

Plasminogen Activator Inhibitor-1 Levels in Non-alcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis

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ABSTRACT

Background & Aims: Several studies have investigated the role of multiple proteins in nonalcoholic fatty liver disease (NAFLD); one that has recently gained attention is plasminogen activator inhibitor-1 (PAI-1). However, studies evaluating PAI-1 levels in NAFLD demonstrated conflicting results. Our objective was to understand the role of PAI-1 in NAFLD more clearly by carrying out a systematic review and meta-analysis.

Methods: We gathered evidence by performing a systematic search on PubMed, EMBASE, and Cochrane Library, through using a predefined search string. The included studies diagnosed NAFLD through either liver biopsy, ultrasonography, computed tomography (CT), magnetic resonance spectroscopy, or using one of the latter methods with blood parameters. Studies had to fulfill predefined inclusion and exclusion criteria. To assess the quality of the studies included, we used the NHLBI quality assessment tools. The main summary outcome was the mean difference (MD) in serum PAI-1 levels reported as ng/mL

Results: 33 articles involving 10,840 subjects fulfilled our inclusion criteria and were systematically reviewed. 11 studies were included in our meta-analyses. We found a significant MD in PAI-1 levels in NAFLD patients vs. controls [17.147 (95%CI: 7.720–26.574)]. Moreover, subgroup analysis evaluating PAI-1 levels in biopsy-proven NAFLD vs. controls remained significant [24.086 (95%CI: 3.812–44.361)], as well as in CT-diagnosed NAFLD [15.523 (95%CI: 7.163–23.883)]. However, no significant MD in PAI-1 levels was found in ultrasound-diagnosed NAFLD patients vs. controls [10.394 (95%CI: -13.335–34.123)]. No significant MD in PAI-1 levels in NASH patients vs. controls was observed [26.835 (95%CI: -0.879–54.549)].

Conclusions: In summary, elevated serum PAI-1 levels are associated with adult NAFLD (biopsy-proven and CT-diagnosed). However, no significant difference was found in ultrasound-diagnosed NAFLD and NASH patients. Nonetheless, the included studies have methodological variance, dictating that the obtained results should be carefully interpreted.

Key words: non-alcoholic fatty liver disease – non-alcoholic steatohepatitis – plasminogen activator inhibitor-1 – systematic review - meta-analysis.

Abbreviations: aPAI-1: activated plasminogen activator inhibitor-1; CI: confidence interval; CT: computed tomography; MD: mean difference; MRS: magnetic resonance spectroscopy; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; PAI-1: plasminogen activator inhibitor-1; SD: standard deviation; tPAI-1: total PAI-1.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined as a sum of findings that arise due to hepatosteatosis, which are diagnosed either through various imaging techniques or histologically. It is the most common liver disease in the world, and its prevalence is

increasing. It commonly presents with several conditions, including obesity, diabetes mellitus, metabolic syndrome, and cardiovascular manifestations. Patients with NAFLD are at risk of developing liver cirrhosis and hepatocellular carcinoma [1, 2]. Furthermore, NAFLD patients have increased mortality and mortality rates, mainly due to hepatic involvement as well as associated extrahepatic manifestations such as cardiovascular events [3].

There are multiple hypotheses regarding the pathophysiology of NAFLD, the initial one being the 2-hit hypothesis which states that to develop NAFLD, the presence of 2 hits is required

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(such as the first being insulin resistance and the second being oxidative stress, for example) [4]. However, this hypothesis has proven insufficient in explaining a plethora of molecular and metabolic events that occur during the development of NAFLD; therefore, a new, more widely accepted theory has replaced the 2-hit hypothesis. The „multiple-hit hypothesis” stipulates that there must be multiple hits for NAFLD to occur [5]. One factor that has been recognized in the pathogenesis of NAFLD is the dysfunction of adipose tissue, and throughout its progression, its hallmark is considered to be the cytoplasmic accumulations of triglycerides in hepatocytes [6].

Plasminogen activator inhibitor-1 (PAI-1) is a single chain molecule that is secreted in endothelial cells, smooth muscle cells, cells of immune origin, myocytes, hepatocytes, renal tissue, adipose tissue, and various neoplastic cells [7]. PAI-1 has been observed to demonstrate pleiotropic functions and roles in various physiological and pathological instances, including stress, neoangiogenesis, fibrosis, obesity, insulin resistance, thrombosis, as well as numerous other situations [8, 9].

The pathogenesis of NAFLD involves multiple molecular pathways, some of which are still controversial. A protein that has recently gained more attention regarding the pathophysiology of NAFLD is PAI-1. As disproportionate fat distribution was observed to be linked to NAFLD, studying the relationship between NAFLD and PAI-1 seems necessary [10]. Numerous studies have reported an increase of PAI-1 levels in several conditions that could be associated with NAFLD. These conditions included metabolic syndrome, diabetes mellitus, insulin resistance, cardiovascular events, obesity, and inflammation, all of which have increased comorbidities with NAFLD, which might indicate that PAI-1 is associated with the disease [11–14]. Of these conditions, type 2 diabetes mellitus seems to have the closest relationship with NAFLD [15]. Evidence suggests that liver lipid accumulation is likely linked to hepatic, adipose and muscle tissue insulin resistance [16].

Different studies have attempted to understand the role of PAI-1 in the pathophysiology of NAFLD. Animal studies have shown that the targeted inhibition of PAI-1 prevents hepatic steatosis in mice [17, 18]. Therefore, we conducted this systematic review and meta-analysis to evaluate the association between PAI-1 and NAFLD in humans.

METHODS

This systematic review and meta-analysis was written according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist 2020 [19].

Data Sources and Data Extraction

Our objective was to review all the current research published on PubMed, EMBASE, and Cochrane Library, including observational and interventional studies assessing the association between PAI-1 and NAFLD and NASH. The description of the search strategy has been provided in the Supplementary file. We searched for published articles from inception until 12 October 2020 without using any search filters regarding duration, country, or language. We performed a screening evaluation by assessing titles and abstracts for appropriateness. Selected research matching the inclusion and

exclusion criteria underwent a full-text review. Eligibility of the selected studies and data extraction from eligible studies was performed by two authors (H.A. and A.I.) independently while resolving any discrepancies through mutual consensus.

Eligibility Criteria

Inclusion criteria of original articles were as follows: (1) full-text article studies of interventional studies and observational cohort population-based/ hospital-based, cross-sectional, or case-control designs that evaluated the association between PAI-1 and NAFLD, and NASH; (2) hepatic steatosis evaluated through biopsy or imaging techniques such as ultrasonography, computed tomography (CT), and magnetic resonance spectroscopy (MRS) while excluding secondary causes of hepatic steatosis or significant alcohol consumption; (3) human studies only; and (4) studies published in English, German, or Romanian languages.

Exclusion criteria were as follows: (1) significant consumption of alcohol, or presence of other secondary causes of hepatic steatosis; (2) presence of hepatitis virus of any etiology; (3) other known causes of chronic liver disease; (4) liver cirrhosis of any etiology; (5) subjects that present with hepatic pathologies awaiting liver transplantation or who received a liver transplant; and (6) case reports, conference abstracts, letters to the editor, commentaries, editorials, literature and systematic reviews, practice guidelines, and abstracts published without an entire article.

Risk of Bias Assessment in Individual Studies

The risk of bias in individual studies was assessed using quality assessment tools from the National Heart, Lung, and Blood Institute (NHLBI) [20]. Four tools were used for observational cohort and cross-sectional studies, case-control studies, pre-post intervention studies, and controlled intervention studies. The use of these tools was performed to evaluate the risk of bias as well as the internal validity in individual studies. Two authors (H.A. and S.L.P.) performed the evaluation independently. In case of disagreement, a consensus was reached through a discussion.

Summary Measures and Synthesis of Results

The primary summary outcome was the mean difference (MD) of PAI-1 levels. The data analyses of the quantitative assessment of included studies in our review were performed using R with Metafor package (OpenMeta [Analyst]) [21, 22]. The χ^2 based Q-test and I^2 were performed to assess between-study heterogeneity. The evaluation of the estimated total effect size was done using the random-effects model and MD. We calculated the mean and standard deviation (SD) in studies that stated medians and interquartile ranges, as well as combined groups in studies that had a number of subgroups of NAFLD patients or control subjects without a total group, in line with the Cochrane Handbook recommendations. In case the studies did not report the PAI-1 levels in ng/mL, the values were converted into ng/mL in order to allow the pooling of the obtained data from the included studies. Subgroup assessment was performed according to the method used to evaluate hepatic steatosis, in addition to the presence or absence of NAFLD, and NASH. Furthermore, if a study reported two

values taken for the same variable, we used the initial baseline value. Also, because a number of studies reported the use of total PAI-1 (tPAI-1) and activated PAI-1 (aPAI-1), we used aPAI-1 values. Moreover, one study reported an invalid unit, for which we assumed the use of the standard unit of measurement of PAI-1 which is ng/ml. The data was reported as the estimated MD with 95% confidence interval (CI). A p-value of < 0.05 was considered statistically significant.

RESULTS

The preliminary search returned 299 results (PubMed = 86 articles, EMBASE = 206 articles, Cochrane Library = 7 articles) as shown in Fig. 1. A total of 60 duplicate articles were detected and removed. Afterward, 239 articles were assessed according to the eligibility criteria by evaluating the titles and abstracts of the articles. Screening the articles yielded the following results: 111 irrelevant articles, 41 experimental studies, 20 editorials, letters, commentaries and notes, 12 conference abstracts, and 1 literature review. Therefore, a total of 185 articles were excluded

throughout the initial assessment. A thorough assessment of the full-texts was performed afterward for the remaining total of 54 articles. Of these, 21 were excluded as follows: 17 studies were conference abstracts or papers [23-39], 2 articles were conducted in languages other than English, Romanian or German (1 in Spanish and 1 in Russian language) [40, 41], 1 article was a study involving HIV patients [42], 1 study was a letter [43]. A total of 33 articles were included in our qualitative synthesis, 11 of which were included in the quantitative analysis [44-76].

Study Characteristics

The main study characteristics of the included articles were summarized as shown in Supplementary file (Tables S1 – S4), divided according to observational and interventional studies, as well as adults and pediatrics. This systematic review and meta-analysis included 10,840 individuals (6,581 individuals in cross-sectional studies, 2,301 individuals in cohorts, and 941 individuals in case-control studies). The gender distribution was higher for females [males: 4,881 (46%), females: 5,880

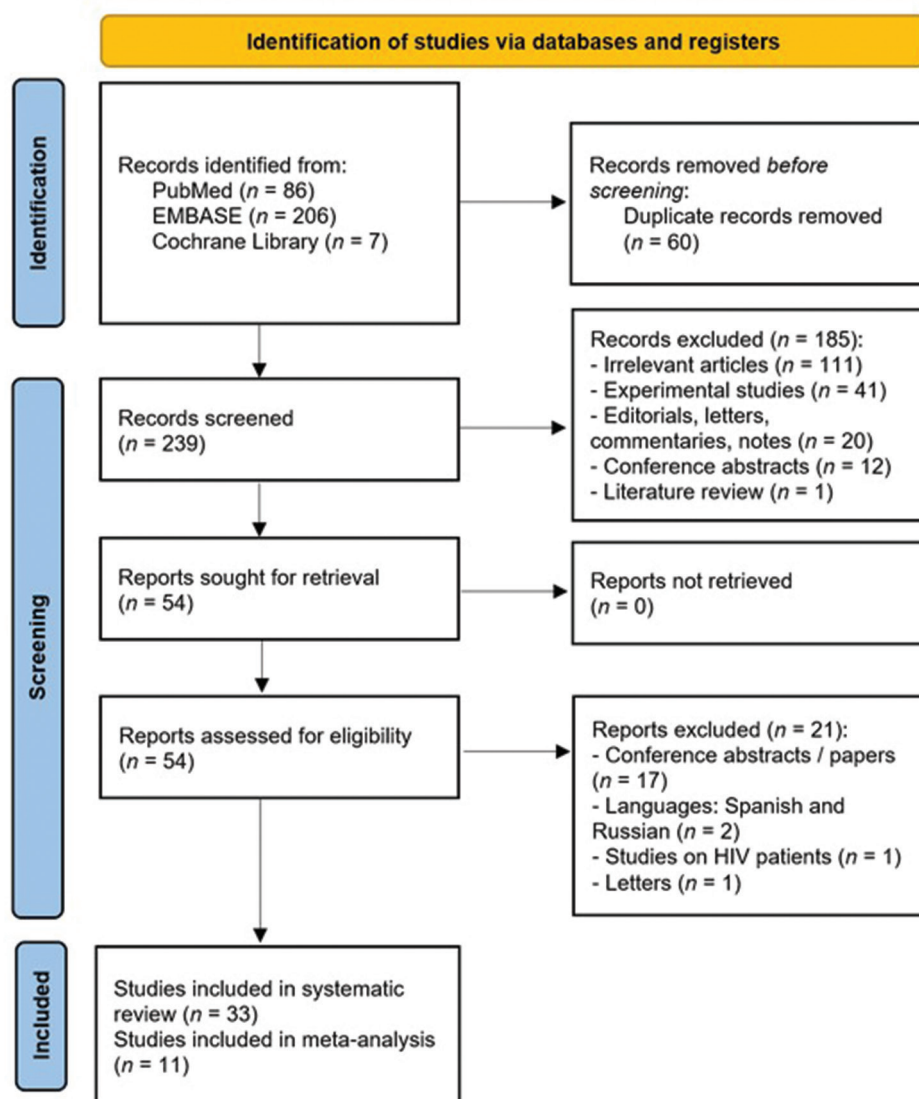


Fig. 1. Summary of the identification, screening, and inclusion phases of our systematic review and meta-analysis according to the PRISMA flow diagram.

(54%)). Of the total population, 3,343 subjects (31%) had NAFLD.

Seven studies were designed as cross-sectional studies, while five were cohort studies and four studies had a case-control design. Thirteen studies took place in Europe (5 multi-center study in Europe, 4 in Germany, 2 in UK, 1 in Italy, 1 in Romania, 1 in Spain), eight studies in the USA, six in Asia (3 in Turkey, 1 in China, 1 in Taiwan, 1 in Japan), five in South America (2 in Argentina, 2 in Brazil, 1 multi-center study in South America).

Definition of NAFLD

Hepatic steatosis was evaluated through liver biopsy in fifteen studies [44, 46, 48, 50, 51, 53, 56, 57, 60, 62, 63, 71, 72, 75, 76], while eleven studies used ultrasonography [49, 54, 55, 58, 61, 64-67, 70, 73], and three studies used CT [45, 47, 59]. Furthermore, two studies used surrogate markers to evaluate hepatic steatosis, along with ultrasound [55, 65]. One study used MRS [52]. Although one study used CT to detect liver fat, it did not specifically test for NAFLD [68].

Serum PAI-1 Levels in NAFLD vs. Controls

From the studies included in our meta-analysis, eight compared NAFLD patients with controls and assessed serum PAI-1 levels [44, 45, 47, 49, 52, 54, 57, 60]. As outlined in Fig. 2, the combined studies for the analysis evaluating serum PAI-1 levels in NAFLD patients vs. controls showed an overall MD of 17.147 (95%CI: 7.720-26.574), while the heterogeneity was considerable ($I^2=99.84\%$ and $p<0.001$).

Moreover, a subgroup analysis was also performed according to the method used to evaluate hepatic steatosis, using liver biopsy, ultrasonography, and CT, as shown in Fig. 3. In three studies involving liver-biopsy proven NAFLD vs. controls, we observed PAI-1 levels to be higher in NAFLD subjects than in controls, as the analysis returned a MD of 24.086 (95%CI: 3.812-44.361), and a considerable reported heterogeneity ($I^2=99.83\%$ and $p<0.001$). Two studies involving ultrasound-diagnosed NAFLD were included in the analysis, with a MD of 10.394 (95%CI: -13.335-34.123), reported heterogeneity was substantial ($I^2=83.67\%$ and $p=0.013$). Two studies involving CT diagnosed NAFLD were included in the analysis with a MD of 15.523 (95%CI: 7.163-23.883), reported heterogeneity was substantial ($I^2=84.8\%$ and $p=0.01$).

Serum PAI-1 Levels in NASH vs. Controls

Three studies compared serum PAI-1 levels in NASH patients and controls [48, 61, 63]. As shown in Fig. 4, the pooled analysis evaluating serum PAI-1 levels in NASH patients vs. controls showed a MD of 26.835 (95%CI: -0.879-54.549), while the heterogeneity was considerable ($I^2=97.64\%$ and $p<0.001$).

Quality Assessment

To assess the methodological quality of the studies included in our systematic review and meta-analysis, the NHLBI quality assessment tools were used, as described in Supplementary file (Tables S5-S9). A total of 23 articles were assessed through the NHLBI quality assessment tool for observational cohort and cross-sectional studies [44-48, 50, 51, 53-55, 57-59, 61-63, 69-72, 74-76], 4 articles were assessed through the NHLBI quality assessment tool of case-control studies [49, 52, 56, 60], 4 articles were assessed through the NHLBI quality assessment tool for before-after (pre-post) studies with no control group [64, 65, 67, 68], and 1 article was assessed through the NHLBI quality assessment tool for controlled intervention studies [66].

Several issues were found concerning the presence of bias in the assessed studies. Sixteen articles were rated as "fair" [44, 48-50, 52, 54, 58, 61, 63, 66-68, 70, 71, 73, 76], fifteen articles were rated as "good" [45-47, 51, 53, 56, 57, 59, 62, 64, 65, 69, 72, 74, 75], and two articles were rated as "poor" [55, 60]. Overall, all studies had a clearly stated research aim or question. 13 studies did not specify or define the study population clearly. None of the investigators in all the case-control studies were able to confirm that the exposure or risk occurred prior to the development of the conditions. Only one study had the assessors of exposure blinded within the case-control studies [49]. One case-control study did not use concurrent controls [60]. All case-control studies adjusted for confounding factors except one [60]. Four of the cross-sectional studies did not adjust for key confounding variables [44, 46, 55, 70]. Only six cross-sectional and cohort studies assessed exposures more than once over a period [45, 47, 51, 59, 69, 70]. Out of the four pre-post intervention studies, only one had the assessors blinded to the participants' exposures [64]. All of the pre-post intervention studies had representative samples of the clinical population of interest, and all their participants that met the prespecified entry criteria were enrolled.

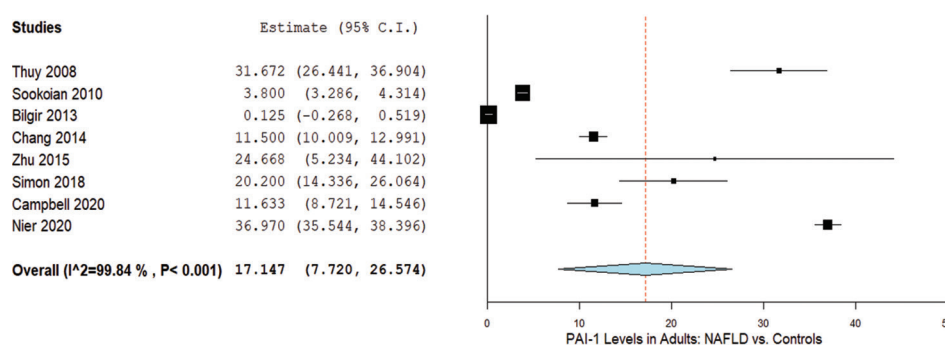


Fig. 2. Studies evaluating PAI-1 levels in NAFLD patients vs. controls.

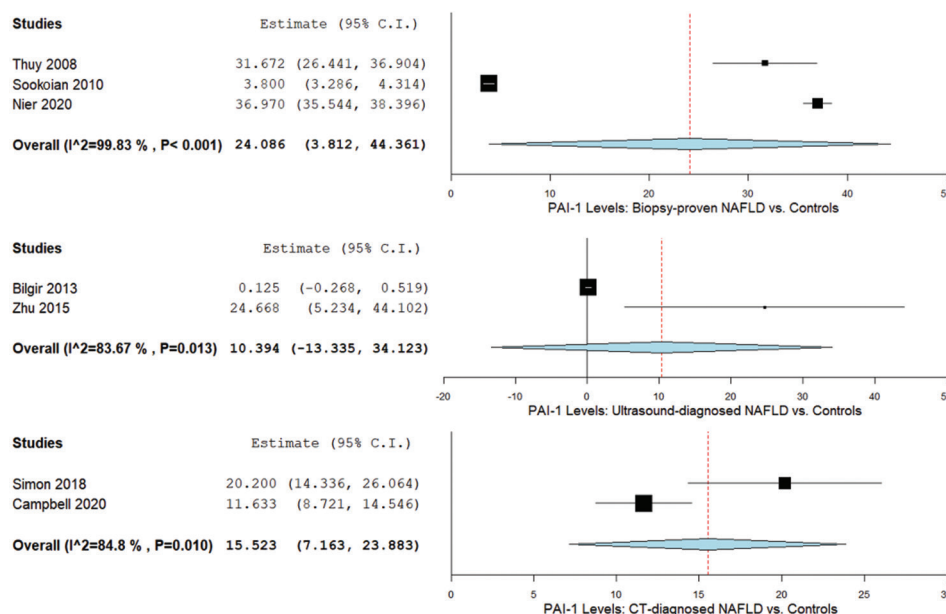


Fig. 3. Studies evaluating PAI-1 levels according to the diagnosis methods of NAFLD (biopsy-proven, ultrasonography, CT) vs. controls.

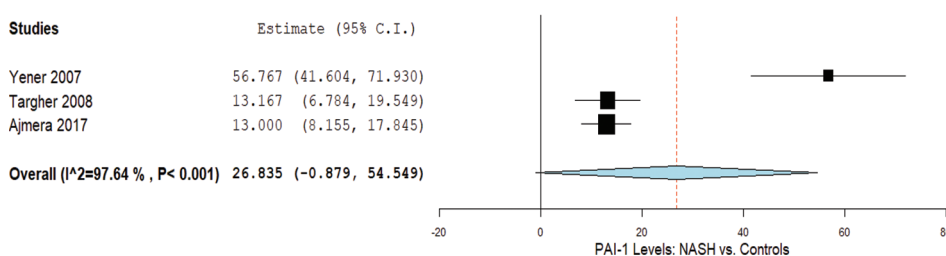


Fig. 4. Studies evaluating PAI-1 levels in NASH patients vs. controls.

DISCUSSION

The use of liver biopsy, the gold standard of diagnosing NAFLD, is associated with several limitations, including higher risk of complications, higher costs, and the inability to perform a biopsy on some patients [77-79]. Therefore, recent literature has been directing more attention towards non-invasive biological markers associated with NAFLD, ranging from nonspecific markers such as inflammation, oxidative stress, degree of steatosis or fibrosis, to more specific markers including adipokines such as visfatin and adiponectin. However, the research remains inconclusive [80-83]. Even though the current published research includes several systematic reviews and meta-analyses, none of them assessed serum PAI-1 levels in NAFLD. To our best knowledge, this is the first systematic review and meta-analysis evaluating the association between PAI-1 and NAFLD, and NASH. Thirty-three articles were included in the qualitative synthesis with a population of 10,840 multiracial subjects from numerous backgrounds who participated in 15 various observational studies, 8 cross-sectional studies, 4 case-control studies, 4 prospective studies, 1 cohort study, and 1 controlled trial study that were conducted in Europe, North America, South America, and Asia. Furthermore, 11 studies were included in our quantitative synthesis. We have shown that serum PAI-1

levels are significantly associated with biopsy-proven and CT-diagnosed NAFLD. However, no significant association was found in ultrasound-diagnosed NAFLD and NASH.

There are several results within our study that require further discussion. First of all, NAFLD prevalence in the studies within our meta-analysis was around 30%, with a gender distribution of approximately 50%, which is likely due to the sampling methodology carried in the analyzed literature. As NAFLD has been demonstrated to have a varied pathophysiology associated with different racial and ethnic backgrounds, the variety of the studied population would provide more reliable data and generalizable results.

Furthermore, the studies included in our systematic review and meta-analysis have reported the use of a plethora of methods to assess liver fat. As the confirmation of NAFLD diagnosis is done through a liver biopsy, or imaging methods from ultrasound, CT, and MRS, in addition to surrogate markers [84]. Around half of the included studies in our review performed histopathological assessment through liver biopsy to confirm hepatic steatosis, with the other half carrying out other methods such as ultrasonography, CT, and MRS, as well as surrogate markers.

Additionally, a number of biochemical blood markers associated with NAFLD have been thoroughly researched, with the most being more specific to NASH, such as adipokines,

inflammatory markers, lipid oxidation products, and apoptosis markers, as well as lysosomal enzymes, since they carry a diagnostic value [85]. However, most are regarded as suboptimal methods of diagnosis at best and have several limitations, including reproducibility, accuracy, sensitivity, and specificity [86].

Our findings show that increased serum PAI-1 levels are associated with NAFLD (biopsy-proven and CT-diagnosed) in comparison to controls. However, this does not seem to be the case for ultrasound-diagnosed NAFLD, which is most likely because hepatic steatosis can be detected using ultrasound when there is more than 20% fat, with a sensitivity and specificity up to 100% and 90%, respectively [84, 87]. Thus, possibly underestimating the prevalence of NAFLD in studies evaluating hepatic steatosis using ultrasonography. Our results are in line with several other studies that confirm that PAI-1 has a role in inflammation and hepatic steatosis, as well as in vitro liposomal functions [14, 74, 88, 89]. Moreover, we did not observe a significant MD between PAI-1 levels in NASH patients.

PAI-1 dysregulation is also observed in a number of varying conditions, from cerebrovascular diseases such as atherosclerosis, vascular thrombosis, and hypertension, to metabolic pathologies such as type 2 diabetes mellitus, metabolic syndrome, and obesity [90]. Several of these conditions are associated with a higher risk of mortality when comorbid with NAFLD. Moreover, NAFLD's increased mortality is due to intrahepatic causes such as progressing to liver cirrhosis, as well as extrahepatic manifestations, mainly cardiovascular disease [91, 92].

There are limitations to our study. Firstly, we could not confirm the causal nature between PAI-1 and NAFLD, which is mainly due to the design of the included studies, which is primarily observational. Secondly, since not all the included studies used liver biopsies to confirm NAFLD, around half resorted to using alternative methods such as ultrasonography, CT, MRS, and blood parameters, which may have affected the reported prevalence of NAFLD in these studies and the results obtained, by inducing misclassification bias. Thirdly, we reported important heterogeneity among several included studies in our review. Fourthly, we could not perform a meta-analysis on PAI-1 levels in NAFLD pediatric patients due to insufficient data. Fifthly, since sixteen of the studies included were rated as „fair“, and two as „poor“, and since around five studies did not adjust their results for confounding factors, results must be cautiously interpreted due to risk of confounding. Moreover, as PAI-1 levels were evaluated using different ELISA kits, possibly leading to differences in the obtained values between different kits.

Overall, despite the limitations of this systematic review and meta-analysis, it includes several important points. As the clinical significance of the key points discussed in this review is increasing due to the rise in prevalence and mortality associated with NAFLD. A comprehensive search strategy was conducted, summarizing the currently published data in a non-biased manner, in addition to pointing out gaps in evidence that need to be further assessed in future studies regarding this topic. We believe that it is important to do further research regarding PAI-1 as it seems to play an important role in the

pathology discussed in this review as well as several other diseases with increasing prevalence.

CONCLUSIONS

Elevated serum PAI-1 levels are associated with adult NAFLD (biopsy-proven and CT-diagnosed). However, no significant mean difference in PAI-1 levels was found in ultrasound-diagnosed NAFLD and NASH patients. These results need to be considered with caution due to the imperfect methodological quality and the low number of the included studies in the subgroup analyses. Moreover, there was inadequate data assessing hepatic levels of PAI-1. Further studies are also required to understand the role of PAI-1 in liver cirrhosis.

Conflicts of interest: None to declare.

Authors' contribution: A.I. had the idea of the manuscript. H.A. and A.I. independently applied the search strategy and performed the study selection. H.A. and S.L.P. performed risk of bias assessment and data extraction. A.I. and D.C.L. conducted the statistical analysis. H.A. drafted the manuscript. A.I., D.C.L., S.L.P., D.L.D. contributed to the writing of the manuscript. A.I., D.C.L., and D.L.D. made substantial contributions to the conception and critically revised the manuscript for important intellectual content. All authors revised the final manuscript, approved the final version, and agree to be accountable for all aspects of the work.

Supplementary material: To access the supplementary material visit the online version of the *J Gastrointest Liver Dis* at <http://dx.doi.org/10.15403/jgld-4091>

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