Original Article

Serum uric acid levels in non-alcoholic steatosis patients: a meta-analysis

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Background and Objectives: Experimental and observational studies suggest a role for increased uric acid in non-alcoholic fatty liver disease (NAFLD). This study aimed to systematically review the association between serum uric acid (SUA) levels and NAFLD. **Method and Study Design:** We used PubMed, and the EMBASE database to identify all applicable studies through November 2015. We used the weighted mean difference (WMD) to demonstrate the differences between the control and NAFLD groups in continuous data. We calculated the odds ratios (ORs) for dichotomous data using the Mantel-Haenszel method. A total of 16 observational studies were identified and used for the analysis of continuous data, and 4 studies were analyzed for dichotomous data. **Results:** The WMD was 52.3 (95% CI: 39.0, 65.5, $p<0.00001$). The pooled OR in observational studies was 2.08 (95% CI: 1.93-2.24, $p<0.00001$). The results were heterogeneous for the comparison of continuous data and homogeneous for the comparison of dichotomous data. The SUA cutoff value for the occurrence of NAFLD was 308, with a sensitivity of 94.12% [71.3-99.9] and specificity of 70.6% [44.0-89.7]. **Conclusion:** We observed a positive association between increased SUA levels and the diagnosis of NAFLD in all analyses. Our results suggest that SUA is upregulated in patients with NAFLD and might be related to the pathogenesis of NAFLD.

Key Words: serum uric acid, non-alcoholic fatty liver disease, fatty liver, SUA, NAFLD

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most important global public health problems of the twenty-first century; it affects approximately 20–30% of the general population, and its prevalence is increasing worldwide.[1] NAFLD comprises a histological spectrum of liver disease from nonalcoholic fatty liver or simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis.[1] Although the majority (70-90%) of patients with NAFLD follow a benign, non-progressive clinical course,[2,3] a significant minority have NASH and are at greater risk for progression to cirrhosis and hepatocellular carcinoma.[4,5] Hence, distinguishing NASH from NAFLD has important prognostic and management implications.

At present, liver biopsy remains the criterion standard for diagnosing NAFLD[6] because it allows other causes of liver damage to be excluded and the severity of the fatty infiltration into the hepatocyte, lobular inflammation, hepatocyte ballooning and fibrosis to be estimated.[7] However, liver biopsy is poorly suited as a diagnostic test for such a prevalent condition due to its invasiveness, sampling variability and cost. The noninvasive tests available for distinguishing NAFLD include an assessment of clinical signs and symptoms, routine laboratory and radiological imaging tests, and combinations of clinical and blood test results.[8,9] Unfortunately, these tests are of limited use.[8-10] A number of investigators have attempted to identify potential noninvasive markers for NASH diagnosis, such as biochemical markers (the Nash Test),[11] ferritin[12] and serum fragment of cytokeratin-18;[13] however, these markers have not been externally validated.

Serum uric acid (SUA) is the major end product of urine metabolism in humans and higher primates, and the level of SUA is rigorously controlled by the balance between uric acid production and excretion. Hyperuricemia has long been recognized as a cause of gouty arthritis and kidney stones. There is mounting evidence that SUA may also have an important role in the development of vascular conditions, such as hypertension, kidney disease, metabolic syndrome, and cardiovascular disease.[14-16] Recently, emerging data suggest that elevated SUA is frequently associated with the development or progression of NAFLD and that elevated SUA levels are an independent risk factor for NAFLD regardless of insulin resistance,

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components of metabolic syndrome (MetS), or indexes of liver and kidney function.[17-21] Hwang et al suggested that increased SUA concentrations, even within the normal range, are independently associated with the presence of NAFLD.[22] A better understanding of the SUA levels in NAFLD patients will provide a more accurate interpretation of the SUA-NAFLD relationship and has potential implications for NAFLD treatment in the population. Therefore, we performed a meta-analysis to explore the potential diagnostic value of SUA.

METHOD

Data sources

We identified studies that were published in the English language by searching the PubMed and EMBASE databases. Studies that were eligible for this analysis were updated on November 2015 with the following keywords: "nonalcoholic fatty liver disease", "NAFLD" or "nonalcoholic steatohepatitis" plus "uric acid" or "clinical chemistry" (Supplementary table 1). All eligible studies were retrieved, and their bibliographies were reviewed for other relevant publications. Additional papers and book chapters were identified by a manual search of the references from the original manuscripts and reviews. The scientific analysis was approved by the ethics committee of Anhui Medical University.

Study selection

To be included, a study had to fulfill the following criteria: (1) patients with a distinct NAFLD diagnosis by ultrasonography or liver biopsy and (2) participant population of any sex or ethnicity with NAFLD.

The following studies were excluded: (1) those with overlapping articles or duplicate data; (2) articles about animal experiments; (3) studies with pregnant individuals; (4) studies with NAFLD patients with other competing causes of steatosis, such as excessive alcohol, viral hepatitis, autoimmune hepatitis, and hemochromatosis; and (5) conference report, letters, and case reports.

All reports were independently conducted by two investigators. The results were compared, and any questions or discrepancies were resolved through iteration and consensus.

Data extraction and quality assessment

Data extraction was independently conducted by two researchers (Huang F and Yao L). The following data were extracted: year of publication, name of the first author, country, participant characteristics (age, gender, ethnicity and body mass index), method of diagnosing NAFLD, SUA measurement methods, and serum SUA levels. Discrepancies were resolved by discussion and consensus.

The present study followed the quality standard of reports for observational studies in epidemiology guidelines (PRISMA) for observational studies. Selection criteria were established before the search to avoid selection bias. The search results were double-checked by a third investigator (Liu A).

Statistical analysis

For studies that reported means and standard deviations of SUA levels, we analyzed the levels in NAFLD patients and in a non-NAFLD control group for each study using weighted mean differences (WMDs) and 95% confidence intervals (CIs). For studies that reported dichotomous and categorical data, we separated the cohorts into hyperuricemia and un-hyperuricemia (cut-off value: men = 7 mg/dL or 420 μmol/L; women = 6 mg/dL or 360 μmol/L).[23] We used τ^2 to analyze the heterogeneity. If the studies were found to be heterogeneous, a random-effects model rather than fixed-effects model was used to analyze the pooled estimates.[24] Subgroup analysis was performed to investigate the factors that affected the pooled estimates and the source of heterogeneity. Sensitivity analysis was conducted to assess the influence of a single study on the analysis. We used Harbord's regression test for funnel-plot asymmetry and Egger's test to assess the publication bias. Receiver operating characteristics (ROC) curves were generated to determine the cut-off values for the optimal sensitivity and specificity of the relationship between SUA levels and the occurrence of NAFLD. Meta-analysis was performed using Review Manager Version 5 (Cochrane Collaboration and Update Software) and Stata Version 13 for all analyses. Subgroup analysis was performed to evaluate the factors that might impact the pooled effects and to identify the source of any heterogeneity.

RESULTS

Literature search

In the present analysis, a total of 2,461 candidate articles were identified regarding the SUA levels and the diagnosis of NAFLD. In these articles, 1,210 were from PubMed and 1,251 were from EMBASE. After the duplicates were removed, 1,680 articles remained, of which 1,642 were excluded because they did not fit our selection criteria. After the full texts of the remaining 38 studies were reviewed, a total of 22 publications were identified, including 16 articles that used continuous data and 6 that used dichotomous data. A flow diagram showing the methodology used to select relevant studies is presented in Supplementary figure 1.

Study characteristics

Of the 17 independent cohort studies[22,23,25-39] that used continuous data, 35,936 participants and 12,374 NAFLD cases were identified. Four studies were conducted in Western countries, and 13 were conducted in Asian countries. Nine studies were conducted in general population settings, seven were conducted in inpatients settings, one was conducted with postmenopausal women,[25] and one was conducted with obese women.[35] The selected studies were published from 2001 to 2015, and the number of participants per study ranged from 95 to 9,019. The mean SUA levels for the NAFLD subjects ranged from 279 to 449 μmol/L. The mean ages of the subjects were similar among the studies except for the study by Xu et al,[33] which focused on elderly patients. NAFLD was diagnosed by ultrasound in 15 studies,[22,23,25,27-29,31-39] by liver biopsy in 1 study,[30] and by CT in 1 study[26] (Table 1). All studies showed significantly higher SUA levels in NAFLD subjects compared with the controls. The SUA levels were typically presented in μmol/L, but those that were measured in mg/dL were converted to μmol/L by

Table 1. Observational Studies involved in the meta-analysis

First Author, Year	Region	Diagnostic method	NAFLD (N)/ Total (N)	NAFLD, males (%)	NAFLD age	Assay method	SUA in NAFLD patients (μmol/L)	SUA in controls (μmol/L)	*p*-value
Cai, 2014	China	Ultrasonography	934/2191	72.4	46.0±10.0	Automated Analyzer	320±88	254±80	<0.05
Cao, 2013	China	Liver biopsy	44/95	22.7	45.5±12.59	NR	330.4±113.63	295.8±78.47	0.035
Chang, 2014	Taiwan	Magnetic resonance spectroscopy.	210/420	62.8	44.1±12.7	Automated Analyzer	392.63±83.28	326.97±71.38	<0.001
Fenkci, 2007	Turkey	NR	84/105	NR	44.9±11.5	Enzymatic method	313.71±98.84	279.64±65.44	NR
Hu, 2012	China	Ultrasonography	2730/7152	61.2	48.42±12.94	Automated Analyzer	365.44±82.38	308.09±77.00	<0.01
Hwang, 2011	Korea	Ultrasonography	2124/9019	75.9	44.0±13.0	Automated Analyzer	331.46±88.78	272.27±100.62	<0.001
Kuecuekazman, 2014	Turkey	Ultrasonography	154/211	42.8	46.3±10.7	NR	325.55±76.95	284.11±76.95	0.001
Li, 2009	China	Ultrasonography	1051/8925	66.3	56.8±8.4	Automated Analyzer	370.3±86.6	321.1±82.6	<0.001
Lin, 2015	China	Ultrasonography	1424/4305	31.7	62.7±9.0	Automated Analyzer	301.9±77.4	327.2±76.8	<0.001
Liu, 2014	China	Ultrasonography	121/528	0	54±7.4	Automated Analyzer	279.96±44.98	255.70±51.49	<0.01
Lonardo, 2001	Italy	Ultrasonography	60/120	55.0	51.7±1.44	ELISA kit	316.66±10.06	238.53±10.06	<0.01
Omagari, 2002	Japan	Ultrasonography	141/1264	65.2	48±19.25	NR	337.38±333.21	272.27±346.37	<0.01
Seung, 2003	Korea	Ultrasonography	120/360	100	42.8±0.88	Automated Analyzer	371.71±6.21	344.49±0.402	<0.001
Tarantiono, 2011	Italy	Ultrasonography	85/105	36.5	33.45±13.63	NR	310.15±84.64	207.17±23.68	<0.001
Tian, 2014	China	Ultrasonography	2286/5638	66.1	NR	Automated Analyzer	449.86±83.37	354.87±63.28	NR
Xu, 2011	China	Ultrasonography	227/878	61.2	71.2±3.8	Automated Analyzer	372.60±88.5	336.40±82.80	<0.001
Zhang, 2014	China	Ultrasonography	215/844	59.5	50.36±6.50	Radioimmunoassay	328.6±84.5	301.6±90.6	<0.01

NR: not reported.
Studies with data on SUA levels in NAFLD and controls, alphabetically ordered. SUA levels are reported in μmol/L. Levels that were measured in mg/dL were converted to μmol/L by mal by 59.48.

Table 2. Studies involved in the meta-analysis with dichotomous data

First Author, year	Region	Reagent sources	Assay method	Cut-off	TP	FP	FN	TN
Anajas, 2013	Brazil	serum	Automated analyzer	330 μmol/L	6	10	31	82
Xu, 2010	China	serum	Automated analyzer	Men: 420 μmol/L Women: 360 μmol/L	145	671	726	5870
Ryu, 2010	Korea	serum	Automated analyzer	420 μmol/L	305	365	1412	3559
Lee, 2009	Korea	serum	Automated analyzer	Men: 420 μmol/L Women: 360 μmol/L	954	999	2656	6123

†Studies with data on dichotomous SUA levels (hyperuricemia or not) in NAFLD patients and controls. TP: true positive, NAFLD patients with hyperuricemia; FP: false positive, non-NAFLD.
‡Controls with hyperuricemia; FN: false negative, NAFLD with normal SUA levels; TN: true negative: non-NAFLD controls with normal SUA levels. SUA levels reported in μmol/L. Levels that were measured in mg/dL were converted to μmol/L by mal by 59.48.

Table 3. Subgroup analysis of the observational studies

Group	Number of cohorts	WMD (95% CI)	I^2 (%)	p^1	p^2 (subgroup difference)
Total	16				
Mean age					
<50	11	32.3 (31.3, 33.3)	98	<0.00001	
≥50	5	59.3 (31.3, 87.3)	99	<0.00001	<0.00001
Gender					
Men	2	26.5 (14.3, 38.7)	85	<0.00001	
Women	3	35.1 (26.7, 43.5)	83	<0.00001	0.25
Study location					
Asian	12	49.7 (33.9, 65.5)	99	<0.00001	
Non-Asian	5	65.6 (42.4, 88.7)	86	0.0002	0.27
BMI					
<25	6	31.3 (30.3, 32.3)	99	<0.00001	
≥25	8	74.4 (71.3, 77.4)	85	<0.00001	<0.00001
Measurement method					
Automated analyzer	10	35.9 (34.9, 36.9)	92.5	<0.00001	
Radioimmunoassay	1	NE	NE	NE	
Enzymatic method	2	77.2 (73.6, 80.8)	92	<0.00001	
Unknown	3	71.7 (57.5, 85.9)	89	<0.00001	

WMD: weighted mean difference.
Stratified and meta-regression analyses of the effects of the study characteristics.
p^1-value tested for heterogeneity of subgroups; p^2-value for the heterogeneity between subgroups in the meta-regression analysis.

multiplying by 59.48.[40] In the articles that used dichotomous data, a total of 23,392 participants were identified with 6,212 incident cases; the participants were from Brazil,[41] Korea,[42,43] and China.[18] The characteristics of the subjects are shown in Table 2.

Meta-analyses

The first 17 articles provided data on continuous SUA levels, and a significant positive relationship was observed between SUA levels and the diagnosis of NAFLD (Figure 1A). We tested the heterogeneity of the 17 observational studies, and the τ^2 statistic was 698 (p<0.00001). Therefore, the pooled estimates were assessed using a random-effects model rather than fixed-effects model. The WMD was 52.3, and the 95% CI ranged from 39.0 to 65.5 (p<0.00001) (Figure 1A).

In the dichotomous data, the τ^2 was 34% (p=0.21) using a fixed-effects model for the meta-analysis. The odds ratio (OR) was 2.10, and the 95% CI was between 1.95 and 2.27 (p<0.00001) (Figure 1B). All observations suggested that serum SUA levels were positively associated with the risk of NAFLD.

Subgroup, sensitivity, and bias analysis

Subgroup analysis was performed to investigate the source of heterogeneity and the factors that affected the pooled estimates. The effects of SUA on the NAFLD in the subgroup meta-analyses are presented in Table 3. The observed effect was significant among studies between subgroups by subject characteristics, such as age and BMI. The difference was not significant between subgroups by study location (p=0.27), and gender (p=0.25). To detect the stability of this meta-analysis for observational studies, single studies were excluded. The exclusion of any individual studies did not markedly change the overall effect (Supplementary figure 2). Visual inspection of Begg's funnel plot showed asymmetry (Figure 2) for the analysis of the continuous data. The exclusion of a single study did not alter the combined results. There might have been publication bias in Begg's funnel plot of the dichotomous data due to the limited number of publications.

Using the ROC curves, SUA levels higher than 308 μmol/L had optimal sensitivity and specificity for determining NAFLD (94.1% [71.3-99.9] and 70.6% [44.0-89.7], respectively). The accuracy was 0.834 at the cut-off value (Figure 3).

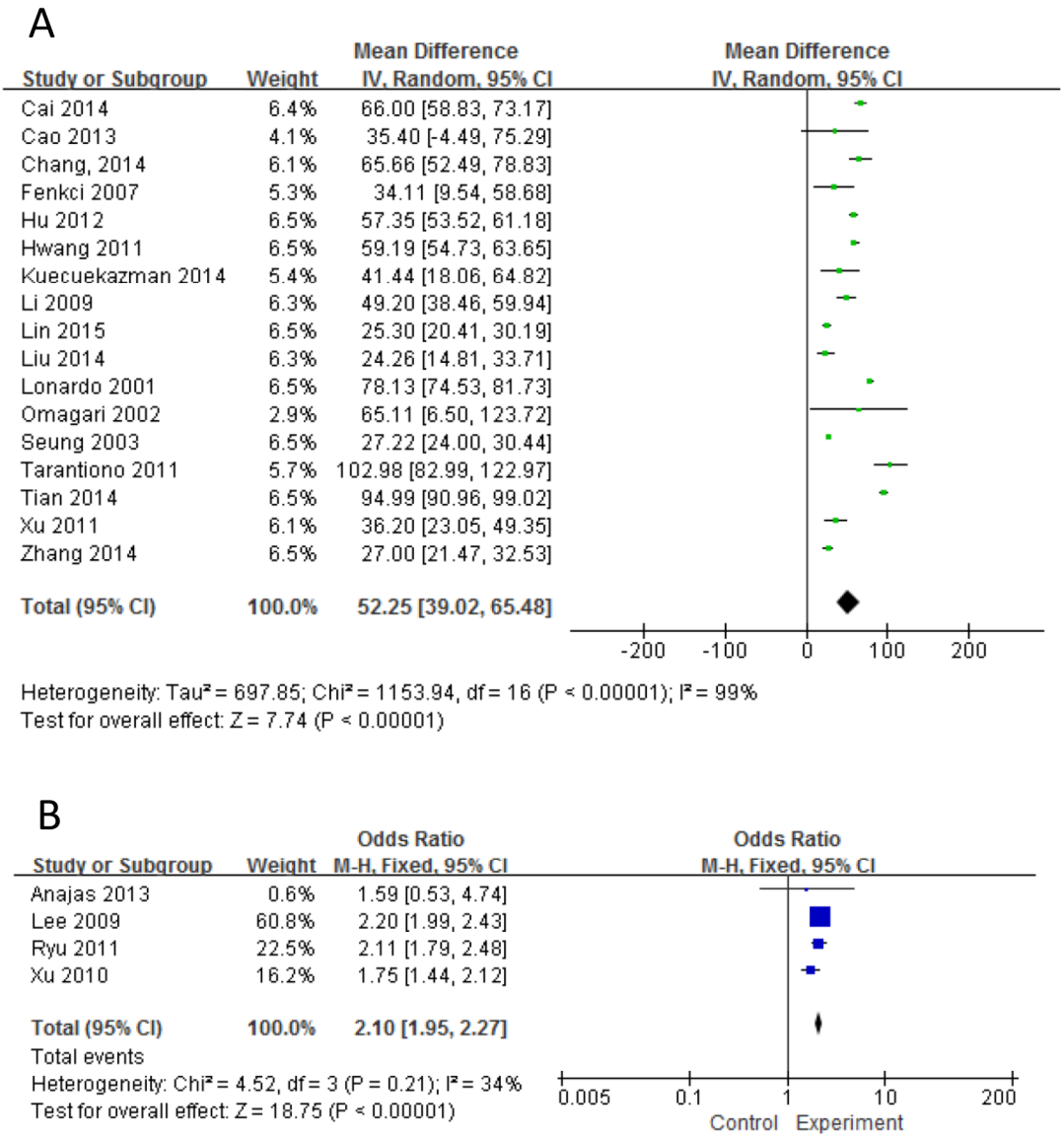

Figure 1. Forest plot of the meta-analysis. (A) Forest plot of the observational studies. IV, Random: Inverse variance heterogeneity random-effects model. Horizontal lines = 95% CI. The size of the data marker corresponds to the weight of that study. The diamond represents the summary estimate. The result favors experimental groups. (B) Forest plot of the dichotomous data. M-H, fixed: Mantel-Haenszel heterogeneity fixed-effects model. Horizontal lines = 95% CI. The size of the data marker corresponds to the weight of that study. The diamond represents the summary estimate. The result favors experimental groups.

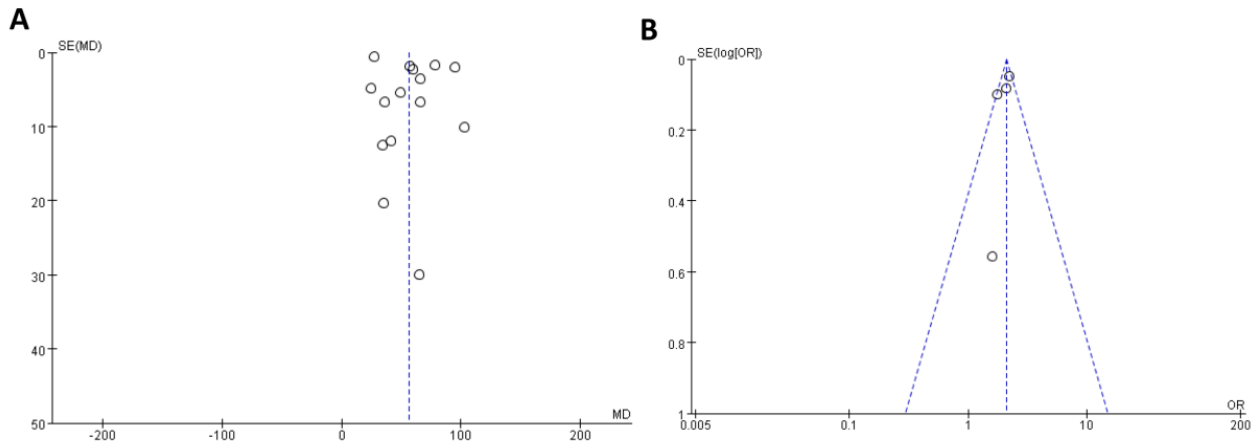

Figure 2. Funnel plots of continuous data (A) and dichotomous data (B). MD: mean difference; OR: odds ratio.

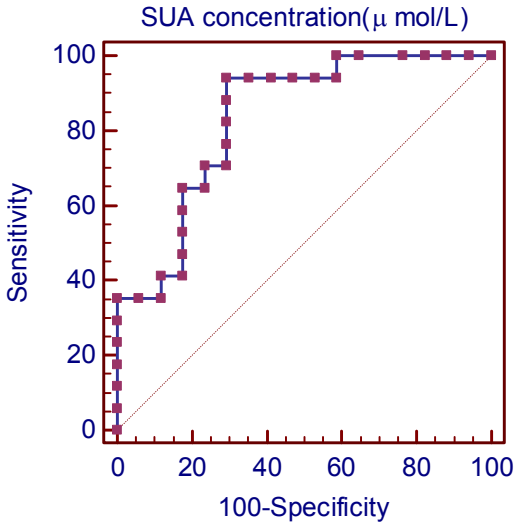

Figure 3. Receiver operating characteristic curves.

DISCUSSION

In this systemic review of 12,374 NAFLD cases and 23,562 controls with continuous data and 23,392 participants and 6,212 incident cases with dichotomous data, we observed a significant positive relationship between SUA levels and the diagnosis of NAFLD. Our results suggested that SUA is upregulated in patients with NAFLD and might be associated with the development of NAFLD.

NAFLD is closely linked to nutrition, including obesity, possibly high-fructose corn syrup consumption and consumption of certain types of fats.[44] Recently, the prevalence of NAFLD has been increasing, and it has become one of the most prevalent forms of chronic liver disease in the world. In many patients, NAFLD is linked with metabolic syndrome, consisting of visceral obesity, insulin resistance, type II diabetes and dyslipidemia.[45]

Hyperuricemia has been associated with NAFLD. Liu et al considered that elevated SUA might be a strong predictor of the development of NAFLD.[25] They observed that serum uric acid levels were positively and independently associated with the presence of hepatic steatosis in Chinese postmenopausal women with normal BMI. Hwang et al reported that increased SUA levels, even within the normal range, were independently associated with the presence of NAFLD. This conclusion was consistent with our finding that the SUA cut-off level for NAFLD was 308 μmol/L. Petta et al observed that in NAFLD patients, hyperuricemia was independently associated with the severity of liver damage and might be a potential new therapeutic target in future intervention trials.[46] Sirota et al reported that serum SUA levels were higher in patients in the United States with ultrasound-diagnosed NAFLD but without diabetes.[20] Moreover, a prospective study from China indicated that the prevalence of NAFLD was 11.8% over 3 years and was positively associated with increased SUA levels.[18] Lee et al assessed the relationship between hyperuricemia and NAFLD by comparing the incidence rates of NAFLD in relation to SUA levels over a 5-year period.[47] Their data indicated that high SUA levels appear to be associated with an increased risk of NAFLD.

Recent experimental evidence has suggested that uric acid may have a causative role in the pathogenesis of NAFLD, which can lead to liver cirrhosis or even hepatoma. Therefore, it is important to investigate the relationship between SUA and NAFLD. Uric acid is responsible for clearing free radicals from the body and acts as an antioxidant in the cardiovascular system.[48] SUA levels and NAFLD are associated in that most patients with NAFLD also have insulin resistance,[28] which increases the synthesis of uric acid.[49] Moreover, uric acid excretion is also lower in insulin-resistant patients.[50] Choi Y et al demonstrated that uric acid promoted hepatic fat synthesis by activating SREBP-1c under endoplasmic reticulum stress[51] and that severe hepatic steatosis could be induced by injecting uric acid into ob/ob mice. Moreover, the impaired oxidation process might be related to the pathogenesis of uric acid in the development of NAFLD.[52] The association between SUA level and NAFLD suggested that SUA could play an important role in developing NAFLD that might have revealed the impaired oxidative function of the liver in NAFLD as well as a compensatory mechanism against that increased oxidative stress.[53]

To our knowledge, this is the first meta-analysis to investigate the association of SUA levels with NAFLD. Although the relationship between SUA levels and metabolic syndrome has been widely discussed.[54,55] The information regarding NAFLD has been rather limited in the different analyses. Our search method had no language or date restrictions, and by including EMBASE, we also incorporated grey literature that has been accepted for scientific meetings, which added strength to our study. However, this meta-analysis does have limitations. First, the method of diagnosing NAFLD varied across studies; in most studies, it was identified by ultrasonography, not liver biopsy. Because liver biopsy is the gold standard in NALFLD diagnosis, variations in the diagnostic methods used might have led to measurement error and caused underestimation of the association between SUA and NAFLD.[56] Second, the methods for measuring SUA levels varied across studies, which likely contributed to the heterogeneity in our analysis. Third, the normal range of SUA levels remains a controversial area of research in that the cut-off values varied across studies. Moreover, the SUA levels between genders also led to errors in determining a normal SUA range, especially in mixed-gender cohort studies. Therefore, our data were markedly heterogeneous for all comparisons.

In conclusion, we have demonstrated that increased SUA levels were prevalent in NAFLD subjects, suggesting that SUA might play a role in NAFLD development. Future research should focus on investigating the effect of SUA on the pathogenesis of NAFLD and on exploring the new diagnostic and treatment strategies for NAFLD.

AUTHOR DISCLOSURES

The authors have declared that no competing interests exist.

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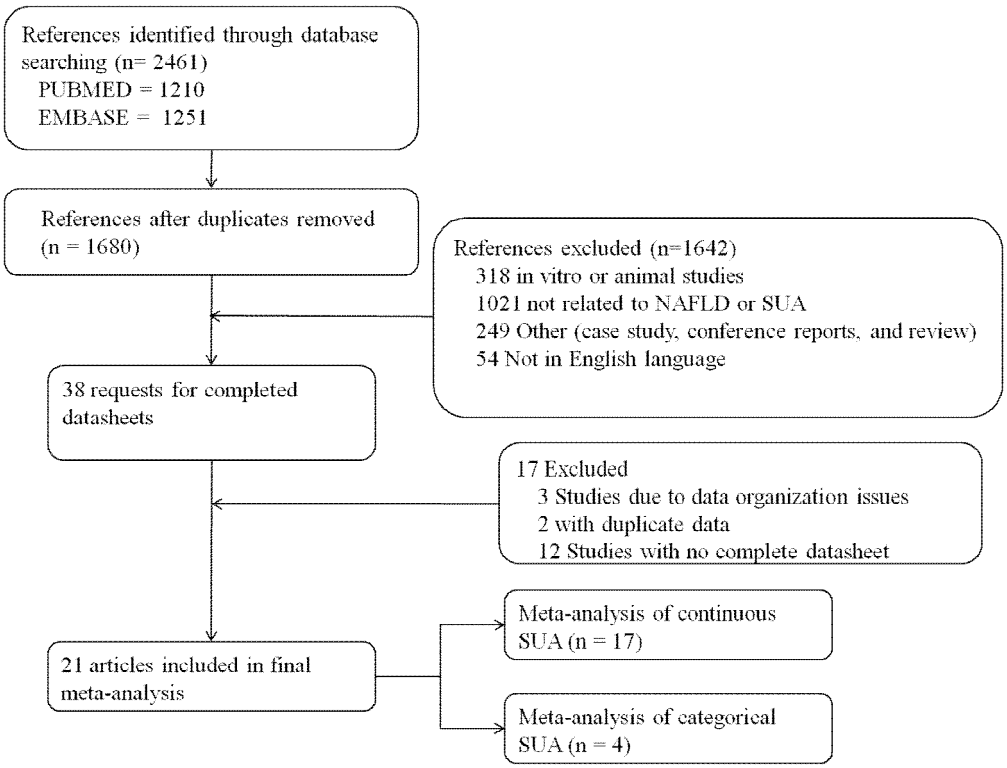
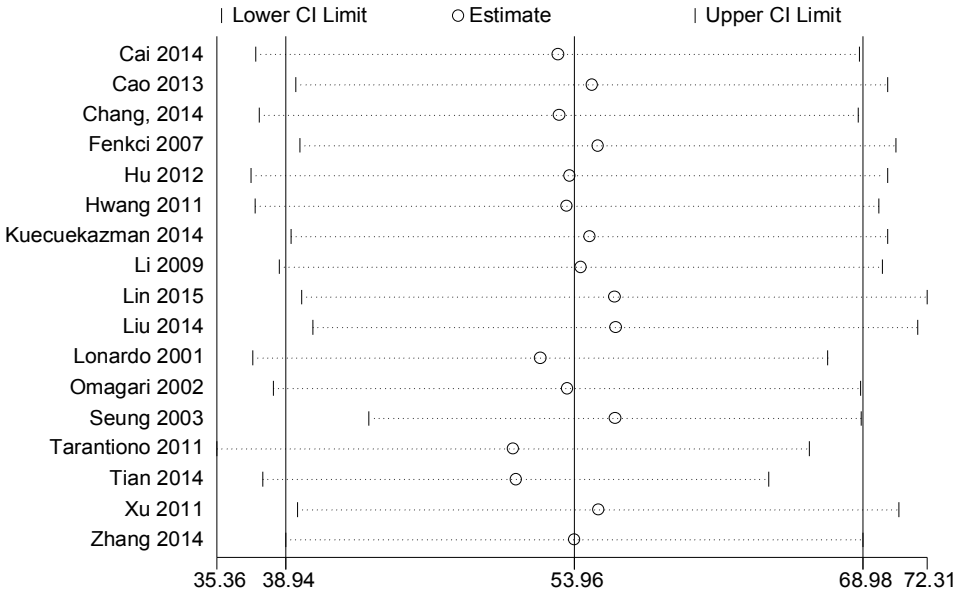
Supplementary table 1. Search Engine up to Nov 2, 2015

PUBMED	(steatosis [All Fields] OR (("fatty liver" [MeSH Terms] OR ("fatty" [All Fields] AND "liver" [All Fields]) OR "fatty liver" [All Fields] OR "steatohepatitis" [All Fields]) AND ("Non-alcoholic Fatty Liver Disease" [Supplementary Concept] OR "Non-alcoholic Fatty Liver Disease" [All Fields] OR "nafld" [All Fields])) OR NASH [All Fields] OR ("fatty liver" [MeSH Terms] OR ("fatty" [All Fields] AND "liver" [All Fields]) OR "fatty liver" [All Fields]) OR "bright liver" [All Fields] OR "transaminases" [All Fields] OR ALT[All Fields] OR ("Proc Int Workshop Autom Softw Test" [Journal] OR "ast" [All Fields]) OR "liver enzymes" [All Fields]) AND ("serum uric acid" [Mesh] OR ("serum uric acid" [MeSH Terms] OR "serum uric acid" [All Fields] OR "SUA" [MeSH Terms] OR "SUA" [All Fields]))
EMBASE	'steatosis'/exp OR steatosis OR 'fatty liver'/exp OR 'fatty liver' OR nafld OR nash OR steatohepatitis OR 'liver enzymes' OR 'transaminase' OR 'alt' OR 'bright liver' AND ('serum uric acid'/exp OR 'serum uric acid' OR 'SUA') AND [embase]/lim



Supplementary figure 1. Flow diagram of the study.



Supplementary figure 2. Sensitivity analysis of the observational studies.