Lysosomal Acid Lipase Activity in Non-alcoholic Fatty Liver Disease as a Novel Diagnostic and Therapeutic Target: A Systematic Literature Review of Current Evidence and Future Directions

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Background and aim: **Non-alcoholic fatty liver disease (NAFLD) presents with the accumulation of excessive intrahepatic fat without significant alcohol intake. Multifactorial pathogenesis is reported to be involved. Reduced lysosomal acid lipase (LAL) activity is suggested as one of the novel-involved pathogenic mechanisms. This review summarizes the available evidence on the role of LAL activity in NAFLD pathogenesis.** ***Methods:*** **Four databases namely, PubMed/Medline, Science direct, Cochrane Library, and Google scholar were searched to identify relevant observational records evaluating the role of LAL activity in the pathogenesis of NAFLD. All studies were assessed for their quality by using Newcastle–Ottawa Scale or The Joanna Briggs Institute Critical Appraisal tools for cohort and cross-sectional studies, respectively. The estimates of LAL activity and other clinical outcomes were expressed as mean (SD) and number (%) as presented in the primary studies.** ***Results:*** **A total of nine good quality studies with 1711 patients with NAFLD and 877 controls from different groups (healthy volunteers, alcoholics, cryptogenic cirrhosis, and HCV-positive) were included. From the NAFLD group, 59.55% were males and the overall mean age ranged between the studies from 12.6 ± 8.5 months in pediatrics to 58.90 ± 13.82 years in adults. In the NAFLD group, the LAL activity varied from 0.53 ± 0.08 to 1.3 ± 0.70 (nmol/spot/hr) between the studies which was less than all control groups except cryptogenic cirrhosis patients (0.5 ± 0.15 nmol/spot/hr). Of the other outcomes of interest, ALT, AST, total cholesterol, triglyceride, and LDL cholesterol were found elevated in NAFLD patients than in controls.** ***Conclusion:*** **The current evidence suggests a potential correlation of reduced LAL activity with NAFLD pathogenesis according to its severity. Large-scale studies are recommended, more importantly in patients with NAFLD having no metabolic or genetic involvement. Further LAL can act as a new non-invasive diagnostic biomarker to identify that specific NAFLD subgroup. (J Clin Exp Hepatol 2022;12:1535–1546)**

NAFLD

Non-alcoholic fatty liver disease (NAFLD) comprises a broad spectrum of disorders characterized by excessive accumulation of hepatic fatty acids (lipid), in the absence of significant alcohol intake or chronic viral infections.[1] The prevalence in the general population for NAFLD is reported to be 20–30%, affecting all age groups; however, it reaches 70–90% in obese, dia-

Key points

- Non-alcoholic fatty liver disease (NAFLD) emerges as a common cause of the chronic liver disease without having a specific pharmacological treatment available.
- A multifactorial pathogenesis is reported to be involved, but still, the mechanisms demand further clarity.
- Reduced lysosomal acid lipase activity is suggested to play its role in the development and progression of NAFLD.
- The available evidence predicts a potential link between reduced lysosomal acid lipase activity and NAFLD pathogenesis as per its severity.
- Further large-scale studies are recommended to further confirm this association.

Keywords: LAL, pathogenesis, NAFLD, NASH, systematic review
Received: 14.1.2022; *Accepted:* 10.4.2022; *Available online 15 April 2022*
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Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CESD: Cholesterol ester storage disease; HCC: Hepatocellular carcinoma; JBI: Joanna Briggs Institute; LAL: Lysosomal acid lipase; MAFLD: Metabolic (dysfunction)-associated fatty liver disease; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; NOS: Newcastle–Ottawa Scale; PNPLA3: Patatin-like phospholipase domain containing 3 protein; WD: Wolman disease
https://doi.org/10.1016/j.jceh.2022.04.011

betic or individuals with other components of metabolic syndrome (MS).[2] With a rapid and continuous global increase in prevalence, NAFLD acts as the third most common indication for liver transplantation after hepatitis C virus and alcoholic liver disease and is further projected to overtake them in the coming years.[3] The spectrum of NAFLD ranges from simple steatosis (presence of fatty liver) to non-alcoholic steatohepatitis (NASH), where steatosis is associated with hepatocellular injury and inflammation.[4] Further, it can progress to fibrosis and eventually NASH-related cirrhosis or/and hepatocellular carcinoma.

So far, the NAFLD pathogenesis appears multifactorial. Several mechanisms are known to be responsible for the infiltration of fat into the liver; however, the underlying mechanisms of disease progression still demand a well-defined clarification. Involvement of MS, insulin resistance, oxidative stress, chronic low-grade inflammation, intestinal microbiota, and toll-like receptor signaling are reported to be linked with the early stages of fatty liver infiltration as well as in liver disease progression.[5] At the same time, the evidence from genetic analyses indicate that genetic factors might be involved to predispose NAFLD, and a polymorphism in the patatin-like phospholipase domain-containing protein 3 gene is the most widely studied in this regard.[6] Meanwhile, the precise pathogenesis and progression of NAFLD are still poorly understood.

Besides the available evidence on the pathogenesis of NAFLD suggesting involvement of multiple parallel hits, insulin resistance is reported to play a central role.[7] Although obesity and other components of MS are thought as leading risk factors for NAFLD development, it can also develop in non-obese and those patients without any component of MS.[8,9] At least 10–15% of patients with NAFLD are seen as lean, and at times, they do not present the metabolic alterations typically associated with NAFLD, suggesting that other factors could also contribute to the NAFLD phenotype.[10] In this particular population, efforts are on from recent years to find the more appropriate underlying pathogenic mechanisms. Of those pathogenic mechanisms, the role of reduced lysosomal acid lipase (LAL) enzyme activity is one of the novel concepts gaining the attention of researchers for assessing its possible pathogenic involvement.[11]

As reported in animal models and humans, LAL is a key hydrolase that plays a pivotal role in lipid homeostasis. It hydrolyses the cholesterol esters and triglycerides in lysosomes to form free cholesterol and free fatty acids.[12] Any reduction in the LAL activity can disturb the lipid metabolism to cause different liver disorders. Genetically determined LAL deficiency is a very rare autosomal recessive disease responsible for two distinct clinical conditions in humans i.e. Wolman disease in infants and cholesteryl ester storage disease (CESD) in children and adults.[13] Both these conditions are characterized by severe liver steatosis, accelerated fibrosis and a rapid liver failure. The genetic studies have suggested the mutation of LIPA-gene, profoundly with E8SJM variant, as the most common genetic alteration which is seen in 50–70% of cases with an ongoing evaluation for more possible mutations.[14]

More recently, a number of studies reported a progressive decrease in LAL activity in patients of NAFLD ranging from NAFL with simple steatosis to those with biopsy-proven NASH. These findings support the speculation that LAL activity reduction might be acting as an unrecognized mechanism in the pathogenesis of NAFLD. Current evidence shows a great variability; and, none of the reports have documented a cumulative report of findings from the individual studies. Therefore, the authors aimed to review and summarize the existing information to present a qualitative synthesis of the role of reduced LAL activity in the development and progression of NAFLD.

MATERIALS AND METHODS

Literature Search Strategy

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines were followed to conduct this systematic review[15] with a prospective PROSPERO database registration (CRD42021274799). Four electronic English databases (PubMed/Medline, Science direct, Cochrane Library, and Google scholar) were searched to identify relevant observational records published up to August 2021. Additionally, the most relevant bibliographic references from the searched records were included. A search strategy with a combination of following relevant medical subject heading terms and key words was applied: "Cholesterol Ester Storage Disease"[MeSH] OR "Wolman Disease"[MeSH] OR "Lysosomal acid lipase deficiency"[tiab] OR "Lysosomal Storage Diseases"[tiab] OR "LAL-D"[tiab] OR "CESD"[tiab] OR "Lysosomal Storage Diseases"[tiab] OR "LIPA deficiency"[tiab] OR "LAL Deficiency"[tiab] OR "Sterol Esterase"[tiab] OR "Reduced LAL activity"[tiab] AND "Non-alcoholic Fatty Liver Disease"[MeSH] OR "NAFLD"[tiab] OR "NASH"[tiab] OR "NAFL/NASH"[tiab] OR "Nonalcoholic Fatty Liver Disease"[tiab] OR "Fatty Liver, Nonalcoholic"[tiab] OR "Liver, Nonalcoholic Fatty"[tiab] OR "Nonalcoholic Fatty Liver"[tiab] OR "Nonalcoholic Fatty Livers"[tiab] OR "Nonalcoholic Steatohepatitis"[tiab] OR "Steatohepatitis, Nonalcoholic"[tiab].

Inclusion and Exclusion Criteria

Two reviewers (AB and AV) screened and identified studies according to predefined inclusion and exclusion criteria. The observational studies that evaluated LAL activity in patients with known NAFLD with or without a control group were eligible. Studies were included if they have reported at least one of the included outcomes essentially

LAL activity from patients with NAFLD. The studies were excluded if they included patients with the clinically significant concurrent disease, absence of LAL activity estimates and had any other liver-related complications. The review articles, case series/reports, abstracts, letters, historical articles, editorials, or non-English language articles were excluded.

Study Selection

Reviewers (AB & AV) independently checked the titles and abstracts after excluding the duplicate and irrelevant studies. The observational studies (cohort, cross-sectional studies) were assessed for eligibility on the basis of a checklist of aims, research questions, and inclusion/exclusion criteria of this review. After screening for eligibility, the reviewers evaluated the full-text articles for data extraction. Two more independent reviewers (PT & AD) assisted to resolve any discrepancies between the reviewers. The literature selection process is presented in Figure 1.

Outcome Measures

Reported LAL activity measurement was the primary outcome of this systematic review. Additionally, markers of liver function (ALT and AST), serum lipid levels (total cholesterol, triglyceride, LDL cholesterol and HDL cholesterol), body mass index, and fasting blood glucose were also included. The data were expressed as mean (SD) and number (%) as presented in the primary studies for the reported outcomes. Those outcomes presented as median (IQR) in primary studies were converted to mean (SD) using a standard tool to maintain uniformity of data.[16]

LAL Activity Estimation

In all the included studies, LAL activity is measured through dried blood spot testing as used by Hamilton J *et al*, using Lalistat-2 as a specific inhibitor, and cardiolipin as an activator for LAL.[17] From all the enrolled patients, 3 mL fresh venous blood sample is being collected into ethylenediaminetetraacetic acid tube, after 12 h of fasting. The blood

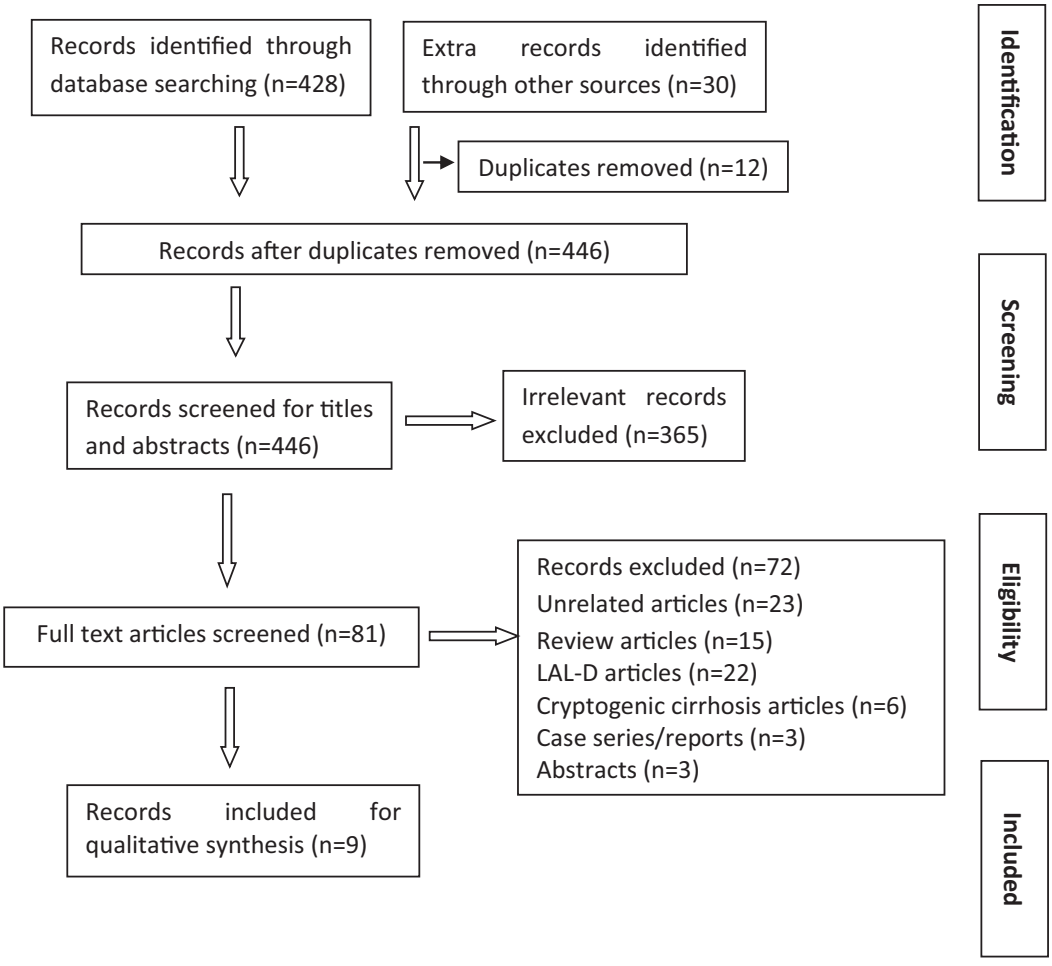

Figure 1 Flowchart of study selection process.

sample is then distributed to fill all four circles in a filter paper [Whatman #903 card] to fulfil the requirements of the National Committee for Clinical Laboratory Standard Protocol. The blood spotted card is dried by keeping it at room temperature for 12 h. Further, the samples are stored in a resealable plastic ziplock bag with a desiccant at −20 °C and are analyzed within 1 week of collection. Uninhibited and inhibited activities with Lalistat 2 are performed and activity is determined by subtracting activity in the inhibited reaction from the uninhibited reaction (total lipase) and expressed as nmol/spot/h of 4MU (methylumbelliferone). The reaction is stopped with 100 μL stop buffer HgCl2 (15 mM). The fluorescence intensity Is measured using the Enspire Multimode plate reader by Perkin Elmer (Waltham, MA, USA) (λ excitation = 355 nm, λ emission = 460 nm). The reference range considered for normal LAL activity is between 0.37 and 2.30 nmol/spot/h. In case LAL activity is found ≤0.40 nmol/spot/h, the samples are subjected to the determination of mutation of the LIPA gene to confirm the cases of autosomal recessive genetic disease.

Data Extraction and Quality Assessment

Two reviewers (AB & AV) independently extracted data from each study and organized it using Microsoft Excel. The following data were extracted; publication characteristics (author, year, country), population characteristics (gender, age), methodological quality (sample size, study design), presence of any MS components and other included patient outcome measures. Any disagreements were resolved by group discussion or by consulting other authors. In case data were missing, the corresponding authors were contacted to assist if possible.

The included studies were assessed for their quality by using Newcastle–Ottawa Scale (NOS) for cohort studies and The Joanna Briggs Institute (JBI) Critical Appraisal tools for cross-sectional studies.[18,19] The NOS tool uses a scoring system to judge the quality of the study on basis of selected populations, the comparability of the groups and the exposure/outcome of interest and the total score ranges from 0 to 9. A study with NOS ≥6 was assigned as of good quality while NOS 3-5 was assigned to be of fair quality. In JBI, a judgment is rated as yes, no, unclear, or not applicable for each of the tool's questions. The total score ranges from 0 to 8. Studies with 8 scores were defined as high score, 6 and 7 scores as medium score, and 5 or less as low score.

Data Synthesis and Analysis

This systematic review presents a comprehensive qualitative synthesis of results from all the included studies. A quantitative meta-analysis was not feasible due to the heterogeneous nature of reported data such as variable control groups, study designs, and effect measures used between the included studies. The data on outcomes of interest from included studies were summarized in Table 2.

RESULTS

Study Selection

The PRISMA flow diagram (Figure 1) presents the detailed literature selection process of included studies. Initially, a total of 458 records were identified from all the data sources. After the removal of duplicate records, 446 records were reviewed for the titles and abstracts. Of these, 365 were not relevant. The remaining 81 records were screened for eligibility in full-text version, 72 were excluded based on inclusion and exclusion criteria. The excluded records were unrelated articles (n = 23), review articles (n = 15), LAL-D articles (n = 22), cryptogenic cirrhosis articles (n = 6), case series/reports (n = 3), abstracts (n = 3). Finally, nine studies qualified for systematic review and were included for qualitative synthesis.

Study Characterization

The individual characteristics of the included studies are presented in Table 1. The review comprises a total of 9 observational records (5 cross-sectional and 4 cohort studies) conducted prospectively.[20–28] The data on outcomes of 1711 patients with NAFLD in comparison to different control groups as healthy control (n = 574), HCV patients (n = 127), alcoholics (n = 116), and cryptogenic cirrhosis (n = 60) are presented. Of the patients with NAFLD, 1019 were males and the overall mean age between the studies varied from 12.6 ± 8.5 months in pediatrics (Selvakumar et al. 2016) to 58.90 ± 13.82 years in adults (Tovoli et al. 2017).[25,26] The NAFLD activity score or MELD/CTP scores were largely not reported in the included studies.

Findings on the presence of MS components show that patients with NAFLD reported by Shteyer et al (2016) had the highest BMI at 33.4 ± 8.5 kg/m^2; and, the lowest BMI was found in the study reported by Selvakumar et al (2016) as 27.1 ± 5.4 kg/m^2.[26,27] Thoen et al (2021) have reported that in patients with metabolic (dysfunction)-associated fatty liver disease (MAFLD) (+steatohepatitis), diabetes was the most common co-morbidity present in 56.2% of the patients.

From other MS components, hypertension was highest (74.5%) seen in patients of Baratta et al (2019).[22,28] Additionally, the same study also reports that 58.3% of patients with cryptogenic cirrhosis had diabetes being the highest among all the controls.[22] Out of nine studies, seven reported the statins as the only option of pharmacotherapy being used among included patients.

Quality Assessment

Both the quality assessment tools used –NOS and JBI– demonstrated that all the included studies were of good quality (Tables 3 and 4); and, none of the studies was excluded on the basis of poor quality. For the included cohort studies, all got NOS scores of above 7 [mean 7.75

Table 1 Baseline Study Characteristics of the Included Studies.

#	Authors (Year)	Country	Type of study	NAFLD/NASH diagnostic criteria	Type of patients	Total (N)	Age	Sex (M/F)	NAFLD activity score N (%)	MELD/CTP score	Metabolic components					Pharmacotherapy N (%)
											BMI (kg/m^2)	Diabetes N (%)	HTN N (%)	Mets N (%)	Dyslipidaemia N (%)	
1	Thoen et al, (2021)[20]	Brazil	Prospective cross-sectional study	Biopsy-proven MAFLD with exclusion of all other causes of CLD	MAFLD (-steatohepatitis)	12	55.07 ± 13.41	4/8	8 (8.0)[a] 25 (24.7)[b] 22 (21.8)[c]	NR	30.64 ± 5.03	3 (25)	6 (50)	5 (41.7)	NR	NR
					MAFLD (+steatohepatitis)	89	56.64 ± 9.79	25/64	6 (11.8)[a] 16 (31.4)[b] 14 (27.6)[c]	NR	31.85 ± 5.65	50 (56.2)	60 (67.4)	48 (54)	NR	NR
2	Ferri et al, (2020)[21]	Italy	Cross sectional study	Ultrasonographic evidence, biopsy-proven and exclusion of all other causes of CLD	NAFLD	118	58.09 ± 12	70/48	NR	12.64 ± 3.75 (NASH-Cir)	27.82 ± 3.89	48 (40.6)	49 (41.5)	NR	72 (61)	Non-Cir -Statins 14 (21) NASH-Cir-Statins 2 (4)
					Alcoholic	116	54.5 ± 9.7	112/4	NR	13.64 ± 5.25 (ALC-Cir)	25.42 ± 2.85	17 (14.6)	57 (49.1)	NR	23 (19.8)	ALC-Non-Cir - statins 2 (4) Cir-ALD - statins 3 (5)
					HCV	49	63.5 ± 14.5	33/16	NR	NR	24.85 ± 3.62 (Non-Cir-HCV)	4 (8.1)	21 (42.8)	NR	18 (36.7)	Non-Cir HCV-statins (0) Cir-HCV- Statins (0)
					Control (Normal)	103	49.83 ± 27.81	47/56	NR	NR	24.36 ± 3.38	6 (6)	30 (29)	NR	56 (54)	Statins 26 (25)
3	Baratta et al, (2019)[22]	Italy	Cross sectional study	Ultrasonographic evidence, biopsy (NASH-suspects), Non-invasive markers (FIB-4, NFS) and exclusion of all other causes of CLD	NAFL	454	57.4 ± 11.3	274/180	(N = 515) FIB-4 <1.3, 360 (69.9) FIB-4 >1.3, 155 (30.09) NFS -<1.455, 222 (43.10) NFS ->=1.455, 293 (56.89)	NR	NR	137 (30.1)	338 (74.5)	NR	NR	Statins 196 (43.2)
					NASH	61	49.5 ± 12.9	38/23		NR	NR	18 (29.5)	34 (55.9)	NR	NR	Statins 13 (21.7)
					Cryptogenic cirrhosis	60	68.6 ± 10.6	43/17	NR	NR	NR	35 (58.3)	30 (50.0)	NR	NR	Statins 4 (7.0)
					Patients without fatty liver	68	59.3 ± 13.9	34/34	NR	NR	NR	6 (9.0)	40 (58.5)	NR	NR	Statins 32 (47.7)
4	Gomaraschi et al, (2019)[23]	Italy	Cohort	Biopsy proven, following EASL guidelines and exclusion of all other causes of CLD	NAFLD	164	52.8 ± 13	116/48	NR	NR	28.7 ± 4.4	40 (24)	78 (48)	78 (48)	NR	On dietary recommendations 96 (58.53) On statins 28 (17.07) On fibrates 20 (12.19 On statins and fibrates in combination 36 (21.95)
					Control (Dyslipidemia)	180	56.5 ± 13.2	144/44	NR	NR	25.7 ± 3.1	14 (8)	54 (30)	45 (25)	NR	NA
5	Polimeni et al, (2017)[24]	Italy	Cross sectional study	Ultrasonographic evidence and exclusion of all other causes of CLD	NAFLD	315	56.2 ± 11.3	189/126	NR	NR	30.2 ± 4.8	93 (29.4)	190 (60.3)	200 (63.5)	NR	Statins 128 (40.6)
					Control (non-NAFLD)	110	57.4 ± 14.4	65/50	NR	NR	26.7 ± 3.7	14 (12.4)	65 (59.3)	25 (22.9)	NR	Statins 43 (39.8)

(Continued on next page)

Table 1 (*Continued*)

#	Authors (Year)	Country	Type of study	NAFLD/NASH diagnostic criteria	Type of patients	Total (N)	Age	Sex (M/F)	NAFLD activity score N (%)	MELD/CTP score	Metabolic components					Pharmacotherapy N (%)
											BMI (kg/m^2)	Diabetes N (%)	HTN N (%)	Mets N (%)	Dyslipidaemia N (%)	
6	Tovoli et al, (2017)[25]	Italy	Cohort	Ultrasonographic evidence, Non-invasive markers (FIB-4, NFS) and exclusion of all other causes of CLD	NAFLD	81	58.90 ± 13.82	53/28	NR	10.51 ± 3.30 Cir -FLD (N = 43)	28.23 ± 3.71	32 (39.5)	NR	NR	NR	Metformin 16 (19.8) Insulin 12 (14.8) Statins 18 (22.2)
					Control (HCV related CLD)	78	62.66 ± 10.58	41/37	NR	9.66 ± 3.32 Cir HCV (N = 46)	23.73 ± 2.15	11 (14.1)	NR	NR	NR	Metformin 2 (2.6) Insulin 5 (6.4) Statins 2 (2.6)
7	Selvakumar et al (2016)[26]	Italy	Prospective cohort	Ultrasonographic evidence, biopsy-proven and exclusion of all other causes of CLD	NAFLD	168	12.6 ± 8.5	101/67	3.5 ± 1.5	NR	27.1 ± 5.4	NR	NR	NR	NR	NR
8	Shteyer et al, (2016)[27]	Israel	Cohort	Biopsy-proven NAFLD with exclusion of all other causes of CLD	NAFLD (Low risk LAL-D)	9	54.3 ± 17.1	4/5	2.2 ± 2.2	NR	33.4 ± 8.5	NR	NR	NR	NR	NR
					Control (high risk LAL-D)	13	17.2 ± 12.3	8/5	2.8 ± 2	NR	22.1 ± 6.8	NR	NR	NR	NR	NR
9	Baratta et al, (2015)[28]	Italy	Cross sectional study	Ultrasonographic evidence, biopsy (NASH-suspects) and exclusion of all other causes of CLD	NAFLD	240	55.4 ± 11.0	145/95	NR	NR	30.5 ± 4.7	76 (31.6)	NR	169 (70.4)	NR	Statins 86 (35.7)
					Control	100	53.0 ± 11.3	55/45	NR	NR	NR	NR	NR	NR	NR	NR

a = NAS < 3, b = NAS ≥ 3 to < 5 and c = NAS ≥ 5.

ALD = Alcoholic liver disease; BMI = Body-mass index; Cir = Cirrhosis, CLD = Chronic liver disease; EASL = The European Association for the Study of the Liver; FIB-4 = Fibrosis-4; FLD = Fatty liver disease; HCV = Hepatitis-C virus, HTN = Hypertension, LAL-D = Lysosomal acid lipase deficiency, MAFLD = metabolic associated fatty liver disease, MELD/CTP = Model for End-Stage Liver Disease/Child-Turcotte-Pugh, Mets = Metabolic syndrome, NAFLD = Non-alcoholic fatty liver disease, NASH = Non-alcoholic steato-hepatitis, NFS = NAFLD fibrosis score, NR = Not reported.

Table 2 Clinical and Biochemical Characteristics of Patients Diagnosed with NAFLD in Comparison with Other Controls.

#	Authors (Year)	Type of patients	LAL activity (nmol/spot/h)	AST (UI/L)	ALT (UI/L)	ALP	TC (mg/dl)	LDL-Cho (mg/dl)	HDL-Cho (mg/dl)	TG (mg/dl)	FBS (mg/dl)	Total bilirubin	Platelets (n/mmc)	Albumin (g/dl)
1	Thoen et al, (2021)[20]	MAFLD (-steatohepatitis)	67.96 ± 55.85 pmol/punch/h	31.49 ± 25.99	32.19 ± 21.80	86.66 ± 51.99	184.66 ± 67.08	103.02 ± 60.79	48.26 ± 3.35	134.18 ± 62.05	105.76 ± 29.35	0.43 ± 0.25	231.30 ± 56.18	4.6 ± 0.4
		MAFLD (+steatohepatitis)	89.51 ± 77.39 pmol/punch/h	36.11 ± 18.08	43.81 ± 25.62	80.16 ± 20.34	174.18 ± 42.19	98.0 ± 34.66	43.0 ± 9.4	154.33 ± 73.84	115.15 ± 37.67	0.43 ± 0.22	214.52 ± 52.74	4.53 ± 0.37
2	Ferri et al, (2020)[21]	NAFLD	0.60 ± 0.27	NR	44.36 ± 25.51	NR	NR	NR	NR	NR	NR	NR	215 ± 33.77 (Non-Cir)	NR
		Alcoholic	0.85 ± 0.42	NR	24.6 ± 13.51	NR	NR	NR	NR	NR	NR	NR	NR	NR
		HCV	NR	NR	33.27 ± 22.53	NR	NR	NR	NR	NR	NR	NR	NR	NR
		Control (Normal)	1.15 ± 0.77	NR	16 ± 6.01	NR	NR	NR	NR	NR	NR	NR	223 ± 59.39	NR
3	Baratta et al, (2019)[22]	NAFL	0.93 ± 0.37	21.20 ± 6.69	26.75 ± 14.13	NR	197.9 ± 39.8	118.2 ± 34.7	48.3 ± 12.9	141.74 ± 58.52	105.9 ± 26.6	NR	239.4 ± 60.5	4.4 ± 0.3
		NASH	0.8 ± 0.3	38 ± 18.6	67.71 ± 47.83	NR	190.7 ± 38.0	112.9 ± 32.3	49.4 ± 19.8	126.08 ± 58.84	100.0 ± 18.0	NR	228.6 ± 54.4	4.4 ± 0.3
		Cryptogenic cirrhosis	0.5 ± 0.15	39.55 ± 22.78	32.0 ± 15.95	NR	153.1 ± 41.7	85.4 ± 32.7	45.7 ± 15.6	95.76 ± 35.70	118.9 ± 40.5	NR	107.7 ± 55.5	3.5 ± 0.7
		Patients without fatty liver	0.96 ± 0.37	18.05 ± 3.78	17.35 ± 6.81	NR	194.6 ± 41.0	117.3 ± 38.7	58.2 ± 12.2	95.58 ± 7.46	95.2 ± 19.2	NR	230.6 ± 57.7	4.5 ± 0.4
4	Gomaraschi et al, (2019)[23]	NAFLD	0.71 ± 0.29	37.6 ± 24	55 ± 33	NR	194.3 ± 43	NR	48.9 ± 13	143.5 ± 69	104.3 ± 32	NR	215 ± 62	4.5 ± 0.4
		Control (Dyslipidemia)	1.20 ± 0.44	25 ± 8.0	32 ± 17	NR	223 ± 45	NR	46.9 ± 14	195.7 ± 118	93.4 ± 25.3	NR	220 ± 77 (only of 50 patients)	NR
5	Polimeni et al, (2017)[24]	NAFLD	0.89 ± 0.08	21.13 ± 1.94)	27.69 ± 4.04	NR	198.6 ± 40.1	119.0 ± 34.7	49.0 ± 14.6	138.12 ± 14.40	105.8 ± 28.2	NR	229.6 ± 59.5	NR
		Control (non-NAFLD)	NR	17.60 ± 0.98	15.15 ± 1.38	NR	204.1 ± 43.3	122.7 ± 39.8	57.3 ± 14.8	115.5 ± 60.6	94.1 ± 20.1	NR	NR	NR
6	Tovoli et al, (2017)[25]	NAFLD	0.53 ± 0.08	36.90 ± 26.82	43.25 ± 36.72	NR	174.48 ± 53.23	103.79 ± 44.15	46.03 ± 18.15	97.54 ± 53.44	NR	1.26 ± 1.65	177.38 ± 74.07	3.82 ± 0.63
		Control (HCV related CLD)	0.7 ± 0.08	45.71 ± 45.65	58.61 ± 69.71	NR	156.88 ± 39.84	87.75 ± 28.63	50.47 ± 25.73	79.05 ± 44.20	NR	1.16 ± 1.17	153.84 ± 77.60	3.71 ± 0.61
7	Selvakumar et al (2016)[26]	NAFLD	1.3 ± 0.70	26.0 (22.0, 32.0)	26.70 ± 7.47	NR	160.6 ± 37.4	98.6 ± 35.0	44.0 ± 8.97	NR	86.93 ± 114.94	NR	NR	NR
8	Shteyer et al, (2016)[27]	NAFLD (Low risk LAL-D)	0.74 ± 0.28 nmol/punch/h	NR	NR	94 ± 33	NR	NR	NR	NR	NR	NR	NR	NR
		Control (high risk LAL-D)	0.74 ± 0.28 nmol/punch/h	NR	NR	198.5 ± 76	NR	NR	NR	NR	NR	NR	NR	NR
9	Baratta et al, (2015)[28]	NAFLD	0.80 ± 0.29	24.10 ± 10.44	31.75 ± 17.15	NR	198.3 ± 38.8	117.5 ± 33.0	48.4 ± 15.6	146.5 ± 61.15	99.64 ± 14.17	NR	NR	NR
		Control	1.28 ± 0.57	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase, FBS= Fasting blood sugar; HDL-Cho = High density lipoprotein; LDL-Cho = Low density lipoprotein; TC = Total cholesterol, TG = Triglycerides.

Table 3 Newcastle–Ottawa Quality Assessment Form for Cohort Studies.

Domain	List of questions	Gomaraschi et al, (2019) [23]	Tovoli et al, 2017 [25]	Selvakumar et al (2016) [26]	Shteyer et al, (2016) [27]	Outcome
Selection	Representativeness of the exposed cohort	*	*	*	*	Good Quality
	Selection of the non-exposed cohort	*	*		*	Good Quality
	Ascertainment of exposure	*	*	*	*	Good Quality
	Demonstration that outcome of interest was not present at start of study	*	*	*	*	Good Quality
Comparability	Comparability of cohorts on the basis of the design or analysis controlled for confounders	*	**	*	**	Good Quality
Outcome	Assessment of outcome	*	*	*	*	Good Quality
	Was follow-up long enough for outcomes to occur	*	*	*	*	Good Quality
	Adequacy of follow-up of cohorts		*	*		Good Quality
Total score		7	9	7	8	

Table 4 The Joanna Briggs Institute Critical Appraisal Tools for Cross-Sectional Studies.

#	List of questions	Thoen et al, (2021) [20]	Ferri et al, (2020) [21]	Barattaet.al, (2019) [22]	Polimen et al, (2017) [24]	Barattaet.al, (2015) [28]
1	Were the criteria for inclusion in the sample clearly defined?	✔	✔	✔	✔	✔
2	Were the study subjects and the setting described in detail?	✔	✔	✔	✔	✔
3	Was the exposure measured in a valid and reliable way?	✔	✔	✔	✔	✔
4	Were objective, standard criteria used for measurement of the condition?	✔	✔	✔	✔	✔
5	Were confounding factors identified?	Unclear	✔	✔	✔	✔
6	Were strategies to deal with confounding factors stated?	Unclear	✔	✔	✔	✔
7	Were the outcomes measured in a valid and reliable way?	✔	Unclear	✔	Unclear	✔
8	Was appropriate statistical analysis used?	✔	✔	✔	✔	✔
	Overall appraisal	✔	✔	✔	✔	✔

(range 7–9)],[23,25–27] while in the cross-sectional study group, a JBI score of 6 (medium score) was found in 1 study,[20] 7 in 2 studies (medium score)[21,24] and 8 in 2 studies (high score).[22,28]

Clinical Outcomes

The measures of clinical and biochemical outcomes of patients diagnosed with NAFLD in comparison with other controls are presented in Table 2. Of seven studies in this analysis comparing LAL activity with control groups, only four reported a significant reduction in LAL activity in patients with NAFLD compared to any other control group.[21,23,25,28] It is interesting to note that Baratta et al (2019) have reported that patients with cryptogenic cirrhosis (CC) had LAL activity (0.5 ± 0.15) even less than that of NAFLD subcategories, both NAFL (0.93 ± 0.37) and NASH (0.8 ± 0.3).[22] However, Shteyer et al (2016) reported that there was no difference in LAL activity when compared between NAFLD (Low-risk LAL-D) or control (high-risk LAL-D), as both reported the same measures as 0.74 ± 0.28 nmol/punch/hr for LAL activity.[27]

The levels of ALT and AST were found to be elevated in patients with NAFLD than other controls, except for patients with HCV. However, Ferri et al (2020) reported that NAFLD group had elevated ALT levels (44.36 ± 25.51 IU/L) than found in HCV (33.27 ± 22.53 IU/L).[21] In addition, two studies that report data according to disease severity, transaminases were reported to be elevated according to the presence of steatohepatitis i.e. more elevated in NASH (with steatohepatitis) than in NAFL (without steatohepatitis).[20,22]

A disturbed lipid profile is an important indication associated with almost all liver diseases; and, NAFLD is no exception. In the present review, Polimeni et al (2017) reported the maximum level of total cholesterol as 198.6 ± 40.1, LDL-C as 119.0 ± 34.7, and HDL-C as 49.0 ± 14.6 among the patients with NAFLD while the lowest values were reported from study patients of Selvakumar et al (2016) as 160.6 ± 37.4, 98.6 ± 35.0, and 44.0 ± 8.97, respectively.[24,26] For triglycerides, Thoen et al (2021) reported the highest value among MAFLD (+steatohepatitis) as 154.33 ± 73.84 in comparison to Tovoli et al (2017) who showed lowest levels in the included patients with NAFLD as 97.54 ± 53.44.[20,25]

Among the studies that focused on fasting blood sugar (FBS), Thoen et al (2021) have reported the highest blood sugar among patients with MAFLD (+steatohepatitis) as 115.15 ± 37.67. In contrast to this, Selvakumar et al (2016) reported the minimum level as 86.93 ± 114.94 in the included NAFLD patients.[20,26] Further, the highest level for platelets was reported by Baratta et al (2019)[21] as 239.4 ± 60.5; and, for albumin Thoen et al (2021)[20] reported 4.6 ± 0.4 as maximum while Tovoli et al (2017) reported both these outcomes as minimum as 177.38 ± 74.07 and 3.82 ± 0.63, respectively.[25]

DISCUSSION

NAFLD is an emerging common cause of chronic liver disease without having a specific pharmacological treatment available. Presence of MS components is suggested as the main reason of steatosis and it has been very well supported by evidence.

In this systematic review, we aimed to provide evidence of available reports which have evaluated the role of LAL activity in pathogenesis of NAFLD and also compared it with different control groups. Out of total 9 studies, only 7 had comparison groups while 2 studies reported the LAL activity estimates from NAFLD patients only. From those seven having controls, four have reported a significant reduction in LAL activity among NAFLD patients in comparison to other control groups.[21,23,25,28] These findings collectively suggest that a progressive reduction of LAL activity is seen in NAFLD and that varies according to the severity i.e. at higher severity stages of NAFLD, more reduction in LAL activity is observed. Meanwhile, in study of Baratta et al (2019), patients with CC showed LAL activity (0.5 ± 0.15 nmol/spot/hr) less than all other study groups even NAFLD.[22] In line with these LAL activity estimates of CC patients, two more studies (not included in this review) by Angelico et al (2017) and Vespasiani-Gentilucci et al (2016) had also reported a similar level of LAL activity as 0.54 ± 0.27 and 0.64 ± 0.31 respectively.[29,30] These findings suggest that reduced LAL activity might be an unrecognized pathogenic cause of CC too.

Thoen et al, in 2021, have compared LAL activity data between MAFLD patient subgroups.[20] The term MAFLD (metabolic (dysfunction) associated fatty liver disease) was recently suggested by The International Consensus Panel as more appropriate terminology for NAFLD to define fatty liver patients with MS components.[31] However, its adoption is still a matter of debate and experts share divergent views to its use.

In their study, Thoen et al have reported that in patients of MAFLD (without steatohepatitis), the LAL-activity is reduced more (67.96 ± 55.85 pmol/punch/hr) than that of MAFLD (with steatohepatitis) (89.51 ± 77.39 pmol/punch/hr). These findings were inconsistent with results of Baratta et al (2019) as they report NAFL (without steatohepatitis) have higher LAL activity (0.93 ± 0.37 nmol/spot/hr) than patients with NASH (with steatohepatitis) seen as 0.8 ± 0.3 nmol/spot/hr. Therefore, such results need to be validated on the basis of status of steatosis among NAFLD.

The LAL activity measurements were further confirmed by Selvakumar et al (2016) who had evaluated it in the

pediatric population.[26] The authors reported that reduced blood LAL activity is related to the severity of liver fibrosis in children having NAFLD. However, when compared to adults, LAL activity found in children of this study was not reduced to that extent but showed a level that was comparable with controls used in other studies. Such findings further suggest future studies to evaluate and compare LAL activity between children and adult patients diagnosed with NAFLD.

Meanwhile, out of all the nine included studies, only two have assessed LIPA gene sequencing to exclude the genetic LAL deficiency[22,23] while one study by Ferri F et al (2020) had analyzed their patients for patatin-like phospholipase domain-containing protein 3 gene mutation.[21] As discussed, all the patients with highly reduced LAL activity must be subjected to genetic analysis to confirm whether the disease is of the genetic cause or could be labelled as NAFLD.

Overall, patients with higher BMI or with coexistent MS components were found to have significantly reduced LAL-activity among NAFLD. Furthermore, these patients were seen with deregulated lipid profiles with a moderate elevation of triglycerides, total and LDL-C while no differences were reported in HDL-C among them. As there is no specific pharmacotherapy to treat NAFLD patients, the statins were the only option of pharmacotherapy used to deal with the dyslipidemia.[32]

While reporting the trends on serum transaminases, patients with NAFLD were found to have elevated levels of ALT and AST than other controls like alcoholics followed by CC and healthy controls but lower levels than in the disease control group of HCV. In contrast, Ferri et al (2020) showed elevated ALT levels in the NAFLD group than found in HCV patients.[21] In addition, both transaminases were seen elevated when the data were presented on basis of disease severity from NAFL (without steatohepatitis) to NASH (with steatohepatitis) as reported by Thoen et al (2021) and Baratta et al (2019).[20,22] These findings were complementary to the earlier reported aminotransferase levels through a previous review by Baratta et al (2015) who suggested that patient with NAFLD with reduced LAL activity present with elevated liver enzymes.[11]

Interestingly, Witeck C et al (2022) recently did a scoping review of genetic LAL-D for its both variants i.e. Wolman disease and CESD by including all the diverse study designs of observational studies.[33] The authors found that the LAL activity is dramatically reduced in Wolman disease with a mean reported level of approximately 4.91% compared to 7.9% in CESD group. However, the authors presented LAL activity as a percentage of normal activity which could not be compared with our study findings. Therefore, to get a comparative picture of differences in definitive LAL activity estimates between genetic LAL-D and NAFLD, future studies with comparable data are required.

To summarize, the current findings suggest that reduced LAL activity is an important pathogenic mechanism in the development and progression of NAFLD. As of now, very few studies are available to explain this relation; therefore, more long-term, well designed real-world studies are needed to confirm the clinical relevance of these results and further to extend the literature and better understanding of NAFLD pathogenesis. Reduced LAL activity should always be suspected in non-obese patients presenting with NAFLD and/or CC after excluding the potential causes contributing to fatty liver, such as viral causes, alcohol abuse, or the presence of familial hypercholesterolemia. Those patients must be indicated to test LAL activity for the confirmation of underlying pathology.

Limitations

This study provides first cumulative evidence as a systematic review over the pathogenic role of LAL activity in NAFLD. Despite that, the study possesses several limitations that are worth to mention. First, we have summarized results of different study designs including cross-sectional and cohort together. Due to heterogeneity among the studies, a formal statistical analysis was not performed. Second, given the small number of published studies available for investigation, our results may be underpowered to give a general recommendation regarding the role of LAL activity in NAFLD development. In addition, none of the studies had presented the follow-up data for LAL activity estimates which may vary as per different severity stages of NAFLD. Third, as primary studies have not well reported, we have included a limited number of outcomes among several others like, MELD score, liver fibrosis markers (AST/ALT ratio, FIB-4 score, NFS) measures of FibroScan (LSM, CAP) etc. These important outcomes must be evaluated through future studies. Finally, we converted median (range) into mean (SD) by using appropriate tools[16] from original measurements presented in original studies, which might give some variation in pooled results.

The current evidence suggests a potential role of reduced LAL activity in hepatic steatosis/dysfunction and also its association with the severity of NAFLD. The confirmation of available findings will need further analysis through large-scale studies, more importantly in patients with NAFLD having no metabolic or genetic involvement. Furthermore, the testing of all patients with NAFLD for LAL activity can be recommended as a new non-invasive diagnostic marker to identify that specific proportion of patients within NAFLD who had involvement of reduced LAL activity as a pathogenic component.

Future Directions

The initial reports indicate a definite role of reduced LAL activity in NAFLD development. Just like exploring the

role of metabolically associated factors in NAFLD, the reduced LAL activity needs to be assessed to understand its contribution in its pathogenesis. As there is a scarcity of correlation data between the LAL activity and individual pathogenic mechanisms, future research should evaluate the LAL activity in different specified paradigms of patient subgroups with NAFLD like, obese/non-obese, with/without MS, cirrhotic/non-cirrhotic, based on gender and even at different NAFLD severity stages. In addition, the efforts must be enhanced in already initiated fields of evaluating the genetic and epigenetic implications associated with LAL activity reduction to cause NAFLD. The findings would particularly help in better understanding of association between impaired LAL with NAFLD pathogenesis and progression. Another essential area of research which needs an attention is the treatment of reduced LAL activity in NAFLD. As in genetic LAL deficiency (LAL-D), the recombinant enzyme replacement therapy (Sebelipase-alfa) is recommended as it showed a significantly safe and effective profile[34]; the therapy can be studied in NAFLD and CC. Interestingly, if the results favor and fat in liver responds, it might be a breakthrough in treating NAFLD, where there is no specific treatment available yet.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

AB and AV contributed in literature search and selection process. AB, AV, AD, PT and AD contributed towards the conception of the article, drafted and reviewed the manuscript, performed critical revisions related to important intellectual content of the manuscript. PT, AD and AD contributed to the final revision of the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The author declares that they have no competing interests.

ACKNOWLEDGEMENTS

Not applicable.

FUNDING

No funding.

AVAILABILITY OF DATA AND MATERIALS

Data available on reasonable requests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

PROSPERO DATABASE REGISTRATION NUMBER

CRD42021274799.

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