stepanova M, et al. A novel diagnostic biomarker panel for obesity-related nonalcoholic steatohepatitis (NASH). *Obes Surg*. Nov; 2008 18(11):1430–7. [PubMed: 18500507]
22. Tiniakos D, Vos M, Brunt E. Nonalcoholic Fatty Liver Disease: Pathology and Pathogenesis. *Annu Rev Pathol Mech Dis*. 2010; (5):145–71.
23. Brunt E, Janney C, AM DB, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic Steatohepatitis: A proposal for grading and staging the histological lesions. *American Journal of Gastroenterology*. 1999; 94:2467–74. [PubMed: 10484010]
24. Lackner C, Gogg-Kamerer M, Zatloukal K, Stumptner C, Brunt EM, Denk H. Ballooned hepatocytes in steatohepatitis: the value of keratin immunohistochemistry for diagnosis. *Journal of hepatology*. May; 2008 48(5):821–8. [PubMed: 18329127]
25. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease. *Hepatology*. 2005; 41:1313–21. [PubMed: 15915461]
26. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics*. 2006; 118(4):1388–93. [PubMed: 17015527]
27. Strauss R, Barlow S, Dietz W. Prevalence of abnormal serum aminotransferase values in overweight and obese adolescents. *The Journal of Pediatrics*. 2000; 136:727–33. [PubMed: 10839867]
28. Fraser A, Longnecker MP, Lawlor DA. Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999–2004. *Gastroenterology*. Dec; 2007 133(6):1814–20. [PubMed: 18054554]
29. Schwimmer JB, McGreal N, Deutsch R, Finegold MJ, Lavine JE. Influence of gender, race, and ethnicity on suspected fatty liver in obese adolescents. *Pediatrics*. May; 2005 115(5):e561–5. [PubMed: 15867021]
30. Louthan MV, Theriot JA, Zimmerman E, Stutts JT, McClain CJ. Decreased prevalence of nonalcoholic fatty liver disease in black obese children. *J Pediatr Gastroenterol Nutr*. Oct; 2005 41(4):426–9. [PubMed: 16205510]
31. Leung DH, Williams K, Fraley JK, Klish WJ. Age- and Ethnic-Specific Elevation of ALT Among Obese Children at Risk for Nonalcoholic Steatohepatitis (NASH): Implications for Screening. *Clin Pediatr (Phila)*. Oct.2008 2
32. Denzer C, Thiere D, Mucbe R, Koenig W, Mayer H, Kratzer W, et al. Gender-specific prevalences of fatty liver in obese children and adolescents: roles of body fat distribution, sex steroids, and insulin resistance. *J Clin Endocrinol Metab*. Oct; 2009 94(10):3872–81. [PubMed: 19773396]

33. De Bruyne RM, Fitzpatrick E, Dhawan A. Fatty liver disease in children: eat now pay later. *Hepatol Int.* 2010; 4(1):375–85. [PubMed: 20305757]
34. Bayard M, Holt J, Boroughs E. Nonalcoholic fatty liver disease. *Am Fam Physician.* Jun 1; 2006 73(11):1961–8. [PubMed: 16770927]
35. Cohen MB, HH AK, Lambers D, Heubi JE. Complications of percutaneous liver biopsy in children. *Gastroenterology.* Feb; 1992 102(2):629–32. [PubMed: 1732131]
36. Lachaux A, Le Gall C, Chambon M, Regnier F, Loras-Duclaux I, Bouvier R, et al. Complications of percutaneous liver biopsy in infants and children. *Eur J Pediatr.* Aug; 1995 154(8):621–3. [PubMed: 7588960]
37. Schwenzer NF, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. *J Hepatol.* Sep; 2009 51(3):433–45. [PubMed: 19604596]
38. Gaia S, Carenzi S, Barilli AL, Bugianesi E, Smedile A, Brunello F, et al. Reliability of transient elastography for the detection of fibrosis in Non-Alcoholic Fatty Liver Disease and chronic viral hepatitis. *J Hepatol.* Jan; 2011 54(1):64–71. [PubMed: 20932598]
39. Nobili V, Vizzutti F, Arena U, Abraldes JG, Marra F, Pietrobattista A, et al. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. *Hepatology.* Aug; 2008 48(2):442–8. [PubMed: 18563842]
40. Wieckowska A, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. *Semin Liver Dis.* Nov; 2008 28(4):386–95. [PubMed: 18956295]
41. Feldstein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. *Gastroenterology.* Aug; 2003 125(2):437–43. [PubMed: 12891546]
42. Valva P, De Matteo E, Galoppo M, Pedreira A, Giacove G, Lezama C, et al. Apoptosis markers in liver biopsy of nonalcoholic steatohepatitis in pediatric patients. *Hum Pathol.* Dec; 2008 39(12):1816–22. [PubMed: 18715620]
43. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer.* Aug; 1972 26(4):239–57. [PubMed: 4561027]
44. Schattenberg JM, Galle PR, Schuchmann M. Apoptosis in liver disease. *Liver Int.* Oct; 2006 26(8):904–11. [PubMed: 16953829]
45. Guicciardi ME, Gores GJ. Apoptosis as a mechanism for liver disease progression. *Semin Liver Dis.* Nov; 2010 30(4):402–10. [PubMed: 20960379]
46. Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology.* Jul; 2006 44(1):27–33. [PubMed: 16799979]
47. Wieckowska A, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. *Hepatology.* Aug; 2007 46(2):582–9. [PubMed: 17661414]
48. Papatheodoridis GV, Hadziyannis E, Tsochatzis E, Georgiou A, Kafiri G, Tiniakos DG, et al. Serum apoptotic caspase activity in chronic hepatitis C and nonalcoholic Fatty liver disease. *J Clin Gastroenterol.* Apr; 2010 44(4):e87–95. [PubMed: 19881359]
49. Valva P, De Matteo E, Galoppo MC, Gismondi MI, Preciado MV. Apoptosis markers related to pathogenesis of pediatric chronic hepatitis C virus infection: M30 mirrors the severity of steatosis. *J Med Virol.* May; 2010 82(6):949–57. [PubMed: 20419808]
50. Vos MB, Barve S, Joshi-Barve S, Carew JD, Whittington PF, McClain CJ. Cytokeratin 18, a marker of cell death, is increased in children with suspected nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr.* Oct; 2008 47(4):481–5. [PubMed: 18852641]
51. Fitzpatrick E, Mitry RR, Quaglia A, Hussain MJ, DeBruyne R, Dhawan A. Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD. *J Pediatr Gastroenterol Nutr.* Oct; 2010 51(4):500–6. [PubMed: 20808246]
52. Bantel H, Lugerling A, Heidemann J, Volkmann X, Poremba C, Strassburg CP, et al. Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. *Hepatology.* Nov; 2004 40(5):1078–87. [PubMed: 15486927]

53. Eren F, Yilmaz Y, Kose S, Ozdemir FT, Yonal O, Kurt R, et al. Caspase-cleaved fragments of cytokeratin 18 in patients with chronic hepatitis B. *Clin Chim Acta*. Dec 14; 2010 411(23–24): 2029–32. [PubMed: 20807521]
54. Gonzalez-Quintela A, Mallo N, Mella C, Campos J, Perez LF, Lopez-Rodriguez R, et al. Serum levels of cytokeratin-18 (tissue polypeptide-specific antigen) in liver diseases. *Liver Int*. Dec; 2006 26(10):1217–24. [PubMed: 17105587]
55. Yagmur E, Trautwein C, Leers MP, Gressner AM, Tacke F. Elevated apoptosis-associated cytokeratin 18 fragments (CK18Asp386) in serum of patients with chronic liver diseases indicate hepatic and biliary inflammation. *Clin Biochem*. Jun; 2007 40(9–10):651–5. [PubMed: 17306787]