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Open Access Systematic Review

Effects of Acetylsalicylic Acid on Non-Alcoholic Fatty Liver Disease – Systematic Review

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***Abstract*-- Background:** Several studies have assessed the effects of acetylsalicylic acid (ASA), a widely used drug worldwide, in non-alcoholic fatty liver disease (NAFLD) patients. In this systematic review, we aim to evaluate the effect of ASA use on the prevalence of NAFLD, as well as hepatic steatosis and fibrosis in NAFLD patients. **Methods:** We performed a systematic search on PubMed and Embase electronic databases with predefined keywords searching for observational and experimental studies published from inception and till 19 July 2019. The diagnosis of NAFLD was based on histology, imaging or surrogate markers. Eligible articles based on inclusion and exclusion criteria were extracted and included in the qualitative assessment using the National Heart, Lung, and Blood Institute (NHLBI) quality assessment tools. **Results:** A total of seven observational studies (5 cross-sectional and 2 cohort) were included involving 18,209 subjects. Three studies evaluated the prevalence of NAFLD in subjects receiving ASA, out of which two studies demonstrated a lower prevalence rate in subjects using ASA for at least ≥ 15 times per month. On the other hand, only one study demonstrated no prevalence decrease in NAFLD with the use of ASA. Moreover, hepatic steatosis and fibrosis was evaluated in four studies with histological confirmation of NAFLD. Two of these studies reported a reduced severity of hepatic steatosis with ASA use. On the other hand, a study demonstrated that a less severe hepatic steatosis but not histological improvement was associated with ASA use. Furthermore, another study reported that ASA use in type 2 diabetic patients wasn't associated with protective effects against advanced hepatic fibrosis. Statistical pooling of included studies wasn't performed due to heterogeneity of the study designs. **Conclusions:** Although most studies demonstrated potential protective effects of ASA use in hepatic steatosis and fibrosis, as well as a reduced prevalence of NAFLD, results from the current literature remain inconsistent with the quality of most studies being rated as fair or poor. Therefore, clear conclusions and recommendations can't be drawn from the current studies in the literature evaluating this association. Further experimental studies are required to confirm the potential protective effects associated with ASA use on NAFLD.

Keywords: Non-alcoholic fatty liver disease, NAFLD, hepatic steatosis, hepatic fibrosis, Acetylsalicylic Acid, Aspirin

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the Western countries [1] with a rapid increase in the worldwide prevalence. The estimated global prevalence of NAFLD in adults ranges between 10%-40% [2]. The highest prevalence of NAFLD was reported to be in the Middle East and South America and lowest being in Africa [3].

NAFLD is defined by the presence of hepatic steatosis identified by either imaging or histology in the absence of other secondary causes of hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders [4, 5]. A "multiple hit" concept is being described in NAFLD, involving insulin resistance (IR) that leads to the first hit being described as reversible liver fat deposition, causing consecutive hits associated with oxidative stress, lipid peroxidation and proinflammatory cytokines release. This can ultimately promote hepatic inflammation and fibrosis leading to non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma [6-9].

One of the important factors determining hepatic and cardiovascular morbidity and mortality, as well as cancers, is the staging of liver fibrosis. Therefore, management strategies addressing hepatic fibrosis is of great significance as they can prevent or reduce the progression of hepatic fibrosis [6]. Currently, there are no pharmacotherapies approved as anti-fibrotic drugs for NAFLD. Consequently, several studies are evaluating several therapies for the prevention or reduction of hepatic fibrosis progression.

Lately, several studies evaluated and discussed the role of platelets in hepatic steatosis and fibrosis [10, 11]. Several animal studies demonstrated that antiplatelet drugs, including acetylsalicylic acid, a commonly used drug worldwide mainly for the secondary prevention of atherosclerotic cardiovascular disease, exert beneficial hepatic effects by decreasing hepatic inflammation, oxidative stress and insulin resistance, thereby potentially preventing or reducing hepatic fibrosis progression in NAFLD [12-16].

Accordingly, we performed a systematic literature search with the aim of studying the effects of acetylsalicylic acid use

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on the prevalence of NAFLD, as well as the exerted effects on hepatic steatosis and fibrosis in NAFLD patients reported in experimental and observational human studies. To the best of our knowledge, this is the first systematic review reporting evidence from studies evaluating the effects of acetylsalicylic acid in NAFLD patients.

2. METHODS

2.1 Data Sources and Search Strategy

We conducted a computerized search using the electronic databases of PubMed and Embase. The search was conducted using the following keywords including nonalcoholic fatty liver disease AND acetylsalicylic acid. To prevent missing relevant articles, we performed a hand-search of the bibliographies of relevant articles. We conducted the literature search with no restrictions, searching for studies in the databases from inception till the 19th of July 2019. We screened the titles and abstracts for appropriateness and to avoid duplication. Moreover, we reviewed the available full text of the selected articles that fulfilled the inclusion and exclusion criteria. Studies that fulfilled the inclusion and exclusion criteria through assessing the abstract but had no available full text were also included in this qualitative assessment. An independent extraction for the data from eligible studies was performed by 2 reviewers (A.I and N.A). Any discrepancies in extracted data were resolved by mutual consensus. Data that was extracted from the eligible studies included authors names, publication year, country of origin, design of the study, total sample size, mean age, detection method for diagnosing NAFLD and evaluating hepatic fibrosis, gender ratio, percentage of NAFLD from the study population, odds of hepatic fibrosis with acetylsalicylic use, reported adverse events if mentioned as well as the main study results. These data were further collected and arranged into a table. Final data was then reported in the text of the review article.

2.2 Eligibility Criteria

Inclusion criteria of original articles were as follows (1) Experimental or observational cohort population-based/ hospital-based, cross-sectional or case-control studies, including studies that were presented as abstract and aren't yet fully published, that evaluated the effects of acetylsalicylic acid in NAFLD patients; (2) NAFLD and hepatic fibrosis definitions and measurements evaluated by methods such as imaging technique, biopsy and histopathology, or commonly accepted surrogate markers of NAFLD, including fatty liver index (FLI) which is derived from an equation of anthropometric variables, serum triglyceride and glucose, γ-glutamyl transpeptidase (GGT) concentrations, in addition to commonly accepted serum markers such as composite INR, platelet, transaminase, albumin levels; (3) The absence of other clear secondary cause of hepatic steatosis such as significant alcohol consumption based on the defining assessment in each study or other causes of chronic liver disease; (4) Adult participants aged ≥18 years without any restrictions related to gender, race or ethnicity; (5) Human studies only (6) Studies published in English, German or Romanian languages.

Exclusion criteria included the following: (1) Editorials, letters to the editor, case reports, literature and systematic reviews, practice guidelines, commentaries; (2) Secondary causes of hepatic steatosis such as significant alcohol consumption, viral infections such as hepatitis C, Wilson's Disease, lipodystrophy, starvation, parenteral nutrition, abetalipoproteinemia, medication induced (amiodarone, methotrexate, tamoxifen, corticosteroids, mipomersen, lomitapide, valproate, antiretroviral drugs), Reye's syndrome, acute fatty liver of pregnancy, HELLP syndrome, metabolic disorders or other known causes of CLD; (3) Subjects with confirmed cirrhosis of any etiology or subjects with end -stage liver disease that are awaiting liver transplantation; (4) Studies evaluating other antiplatelet agents other than acetylsalicylic acid; (5) Studies that didn't describe our primary outcome; (6) Studies performed on animals; and (7) Studies published in languages other than English, German or Romanian languages.

2.3 Quality assessment

The studies' quality was evaluated using the quality assessment tools from the National Heart, Lung, and Blood Institute (NHLBI). One tool was used for observational cohort and cross- sectional studies. This assessment tool was used to evaluate the risk of bias and the internal validity of all included studies in a similar manner. Two authors (A.I and N.A) applied these tools independently by evaluating the items included in the assessment as "yes", "no", "not applicable", "cannot determine", or "not reported". The quality assessment of the included studies was rated after completing the evaluation tool as "good", "fair", or "poor". When disagreement was present between the evaluation of the two authors, a consensus was reached through a discussion. Eligibility of studies wasn't affected by the results of the methodological quality assessment.

3. RESULTS

3.1 General Results

Figure 1 demonstrates the PRISMA flow diagram with the conducted search strategy of our systematic review. The initial search yielded two hundred and forty-one articles, out of which two hundred and twelve were from Embase and twenty-nine from PubMed. Twenty-two articles were identified as duplicates and excluded. Screening of all remaining articles for eligibility was performed by assessing the title and abstract. The results of the two hundred and nineteen screened articles were as follows: (1) one hundred and eight reviews (literature reviews n=107, systematic reviews and meta-analysis n=1), (2) eight studies performed on animals, (3) ten case reports, (4) seventeen editorials, (5) one guidelines, (6) eleven letters to the editor, (7) one article withdrawn because of duplicate publication, (8) fifty five other irrelevant studies to this review topic, (9) eight article abstracts met the primary criteria. The total excluded studies in the first screening were

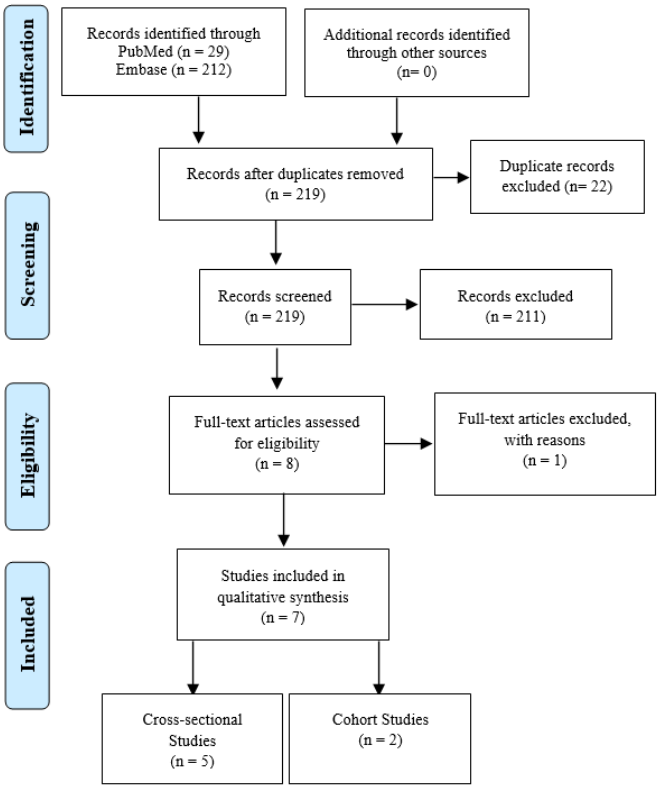

Figure 1. The PRISMA flow diagram for the search and selection processes of this systematic review.

two hundred and eleven studies. The remaining eight articles were further evaluated and their full text was assessed. One article was further excluded due to the presence of secondary hepatic steatosis with alcoholic and viral etiologies [17]. The total number of articles included in this systematic review are seven articles [18-24].

Table 1 summarizes the main characteristics of the included studies in this systematic review. A total number of 18,209 subjects were included in the study population of this systematic review, divided as 17,477 individuals in cross-sectional studies and 732 individuals in prospective cohort studies. The review included five cross-sectional studies and two prospective cohort studies. Six studies were undertaken in USA and one in Moldova.

3.2 Non-alcoholic Fatty Liver Disease Definition

The current gold standard for diagnosing NAFLD remains to be histological examination through liver biopsy. Nevertheless, several non-invasive imagistic investigations are commonly used to evaluate hepatic steatosis such as ultrasonography, CT scan and MRI. Liver stiffness grading can be assessed using transient elastography, in addition to multiple surrogate markers that have been lately used to calculate scores evaluating hepatic fibrosis grading and severity [4]. Histological diagnosis of NAFLD though liver biopsy is considered when 5% or more of the liver cells contain fat. It is considered severe when 30% or more of liver cells contain [25, 26]. On the other hand, diagnosing NAFLD through abdominal ultrasonography is generally considered according to the assessment of the liver echogenicity contrast compared with the right kidney and the diaphragm [27].

In this systematic review, most studies (n=4) diagnosed NAFLD using the gold standard investigation, liver biopsy [18-20, 23]. Moreover, two studies used ultrasonography (n=2) [22, 24] and only one study used surrogate markers by the United States Fatty Liver Index (U.S FLI) (n=1) [21].

3.3 Acetylsalicylic Acid Effects on NAFLD

Several studies assessed the effects of acetylsalicylic acid on the prevalence of NAFLD, hepatic steatosis histological features and hepatic fibrosis severity.

Two prospective cohort studies and two cross-sectional studies evaluated the histological presence of NAFLD through liver biopsy. Mohamed B et al. conducted a cross-sectional study on 186 subjects with biopsy confirmed NAFLD, suggesting that aspirin use may be associated with an improved hepatic steatosis severity, but without other significant improvements of other histological features [23]. Furthermore, Singh A et al. conducted a cross-sectional study on 592 individuals with type 2 diabetes mellitus and biopsy proven NAFLD, concluding that aspirin wasn't associated with protective effects against advanced liver fibrosis in diabetic patients [19]. On the other hand, Simon TG et al. performed a prospective cohort study involving 371 patients with biopsy proven NAFLD and assessed hepatic fibrosis severity using several surrogate markers and scores including Fibrosis-4 (FIB-4), NAFLD Fibrosis Score (NFS) and Aspartate-Aminotransferase-to-Platelet Ratio Index (APRI) [20]. They demonstrated that NAFLD patients who received daily aspirin were associated with a duration-dependent reduction in the risk for developing advanced hepatic fibrosis, mostly seen patients with ≥4 years of aspirin use. Moreover, Simon TG et al. conducted a more recent prospective cohort study on 361 NAFLD patients with histological confirmation, demonstrating that daily aspirin use was found to be associated with better histological features of NAFLD and NASH, in addition to a decreased risk for advanced liver fibrosis progression with time [18].

Two cross-sectional studies assessed hepatic steatosis and diagnosed NAFLD using ultrasonography. Shen H et al. conducted a cross-sectional study on 11,416 subjects, out of which 25% of these subjects had NAFLD diagnosed by ultrasonography, suggested that regular aspirin use of ≥15 times per month may be associated with a decreased NAFLD prevalence, mainly in elderly (>60 years) and male subjects [24]. Moreover, Peltec A et al. reported in a cross-sectional study performed on 625 individuals with ultrasonographic diagnosis of NAFLD in 26.7% that patients with arterial hypertension who received aspirin for ≥15 times per month were associated with a lower NAFLD prevalence suggesting a protective effect in the development of NAFLD [22].

On the other hand, one cross-sectional study conducted by Devaki P et al. assessed hepatic steatosis using the U.S FLI in 4,658 subjects with NAFLD prevalence being 69% [21]. They concluded that daily aspirin use wasn't associated with a lower NAFLD prevalence.

3.4 Quality Assessment of Included Studies

In order to assess the risk of bias and methodological quality of included studies, we performed an evaluation of all included studies using the NHLBI quality assessment tool for

Table 1. Studies Assessing the Effects of Acetylsalicylic Acid on Non-Alcoholic Fatty Liver Disease

First Author / Year / Country	Study Design	Study Characteristics	Main Findings
Shen H et al. / 2014 / USA [24]	Cross-sectional	• **Total Subjects:** 11,416 • **NAFLD:** 25% • **Mean age (years): No NAFLD** 48.7±0.2, **NAFLD** 54.6±0.3 • **NAFLD diagnosis:** Ultrasonography • **Gender (males):** Controls: 49.6% ±0.5, NAFLD: 56.0 % ±0.9 • **Odds Ratio:** Multivariate unconditional logistic regression analysis after controlling age, sex, ethnicity, socioeconomic status, body mass index (BMI), physical activity, smoking status, insulin resistance, systolic/diastolic blood pressure, triglycerides, and high-density lipoprotein (HDL), regular aspirin use and lower prevalence of NAFLD (OR 0.62, 95% CI 0.51-0.74). Lower NAFLD prevalence in males with occasional and regular aspirin use (OR 0.58 and 0.32, 95% CI 0.46-0.74 and 0.23-0.45, respectively) and elderly patients over age 60 years (OR 0.74 and 0.21, 95% CI 0.56-0.99 and 0.14- 0.30, respectively).	Rregular aspirin use (≥ 15 times per month) was associated with a lower prevalence of NAFLD, primarily among males and elderly patients > 60 years. No significant association was found among females or younger patients.
Peltec A et al. / 2015 / Moldova [22]	Cross-sectional	• **Total Subjects:** 625 • **NAFLD:** 26.72% • **Mean age (years):** 48.15±10.5 • **NAFLD diagnosis:** Ultrasonography • **Gender (males):** 72 % • **Odds Ratio:** Multivariate unconditional logistic regression analysis, regular relative to no aspirin use and prevalence of NAFLD (OR 0.23, 95% CI 0.12–0.42; $p < 0.01$).	Regular aspirin use (≥15 times per month) was associated with a lower NAFLD prevalence in arterial hypertentive patients suggesting a protective effect in the development of NAFLD.
Mohamad B et al. / 2015 / USA [23]	Cross-sectional	• **Total Subjects:** 186 subjects • **NAFLD:** 100% • **Mean age (years):** Aspirin use: 55.6, No Aspirin use: 50.1 • **NAFLD diagnosis:** Liver biopsy	Despite the less severe hepatic steatosis that was associated with daily aspirin use in NAFLD patients, no significant histological benefit was demonstrated.
Devaki P et al. / 2017 / USA [21]	Cross-sectional	• **Total Subjects:** 4,658 • **NAFLD:** 30% • **Mean age (years):** NAFLD: 53, Controls: 48 • **NAFLD diagnosis:** U.S FLI • **Gender (males):** NAFLD patients 69% • **Odds Ratio:** Multi-variate logistic regression analysis with Aspirin use (OR 1.31, 95% CI 0.88-1.97, p=0.19).	In a large general US population study, aspirin use was not associated with decreased prevalence of NAFLD.
Simon TG et al. / 2018 / USA [20]	Prospective cohort study	• **Total Subjects:** 371 • **NAFLD:** 100% • **NAFLD diagnosis:** Liver biopsy • **Hepatic Fibrosis Assessment**: FIB4, NFS, and APRI. • **Odds Ratio:** NASH in Aspirin users (aOR 0.68, 95% CI 0.37-0.89) and fibrosis (aOR 0.54, 95% CI 0.30-0.80). Aspirin use and risk of developing advanced hepatic fibrosis after accounting for baseline fibrosis stage and known risk factors for fibrosis progression (aHR 0.60, 95% CI 0.43-0.85). Duration dependent (P-trend=0.024), ≥4 years of Aspirin use (aHR 0.56, 95% CI 0.38-0.78).	A duration-dependent reduced risk for progression to advanced fibrosis was demonstrated in NAFLD patients who received daily aspirin for at least 4 years.
Singh A et al. / 2018 / USA [19]	Cross-sectional	• **Total Subjects:** 592 • **NAFLD:** 100% • **Mean age (years):** 52.2 ± 11.7 years • **NAFLD diagnosis:** Liver biopsy • **Gender (males):** 37.3% • **Odds Ratio:** Unadjusted OR for association of aspirin use with advanced hepatic fibrosis (OR 1.04, 95% CI 0.71-1.5) (p = 0.83) and after adjusting for age, gender, race and body mass index (OR 0.77, 95% CI 0.51-1.2) (p = 0.22).	In type 2 diabetic patients with NAFLD, aspirin use wasn't not protective against advanced liver fibrosis.
Simon TG et al. / 2019 / USA [18]	Prospective cohort study	• **Total Subjects:** 361 • **NAFLD:** 100% • **NAFLD diagnosis:** Liver biopsy • **Odds Ratio:** Daily aspirin and NASH (aOR, 0.68; 95% CI, 0.37-0.89), hepatic fibrosis (aOR, 0.54; 95% CI, 0.31-0.82). Daily aspirin and incident advanced fibrosis (aHR, 0.63; 95% CI. 0.43-0.85). Duration dependent (adjusted P trend=0.026), at least 4 years or more of aspirin use (aHR, 0.50; 95% CI, 0.35-0.73).	Less severe histological features of NAFLD and NASH, as well as a reduced risk for advanced fibrosis progression with time was reported in biopsy-proven NAFLD patients who received aspirin daily.

aHR – **Adjusted hazard ratio;** aOR – **Adjusted Odds Ratio;** APRI – **Aspartate-Aminotransferase-to-Platelet Ratio Index;** CI – **Confidence interval;** FIB-4 – **Fibrosis-4;** NAFLD – **Non-alcoholic fatty liver disease;** NASH – **Non-alcoholic steatohepatitis;** NFS – **NAFLD Fibrosis Score;** OR – **Odds ratio;** U.S FLI – **United States Fatty Liver Index;** US – **United States.**

observational cohort and cross-sectional studies as demonstrated in Table 2. Quality was described as "good", "fair, or "poor". Only one study had an overall rating of "good" [24], while three studies were reported as "fair" [18, 19, 21], and the remaining three studies were rated as "poor" [20, 22, 23]. All studies had a clearly stated research question or objective and all but one study measured key potential confounding variables and performed statistical adjustments [23].

Three studies evaluated the prevalence of NAFLD in subjects receiving acetylsalicylic acid, out of which two studies with NAFLD assessed by ultrasonography, one rated as "good" [24] and one involving hypertensive patients that was rated as "poor" [22] demonstrated a lower prevalence rate of NAFLD in subjects using acetylsalicylic acid for at least ≥ 15 times per month. On the other hand, only one study that was rated as "fair" demonstrated no decrease in NAFLD prevalence assessed by U.S FLI [21].

Moreover, hepatic steatosis and fibrosis was evaluated in four studies with histological confirmation of NAFLD. Two of these studies reported a reduced severity of hepatic steatosis assessed histologically with acetylsalicylic acid use, one study being rated as "poor" [20] and one as "fair" [18]. On the other hand, a study rated as "poor" demonstrated that a less severe hepatic steatosis but not histological improvement was associated with acetylsalicylic use [23]. Furthermore, another study that was rated as "fair" reported that acetylsalicylic acid use in type 2 diabetic patients wasn't associated with

Table 2. NHLBI Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies

Criteria	Shen H et al. [24]	Peltec A et al. [22]	Mohamad B et al. [23]	Devaki P et al. [21]	Simon TG et al. [20]	Singh A et al. [19]	Simon TG et al. [18]
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	No	No	Yes	No	No	No
3. Was the participation rate of eligible persons at least 50%?	Yes	CD	CD	No	CD	Yes	CD
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	CD	CD	Yes	CD	CD	CD
5. Was a sample size justification, power description, or variance and effect estimates provided?	Yes	No	Yes	Yes	No	No	No
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	No	No	No	No	CD	No	No
7. Was the time frame sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	No	No	No	No	Yes	No	Yes
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Yes	Yes	Yes	No	No	Yes	Yes
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	No	No	No	No	Yes	No	Yes
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NR	NR	NR	NR	Yes	NR	Yes
13. Was loss to follow-up after baseline 20% or less?	NA	NA	NA	NA	NR	NA	NR
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	No	Yes	Yes	Yes	Yes
Rating	Good	Poor	Poor	Fair	Poor	Fair	Fair

Available at: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools. CD, cannot determine; NA, not applicable; NR, not reported.

protective effects against advanced hepatic fibrosis [19].

4. Discussion

Currently, to the best of our knowledge, this systematic review is the first to evaluate the effects of acetylsalicylic acid on NAFLD prevalence, as well as hepatic steatosis and fibrosis. Our systematic review included a total of seven studies (five cross-sectional studies and two prospective cohort studies) with a study population of approximately 18,000 individuals and demonstrated that the effects of acetylsalicylic acid on hepatic steatosis and fibrosis in NAFLD patients remains inconsistent in the current literature with further required experimental studies to confirm the suggested benefits demonstrated in the current observational studies.

Our systematic review reported several findings that need to be further discussed. Firstly, we observed that most of the studies evaluating the effects of acetylsalicylic acid in NAFLD patients in the current literature were of cross-sectional design, while only two were prospective cohort studies. This limits the ability to demonstrate and conclude that a clear protective hepatic effect was obtained through the administration and use of acetylsalicylic acid. Randomized controlled clinical trials are required in order to evaluate and establish that these protective effects reported in several observational studies are clearly due to acetylsalicylic use in NAFLD patients.

Secondly, we observed that most studies used liver biopsy and histology for diagnosing NAFLD, which is the current gold standard, being the best method of assessment for hepatic steatosis and fibrosis. On the other hand, a few studies used ultrasonography, which is the most commonly used imagistic assessment for evaluating hepatic steatosis. Ultrasonography was demonstrated to be a reliable method for evaluating hepatic steatosis with a sensitivity of 84.8 % and specificity of 93.6% [28]. Moreover, evaluating hepatic steatosis using liver enzymes solely remains debatable due to possibly normal results in up to 70% of the subjects [29]. The second largest study included in this systematic review used U.S FLI to evaluate hepatic steatosis.

Thirdly, the effect of acetylsalicylic acid on the prevalence of NAFLD was discussed in three studies, out of which two studies that used ultrasonography for diagnosing NAFLD reported a lower prevalence associated with acetylsalicylic acid use, although one of these studies was performed on hypertensive patients which limits generalizability of the results [22, 24] and one study used FLI for assessing hepatic steatosis reporting no significant association [21]. None of the studies evaluating the effects of acetylsalicylic acid on the prevalence of NAFLD used histology for assessing hepatic steatosis which possibly means that patients with hepatic steatosis might have been missed.

Fourthly, hepatic steatosis and fibrosis were evaluated in subjects who received acetylsalicylic acid in a total of four studies with biopsy-proven NAFLD, out of which two prospective studies reported that daily aspirin use was associated with less severe histologic features of NAFLD and NASH, in addition to a duration-dependent reduction in risk

for developing advanced fibrosis [18, 20]. On the other hand, a cross-sectional study with no statistical adjustments for confounding factors concluded that despite the less severe hepatic steatosis that was found in patients using aspirin daily, no significant histological findings were present [23]. Furthermore, a cross-sectional study with type 2 diabetic patients with NAFLD concluded that aspirin use wasn't effective to protect against advanced hepatic fibrosis [19]. This limits the generalizability of the results on the general populations as the study was conducted on diabetic patients, while suggesting that diabetic patients might not have the protective effects associated with hepatic steatosis and fibrosis of acetylsalicylic acid that other studies demonstrated in non-diabetic patients.

Fifthly, quality assessment evaluating the risk of bias and methodology of included studies demonstrated that most studies in the current literature evaluating the effects of acetylsalicylic acid on hepatic steatosis and fibrosis in NAFLD are of "fair" and "poor" quality. This means that results of these studies should be interpreted with caution due to increased risk of bias and methodological flaws.

Sixthly, none of the included studies mentioned or described the indications for which the patients received acetylsalicylic acid.

Our systematic review included several potential limitations which should be mentioned. First, all included studies in this review are observational studies, mostly in abstract form that aren't yet fully published as the current literature is scarce in full articles assessing this topic. No experimental studies are currently available, which limits the possibility of confirming the direct effects of acetylsalicylic acid use in NAFLD patients. Second, we didn't exclude studies that used methods other than histology for confirming NAFLD due to the limited literature studying this topic, which will lead to fewer included studies and a smaller population size with ungeneralizable and less significant results. Third, most included studies are of either fair or poor quality which prevents us from clearly being able to evaluate the effects of acetylsalicylic acid on NAFLD with no risk of bias. Fourth, assessing the included studies through a meta-analysis wasn't conducted. This was mainly due to heterogeneity of the included study designs as studies had different inclusion and exclusion criteria, as well as different diagnostic methods used to assess hepatic steatosis, which doesn't allow a proper statistical analysis to be performed.

Nevertheless, our study also presents several important strengths. Despite the fact that NAFLD is a rapidly growing disease worldwide, no current approved pharmacotherapies for treating NAFLD are available. We believe that this review outlines and summarizes the current literature, as well as points out the missing data that are required to be evaluated in further future studies in relation to the effects of acetylsalicylic acid, a widely used drug worldwide, on hepatic steatosis and fibrosis as well as the prevalence of NAFLD. This systematic review provides the first assessment to the best of our knowledge evaluating the effects of acetylsalicylic acid on NAFLD and demonstrating the heterogeneity of the current published studies.

5. Conclusions and Future Directions

Although most observational studies conducted till the moment demonstrated potential protective effects of acetylsalicylic acid use on hepatic steatosis, fibrosis and the prevalence of NAFLD, results from the current literature remain inconsistent. Moreover, the risk of bias assessment that was conducted demonstrated that only one study was rated as of good quality, while the rest being rated as either fair or poor. Therefore, clear conclusions and recommendations can't be drawn from the current studies in the literature evaluating this association.

In conclusion, acetylsalicylic acid might play a protective role in NAFLD, a rapidly growing disease associated with increasing morbidity and mortality rates with no current approved pharmacotherapies. Further high-quality studies of experimental design are needed in order to confirm the currently reported potential protective effects associated with acetylsalicylic use on NAFLD in order to limit and suppress the expeditious growth of this disease.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions: AI had the study idea, suggested methodology, performed the literature search, data extraction, helped with drafting the tables and wrote the manuscript. NA performed the literature search, data extraction, drafted the tables and contributed to the writing of the manuscript. All authors approved the final version of the manuscript.

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