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Systematic Review

Biomarkers of Non Alcoholic Steatohepatitis: a systematic review and meta-analysis

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Abstract

Introduction: NAFLD is a common cause of chronic liver disease worldwide. Progression of chronic liver disease leads to liver transplant, so theearly detection of the primary stages is of utmost importance. This study aims to perform a systematic review of pertinent literature to determine early stages of NASH and explorediagnostic biomarkers of ck-18 family.

Method: This study is systematic and Meta analysis. We searched articles of different levels published in pub med, Cochrane collaboration library until December 9, 2016. Two independent reviewers assessed articles according to predefined criteria and extracted relevant data. AUC and values related to each biomarker in NASH, NO NAFLD and NAFLD groups were analyzed.

Results: The total population of five studies incorporated in the meta-analysis consisted of 345 subjects with an average age of 69.45 ± 8.40 years. The AUC related to M30 for diagnosis of NASH was reported between 0.60 and 0.88, which was suitable to differentiate NASH from simple steatohepatotitis.

Conclusion: The results showed that M30 was a suitable biomarker for the diagnosis of NASH. It is considering that the use of biomarkers can reduce the need for biopsy for the diagnosis of NASH and consequently decreaserelated costs and risks.

Keywords: Non-alcoholic Fatty Liver Disease, biomarkers, Nonalcoholic Steatohepatitis, NAFLD, systematic review, meta-analysis

Introduction:

Non-alcoholic fatty liver disease (NAFLD),a hepatic manifestation of metabolic syndrome, is the most common cause of chronic liver disease across the world (1). It is estimated that about 30% of the adult population and less than 10% of children in the West suffer from NAFLD (2). Accordingly, 20-25% of people with NAFLD are prone to NASH (3). Patients with NASH have increased morbidity and mortality from cardiovascular disease, cancer and liver-related diseases in the total population (4).

As we know, NAFLD includes a range of simple steatosis and non-alcoholic steatohepatitis (5-6).There are increasing evidences over the past decade that show patients with NASH follows a progressive course (7). Although several biomarkers have been developed for NASH and hepatic fibrosis (8-15), biopsy is considered as an "imperfect gold standard" for diagnosis of NASH and staging of fibrosis area. In addition to being costly, liver biopsy depends on the sample size and incomplete sampling error may be resulted along certain risks (16-18). As a result, the development and discovery of non-invasive diagnostic biomarkers for diagnosis of NASH and its associated fibrosis is of paramount importance inclinical practices (7-21).

Thus, to optimize outcomes of patients with liver disease, physicians and researchers need to performsuitable noninvasive tests for lasting detectionof subclinical liver damage and monitoring liver damage progress and regression. Recent evidences suggest that NASH pathogenesis includesderegulation cytokines, adipokines, insulin resistance and apoptosis markers. (22-24) On the other hand,fibrosis development isassociated with collagen deposition and other fibrogenic pathway. The two pathways may have common components, but they are distinct Thus, it may be difficult to develop a test for the diagnosis of NASH and staging of fibrosis. (24)

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This study offers a systematic review to introduce biomarkers that are activated during apoptosis.

Method:

Studies Selection:

The title and abstract of allarticles and citations were studied by two independent researchers (a pathologist and an MS in Medical Education) and related articles were extracted. All disagreements were resolved by discussion.

Inclusion criteria:

All studies discussing diagnostic biomarkers for NASH were reviewed and included in the study. Abstractspresented at conferences, which were published in journals but their full text was not accessible, were also included in the study. All articles in which the study population included of hepatitis B, hepatitis C and autoimmune liver disease and studies with an animal model were not included.

Sources and search strategies:

We searched all English articles in pubmed and Cochrane collaboration library databases until December 9, 2016. The references of articles were reviewed manually. The search strategy is shown in the following figure 1

Key words were used" Biomarkers", "NASH', "Non-alcoholic Fatty Liver Disease", " Nonalcoholic Steatohepatitis" , " NAFLD". "Biomarkers" [title] AND Non-alcoholic Fatty Liver Disease" [title] , "Non-alcoholic Fatty Liver Disease"" [title] AND Biomarkers , "Nonalcoholic Steatohepatitis" [title] AND Biomarkers [title], "Nonalcoholic Steatohepatitis"in title, abstract, keywords and Biomarker, "Nonalcoholic Steatohepatitis" in title, abstract, keywords , "Non-alcoholic Fatty Liver Disease" in title, abstract, keywords and Biomarker, "NAFLD" in title, abstract, keywords and Biomarker, "NASH" in title, abstract, keywords and Biomarker.

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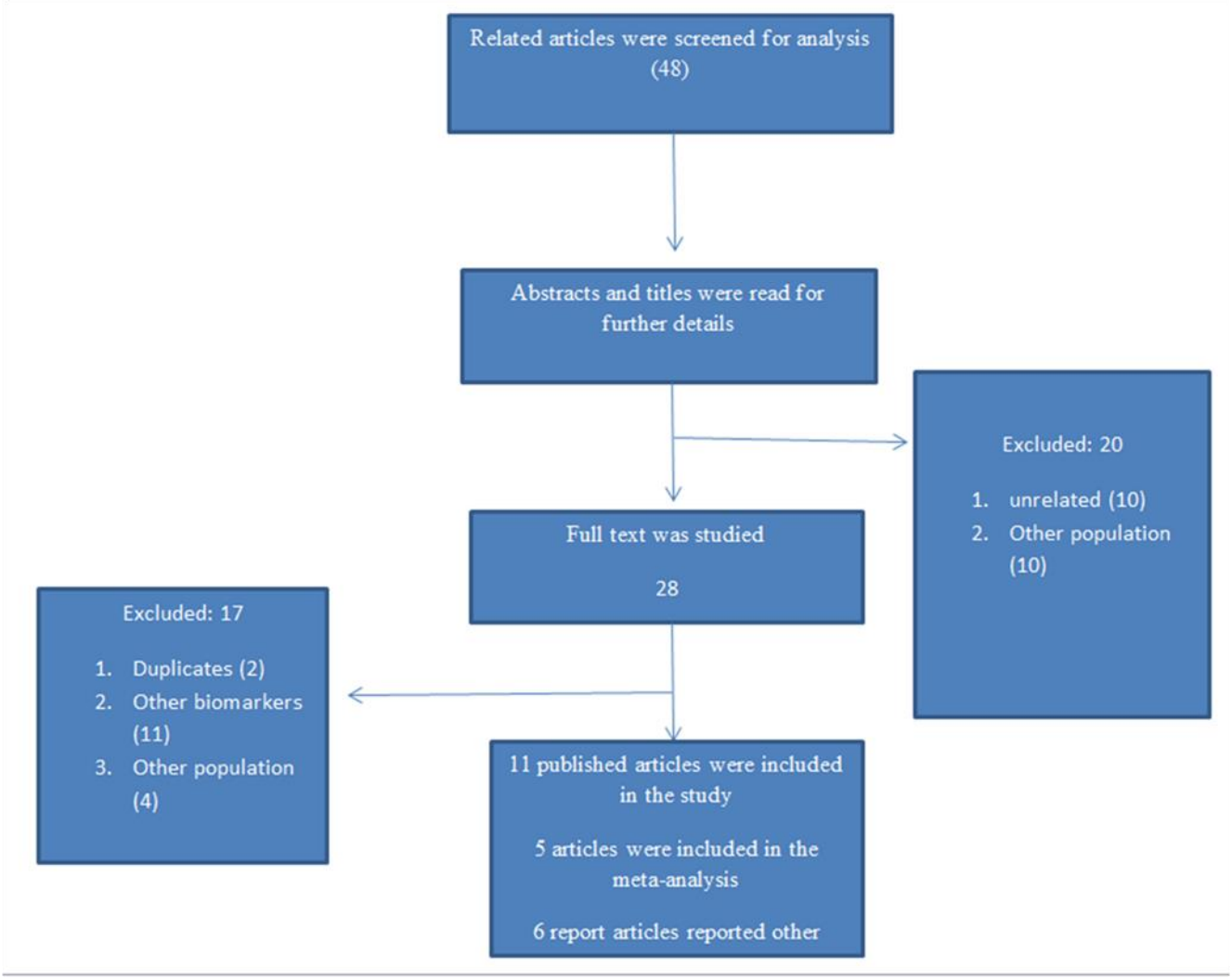
Fig 1. Search strategy



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Data extraction:

In this study, patients with NASH, i.e. patients with steatosis, lobular inflammation, ballooning degeneration with and without Mallory- Denk bodies and /or fibrosis and patients with NAFLD, i.e. patients with simple steatohepatitis and the group with normal biopsy results were enrolled.

Data 1- M30: the total level of CK-18 (M30 antigen released in the process of the caspase cleavage neoepitope and reflecting apoptosis)

2- M65: the total released amount of CK-18 (M65 antigen released from all dying cells and reflecting total cell death including both apoptosis and necrosis)

3- M30-M65: necrosis-reflecting parameter calculated as M65-M30

Quality assessment

For each article, the inclusion criteria were examined by two researchers based on Cochrane collaboration's risk of bias tool for studies incorporated in the meta-analysis (25). Two researchers evaluated the studies independently and differences were resolved through discussion. (Table 1)

study	Adequate sequence generation	Adequate allocation concealment	Blinding	Incomplete outcome data addressed	Free of selective Reporting
M.Younosis,	Low	Low	Low	Low	Low
M.Younossi,	Low	Low	Low	Low	Low
L.Diab, 2008	Low	Low	Low	Low	Low
Yilmaz, 2012 (28)	Low	Low	Low	Low	Low
Kamada, 2013	Low	Low	Low	Low	Low

Table 1. Risk of bias for studied included in the meta-analysis

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Data analysis:

All values of biomarkers were analyzed using the comprehensive meta-analysis software. Results of biomarkers in both groups including the values or ratios were estimated at 95% confidence interval. Mean and standard deviation were calculated and demographic information was also reported.

Results:

48 studies were evaluated out of which a total of 11 studies that met the objectives of the study were extracted. Of these articles, 5 were included in the meta-analysis for the analysis of M30 marker values and 6 other studies introduced related markers for the diagnosis of NASH. A consistency of 98.5% was reported between the two reviewers.

Specifications of studies and subjects under study:

In Table 2, details of 5 studies included in the meta-analysis are presented. The total population of these 5 studies consisted of 345 patients with NAFLD with an average age of 45.69 ± 8.40 years. More than 59% of subjects were female with an average BMI of 37.80 ± 5.8. M30 values were calculated for all studies and specificity and sensitivity was estimated based on the results of liver biopsy. In some studies, values of M65 and M65-M30 were also reported. The highest AUC (Area under curve) for the diagnosis of NASH (i.e. 0.88).was reported in the study of L.Diab in 2008

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Table 2. Details of included studies

study	Study population	PPV,%	NPV%	Specificity, (95% CI)%	Sensitivity, (95% CI)%	AUC (95% CI)
M.Younosis, 2011)	79	55(41.6-67.9)	64.3(35.2-87.1)	25(12.1-42.2)	86.84(71.9-95.5)	0.70(0.59-0.80)
M.Younossi, 2008)	37	88.9	76.7	97.87(88.7-99.6)	36.36(17.2-59.3)	0.711(0.589-0.814)
L.Diab, 2008	35	-	-	77	82	0.88(0.77-0.99)
Yilmaz, 2012	68	87.8	98.2	87	97.8	-
Kamada, 2013	126	-	-	62.5	58	0.62

*PPV: Positive Predictive Value

NPV: Negative Predictive Value

AUC: Area under curve

References

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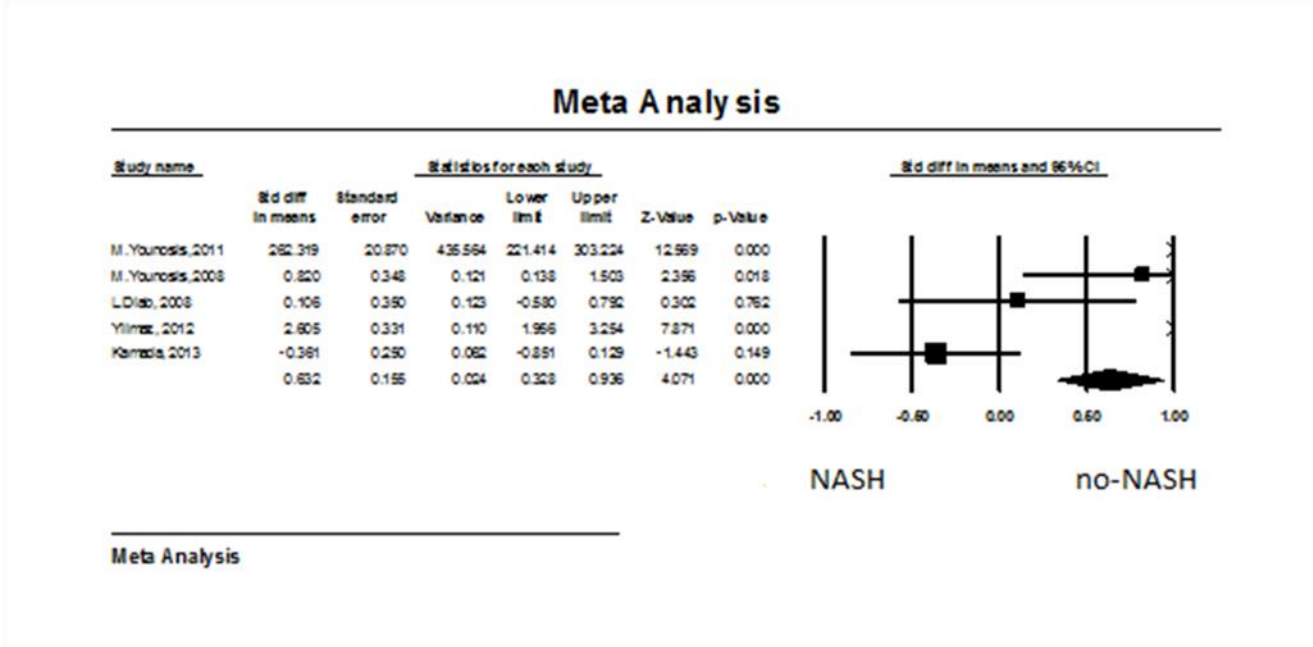
Implications

M30 results: in five studies, the value of M30 was measured and results were compared and analyzed at different cut-offs based on the results of liver biopsy, which served as a golden standard for NASH diagnosis. The sensitivity and specificity and their positive and negative predictive values are also reported in Table 2. M30 values in patients with NASH and NAFLD group were analyzed in various studies, as shown in Figure 2.

In the study of M.Younosis (26), AUC was calculated forboth M30 and M65 (with an estimated value of 0.814 (CI: 0.702-0.897). The AUC related to M30 for diagnosis of NASH was reported between 0.60 and 0.88, which was suitable to differentiate NASH from simple steatohepatotitis The present study also included an estimation of M65-M30 ratio, which was significantly different compared to NAFLD (SIMPLE STEATOHEPATITIS) and subjects with normal liver biopsy This study showed that a panel consisting of adipokines and two biological markers of cell death would be more fitting. Additionally, the value of M65 in the second study of M.Younosis in 2011 was higher in NASH group compared to no-NASH group, and this

Figure 2. Forest plot: summary AMOUNT of M30(the total level of CK-18) IN TWO GROUP (NASH AND NAFLD); pooled estimates are from afixed- effects model



difference was statistically significant, as it was for M65-M30 ratio. (24)

Other reports of biomarkers:

In 2012, Blomme revealed that glyco marker was used especially in obese individualswho needed to be triaged for the development of steatohepatitis and it could differentiate NASH from simplesteatosis. (30) In another study in 2012, NASH was found to be strongly dependent on the level of non-HDL- c, with this value being significantly higher than steatosis in patients without lipid-lowering agents. This biomarker wasnot onlycost effective, but also contributed to the distinction of NASH from steatosis(23).Imajo in 2012 stated that the level of fCh in plasma was related to grade fibrosis and steatosis and it could serve as a predictor of the severity of NASH. Therefore, it acts as a good diagnostic marker for early-stage NASH in clinical practice. According tothese studies, AUC for the diagnosis of NASH with fChand fibrosis staging is 0.811 and 0.816 respectively. In patients with simple steatohepatitis, the fCh level increases. (32) It has been shown that theserum level of ferritin in NASH patients is significantly higher than patients with simple steatohepatotitis with a cut-off value of196 ng /ml and optimal sensitivity (64.2%), specificity (76.5%), PPV (88.19%) and NPV (43.1%) (33). In a paper by Feldstein, CK-18 fragmente was introduced as an independent predictor of NASH after adjusting fibrosis, ALT, AST, age and length of biopsy. The estimated AUC for the diagnosis of NASH was0.83. (34)

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Discussion

As we know, nonalcoholic fatty liver disease is the most common chronic liver disease, which includes a wide range of illnesses associated with excessive accumulation of fat in the liver of people without alcohol abuse. It ranges from simple steatosis to steatohepatitisin terms of severity and can eventually lead to cirrhosis and hepatocellular carcinoma. (35) It has been reported that 14 to 30% of people in Western societies suffer from NAFLD (36). Obesity is strongly connected with NAFLD and it is a predictive of advanced stages of the disease. In light of the increasing prevalence of obesity in Iran and the world, this disease is expected to rise dramatically. (37) Therefore, the introduction of non-invasive diagnostic method can be helpful in the early stages during the process of clinical performance .In studies analyzed in this paper, it was shown that ck-18 (Cytokeratin18) was an epithelial cytoskeleton protein, which was released into the bloodstream when liver cells died. CK-18 is a substrate ofcaspases that are activated during apoptosis. Segments of CK-18 (fragmented ck-18) are accumulated in apoptosis cells and then released into the bloodstream. Many liver damages are associated with increased hepatocyte apoptosis.

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An ELISA test for the detection of caspase-cleaved ck-18 was developed by M30, which producedan antibody that recognized a specific epitope. One of the restrictions related to the application of M30 is its lack of reliability in differentiating different patients with significant hepatic fat from those with steatosis and it cannot be used for staging fibrosis. (38) However, the meta-analysis in this study showed a significant difference in values of M30 between two groups of NASH and simple steatohepatitis. (P = 0.0001) Studies have shown that total ck-18 is a suitable biomarker of liver fibrosis. Although cleaved-ck-18 level is dependent on the stage of fibrosis and liver stiffness, there is a strong correlation between liver fibrosis and total ck-18 (especially M65). It has been posited that total ck-18 is a good indicator of NAFLD / NASH compared to cleaved ck-18. The total ck-18 is used to evaluate differentiate patients based on minimal steatosis (hepatic fat accumulation <10%) from steatosis (hepatic fat> 10%). Therefore, total ck-18 is better at differentiating NAFLD/NASH compared to M30 (39). Given that pathogenesis of NASH has its own complexities, it is possible that cytokines and adipokines pathways also contribute to the progression of NASH. (40) Therefore, a set of biomarkers that containboth apoptosis markers and adipocytokine pathways could be used for differential diagnosis of NASH.

Lack of RCT articles was Limitation of this study. It is recommended to perform clinical trials to determine characteristics of cell death biomarkers for the early detection of NASHFH with enhanced matching and precise classification.

Conclusion:

The results showed that M30 was a suitable biomarker for the diagnosis of NASH. It is considering that the use of biomarkers can reduce the need for biopsy for the diagnosis of NASH and consequently decrease related costs and risks.

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