RESEARCH ARTICLE

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Does Autophagy have a Role in the Pathogenesis of Pediatric Hepatic Steatosis?

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Abstract

Hepatic steatosis has become the most common cause of chronic liver disease among children worldwide. Lipophagy has been considered as a pathway affecting steatosis development and progression. **Objective:** this study aimed to evaluate the immunohistochemical expression of *Beclin1* and *LC3A* in pediatric hepatic tissues with steatosis and to correlate their expression with clinicopathological parameters. **Methods:** this study included 81 Egyptian pediatric patients with hepatic steatosis and 21 pediatric cases without hepatic steatosis. All specimens were stained by *Beclin1* and *LC3A* antibodies. According to final diagnosis obtained from Pediatric Hepatology department, patients were divided into two groups: chronic liver disease (CLD) group that included 45 cases and inborn error of metabolism (IEM) group that included 36 cases. **Results:** higher beclin1 expression was significantly correlated with higher stages of fibrosis and distorted liver architecture in CLD group, (P=0.043) for both. The control group showed higher positivity, percentage, as well as the median values of the H score of *LC3A* expression than did the CLD group or the IEM group (P=0.055, 0.001, and 0.008, respectively). Higher positivity of *LC3A* was significantly associated with higher stages of fibrosis and distorted liver architecture in the studied IEM group (P=0.021) for both. **Conclusions:** Varying intensity grades of *LC3A* and Beclin 1 immunohistochemical expression demonstrate the variation of autophagy at different phases of pediatric hepatic steatosis and varied disease etiology.

Keywords: Beclin 1- Immunohistochemistry- *LC3A*- Lipophagy- Non-alcoholic steatohepatitis

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Introduction

Children's hepatic steatosis has been rising worldwide, making it difficult to discern between basic steatosis and a more complicated condition [1]. Basic steatosis is defined by the accumulation of lipids in more than 5% of hepatocytes and the exclusion of secondary causes of fat accumulation [2]. Causes of steatosis in children are primarily linked to metabolic storage illness, fatty liver disease, viral infection, and cancer [3].

The most frequent cause of liver disease in children is metabolic associated fatty liver disease (MAFLD), a term used to describe a clinical spectrum of liver abnormalities linked to obesity. Since kids don't drink as much alcohol as adults do, the term "non-alcoholic fatty liver disease" (NAFLD) should be replaced with "MAFLD" [4]. According to epidemiology, 3- 10% of pediatric patients may have MAFLD, and the male-to-female ratio is 2:1 [5].

In addition to being an anti-aging strategy, autophagy is a catabolic process that aids the cell in eliminating damaged cytosolic organelles including mitochondria and aggregated proteins [6]. The creation of autophagosomes from membranes that separate the cell structure(s) that must be destroyed is essential for autophagy [7]. The autolysosome is created when late endosomes and lysosomes fuse with the autophagosome to finish the degradation process before continuing metabolism through the endoplasmic reticulum (ER) [8]. Numerous pathological conditions, such as metabolic storage diseases, non-alcoholic fatty liver disease, and cancer, are known to exhibit dysregulation of autophagy [9].

Lipophagy is a specific form of autophagy that controls the breakdown of lipid droplets (LDs) in the liver in order to protect against lipotoxicity. Recently, lipophagy has emerged as a pathway affecting NAFLD development and progression [10].

The primary regulators of the autophagy process are Beclin 1 and *LC3*, however they function differently.

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The early process of autophagy and the development of the autophagosome are both facilitated by mammalian Beclin 1 [11]. The class III phosphatidylinositol 3-kinase (PI3KC3) complexes are affected allosterically by it (PI3KC3-C1 and PI3KC3-C2). At the beginning of the autophagosome development, PI3KC3-C1 performs a role in autophagic vesicle enucleation [12]. The Class III phosphatidylinositol 3-kinase (PI3KC3-C2) participates in the development of autophagolysosomes [13].

A microtubule-associated protein called *LC3* is also arguably the most commonly used indicator of autophagic activity and flow because it has a post-translationally modified, lipidated form that may be connected to continuous phagophore production. The *LC3* gene family has three paralogues in humans: *LC3A*, *LC3B,* and *LC3C*. The specialized targeting of mitochondria for mitophagy has been attributed to *LC3A* [14]. There is some disagreement over the precise function of the autophagic and lipophagic pathways in NAFLD despite the huge number of studies demonstrating a connection between lipophagy/autophagy and NAFLD [10].

The current study aims to evaluate Beclin 1 and *LC3A* autophagy markers' immunohistochemistry expression in fatty liver samples from pediatric patients in Egypt and to connect their expression with clinicopathological parameters to determine its clinical importance.

Materials and Methods

This retrospective case control study included 81 liver biopsy specimens from Egyptian pediatric patients presented with hepatic steatosis. They were divided into two groups according to final diagnosis obtained from Pediatric Hepatology Department into chronic liver disease (CLD) group that included 45 cases and inborn error of metabolism (IEM) group that included 36 cases. Also, 21 cases within the pediatric age group served as control liver tissue without steatosis were included. Specimens were retrieved from the archival material of Pathology Department, National Liver Institute (NLI), Menoufia University, during the period between 2015 and 2020. All samples were collected as part of the standard clinical management of the patients. The study was in accordance to an approved Institutional Review Board (IRB) protocol of the National Liver Institute (NLI) and Faculty of Medicine, Menoufia University

Evaluation of cases

Clinical, laboratory and radiological data were collected from patients' medical reports including age, gender, liver enzymes, viral state (hepatitis A, B, C virus, Epstein Barr virus and cytomegalovirus), Serum Autoantibodies, fatty changes, hepatosplenomegaly, liver fibrosis/cirrhosis, ascites and obesity.

Histopathological evaluation

Hematoxylin and eosin (H&E), Masson trichrom, Orcein , Perl's, PAS and D-PAS stained slides for hepatic steatosis specimens were reevaluated to assess steatosis grading, portal and lobular inflammation, stage of fibrosis, hepatic architecture, hepatocyte ballooning, granulomas, pigments, presence of cholestasis, neutrophils and giant cell change.

Immunohistochemistry

The streptavidin-biotin amplified system using a DAKO automated immunostainer system (Autostainer Link 48) was used in this work. The primary antibodies were polyclonal antibodies raised against beclin 1 (concentrated form (100 ul), rabbit) SNF medical company, catalogue no. YPA1333, China, (dilution 1:50) and *LC3A* (concentrated form (50 ul), rabbit) AM biomedical company, catalogue no. A11438, China (dilution1:50). Ultra V block was applied to block non-specific background staining. Antigen retrieval was performed using tris-EDTA high PH. The detection kit was ultravision detection system antipolyvalent HRP/DAB (ready to use, cat. #TP-015-HD; Lab Vision Corporation, Fremont, California). Finally, the reaction can be visualized by appropriate substrate/chromogen (Diaminobenzidine, DAB) reagent with Mayer's hematoxylin as a counterstain. The staining procedure included positive tissue control (human kidney for beclin 1 and Barrett's esophageal tissue for *LC3A*) and negative tissue control by omitting the primary antibodies.

Interpretation of immunostaining results

Cytoplasmic immunoreactivity in any number of cells is required to assign either beclin 1 [15] or *LC3A* [16] positive expression in hepatocytes.

H score system was applied to evaluate the studied cases according to Fedchenko and Reifenrath [17] where both the intensity (scored 1 to 3 as 1=mild, 2=moderate, and 3=strong) and percentage of positivity were considered using the following formula: H score = (3 strong intensity x percentage%) + (2 moderate intensity X percentage%) + (1 mild intensity x percentage%). The maximum score was 3 × 100 = 300 [17].

Statistical analysis

Data were collected, tabulated, and statistically analyzed using a personal computer with the "Statistical Package for the Social Sciences" (SPSS) version 22 program. Fisher's exact and chi-square tests were used for evaluation of qualitative data while Mann-Whitney and Kruskal Wallis test were used for evaluation of quantitative data. P-value 0.05 was considered statistically significant [18].

Results

Number and Percentage of cases according to etiology of steatosis in CLD and IEM groups were shown in supplementary Table 1. CLD group included 19 case of chronic hepatitis with undetermined etiology as reported by the Pediatric Hepatology department. Histologically, liver tissue from those cases revealed steatosis and hepatocellular injury. Other causes of steatosis seen in CLD group included AIH and DILI.One case revealed simple steatosis with no hepatocellular injury. Another case revealed typical histological features of steatohepatitis such as steatosis, hepatocyte ballooning and lobular inflammatory activity. The

Table 1. Association between *Beclin1* H.score of Expression and Clinicopthological Parameters in Chronic Liver Diseases Group (n = 45)

			H Score of *Beclin1*	
		N	Mean ± SD.	Median
Gender	Male	26	225.0 ± 81.55	200
	Female	19	231.58 ± 82.01	300
	U (p)		234.50 (0.755)	
Liver enzymes	Normal	8	212.50 ± 99.10	250
	High	37	231.08 ± 77.60	200
	U (p)		135.0 (0.716)	
Hepatotropic viruses	No	32	223.44 ± 83.26	200
	Yes	13	238.46 ± 76.79	300
	U (p)		188.50 (0.596)	
Non Hepatotropic viruses	No	41	228.05 ± 80.66	200
	Yes	4	225.0 ± 95.74	250
	U (p)		81.0 (0.985)	
Hepato-splenomegaly	Absent	21	235.71 ± 85.36	300
	Present	24	220.83 ± 77.90	200
	U (p)		221.0 (0.444)	
Obesity	Absent	35	230.0 ± 82.43	300
	Present	10	220.0 ± 78.88	200
	U (p)		160.0 (0.697)	
Grade of steatosis	1	37	225.7 ± 86.30	300
	2	7	242.9 ± 53.45	200
	3	1#	200#	200
	U (p)		122.5 (0.826)	
Inflammation	Minimal	2	200.0 ± 141.42	200
	Mild	35	227.14 ± 77.97	200
	Moderate	8	237.50 ± 91.61	300
	H (p)		0.361 (0.835)	
Fibrosis	Absent	1#	100.0#	
	Minimal + Mild	19	207.89 ± 78.64	200
	Moderate	19	247.37 ± 77.23	300
	Marked	6	250.0 ± 83.67	300
	H (p)		3.382 (0.184)	
	Absent, minimal and mild	20	202.50 ± 80.25	200
	Moderate, marked	25	248.0 ± 77.03	300
	U (p)		168.50* (0.043*)	
Liver architecture	Preserved	20	202.50 ± 80.25	200
	Distorted	25	248.0 ± 77.03	300
	U (p)		168.50*(0.043*)	
Hepatocyte ballooning	Absent	34	227.94 ± 82.75	250
	Present	11	227.27 ± 78.62	200
	U (p)		183.50 (0.927)	
Pigments	Absent	44	231.82 ± 77.08	250
	Present	1#	50.0#	
	U (p)		–	
Cholestasis	Absent	32	229.69 ± 81.18	250
	Present	13	223.08 ± 83.21	200
	U (p)		198.50 (0.796)	
Neutrophils	Absent	35	234.29 ± 76.48	300
	Present	10	205.0 ± 95.60	200
	U (p)		144.50 (0.411)	

H, H for Kruskal Wallis test; U, Mann Whitney test ; p, p value for comparing between the different categories; *, Statistically significant at $p \leq 0.05$

clinicopathological parameters of the studied cases were shown in supplementary Table 2.

Beclin1 immunohistochemical (IHC) results

Beclin 1 IHC expression in all the studied groups

Beclin 1 cytoplasmic expression was identified in 100% of control group and the cases with chronic liver disease while the cases with inborn errors of metabolism 86.1% showed positive beclin 1 expresion. In chronic liver disease group, H score mean ± SD was 227.8±80.87 and the median was 200 while in inborn errors of metabolism (IEM) group the mean± SD was 197.2±110.8 and the median was 200. In control group, the mean± SD was 233.8±60.62 and the median was 200.

Association between Beclin1 H.score of expression and clinicopathological parameters in chronic liver diseases group (n = 45) (Table 1)

Higher H.score of expression was observed to be associated with higher stages of fibrosis and distorted liver architecture in chronic liver diseases group, (P=0.043) for both.

Association between Beclin1 positivity and H.score and clinicopathological parameters in the studied inborn error of metabolism (IEM) group (n = 36)

There was no significant association between *Beclin 1* positivity and H score of expression and any of clinicopathological parameters in inborn error of metabolism (IEM) group (Data was not tabulated).

Comparison between the three studied subgroups according to Beclin1 immunohistochemistry (Table 2)

There was a highly statistical significant difference between the three studied groups regarding *Beclin1* positivity as it was positively expressed in all (100%) chronic hepatitis group and in all (100%) control group. However it was positively expressed in 86.1% of inborn errors of metabolism (IEM) group (P=0.011). Also, There was a statistical significant difference between the three studied groups regarding *Beclin1* intensity and percentage of expression, as both of them were higher in chronic liver diseases group and control groups rather than inborn errors of metabolism (IEM) group (P=0.042, 0.004 respectively).

LC3A immunohistochemical (IHC) results

LC3A immunohistochemical expression in the three studied groups (Figure 1)

LC3A positive expression was seen in 51.1% of chronic liver diseases group and 52.8% of inborn errors of metabolism (IEM) group while 81% of control group showed positive *LC3A* expression. Regarding *LC3A* H-score of expression, in chronic liver diseases group the mean± SD was 48.22 ± 73.15 and the median was 10 while in inborn errors of metabolism (IEM) group the mean± SD was 63.89 ± 84.12 and the median was 15. And in control group, the mean± SD was 106.7 ± 79.90 and the median was 100.

Association between LC3A positivity and H-score of expression and clinicopathological parameters in the studied chronic liver disease group (n = 45)

There was no significant association between *LC3A* positivity and H-score of expression and any of clinicopathological parameters in the studied chronic liver disease group (Data was not tabulated).

Association between LC3A positivity and H-score of expression and clinicopathological parameters in the studied inborn error of metabolism (IEM) group (n = 36) (Table 3)

Higher positivity of *LC3A* was significantly associated

Table 2. Comparison between the Three Studied Subgroups According

Beclin1		CLD (n = 45)		IEM (n = 36)
		No.	%	No.
Positivity	Negative	0	0	5
	Positive	45	100	31
	Sig. bet. grps.	$^{FE}p_1$=0.015*,p_2–,$^{FE}p_3$=0.146		
Intensity	Negative	0	0	5
	Mild	9	20	7
	Moderate	14	31.1	8
	Strong	22	48.9	16
	Sig. bet. grps.	$^{MC}p_1$=0.074,p_2=0.195,$^{MC}p_3$=0.055		
	Median	2		2
	Sig. bet. grps.	p_1>0.05,p_2>0.05,p_3>0.05		
Percentage	Median	100		100
	Sig. bet. grps.	p_1=0.091,p_2=0.001*,p_3=0.076		
H score	Median	200		200
	Sig. bet. grps.	p_1>0.05,p_2>0.05,p_3>0.05		

CLD, Chronic liver diseases; IEM, Inborn errors of metabolism; χ^2, Chi square test; MC, Monte Carlo; H, H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test); p, p value for comparing between the three studied subgroups; p_1, p value for comparing between CLD and IEM; p_2, p value for comparing between CLD and Control; p_3, p value for comparing between IEM and Control; *, Statistically significant at $p \leq 0.05$; **, Statistically highly significant at $p \leq 0.01$.

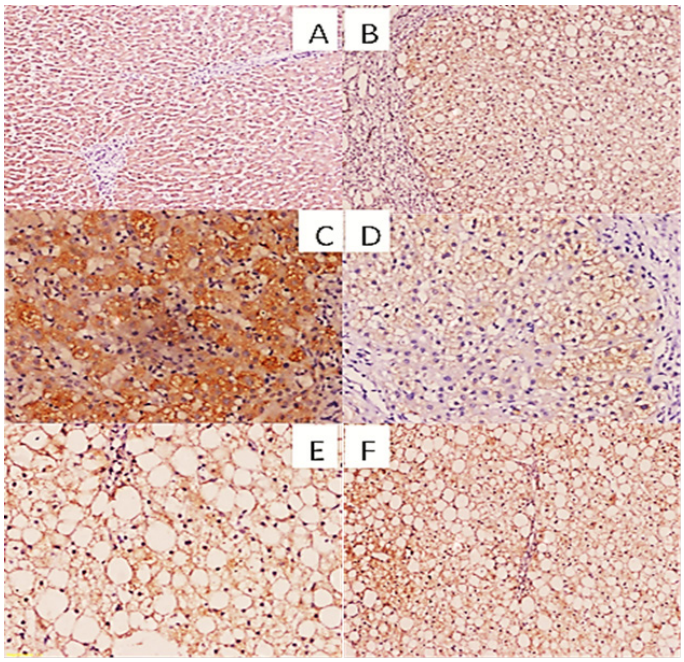

Figure 1. *LC3A* immunohistochemical expression in the studied cases, A: Control liver tissue, showed moderate and diffuse LC3A cytoplasmic staining (IHCx200), B: A case of chronic hepatitis C with marked steatosis, showing mild *LC3A* cytoplasmic staining (IHCx100), C:A case of autoimmune hepatitis with mild steatosis, showing moderate *LC3A* cytoplasmic staining (IHCx400), D: case of chronic hepatitis of undetermined etiology with mild steatosis and marked hepatocyte ballooning, showing moderate *LC3A* cytoplasmic staining (IHCx400), E:A case of steatohepatitis with moderate steatosis, showing diffuse and mild *LC3A* cytoplasmic staining (IHCx400), F:A case of glycogen storage disease with marked steatosis, showing mild and diffuse *LC3A* cytoplasmic staining (IHCx200).

with higher stages of fibrosis and distorted liver architecture in the studied inborn error of metabolism (IEM) group (P=0.021) for both.

Association between intensity and percentage of LC3A positive expression and clinicopathological parameters in all studied cases of pediatric steatosis (n = 42) (Figure 2)

There was a statistical significant association between higher percentage of *LC3A* positive expression and presence of hepatocyte ballooning in all studied cases of pediatric steatosis (p=0.035).

Comparison between all studied groups regarding LC3A immunohistochemical expression (Table 4)

The higher positivity of *LC3A* expression was seen in control group rather than in chronic liver disease group and inborn error of metabolism (IEM) group (P=0.055). as well as higher percentage of *LC3A* expression was significantly observed in control group when compared with its expression in chronic liver disease group and inborn error of metabolism (IEM) group (P=0.001). Additionally, the mean and median values of H score of *LC3A* expression were significantly higher in control group in comparison to chronic liver disease group and inborn error of metabolism (IEM) group (P=0.008).

Discussion

According to the current study, Beclin 1 was expressed

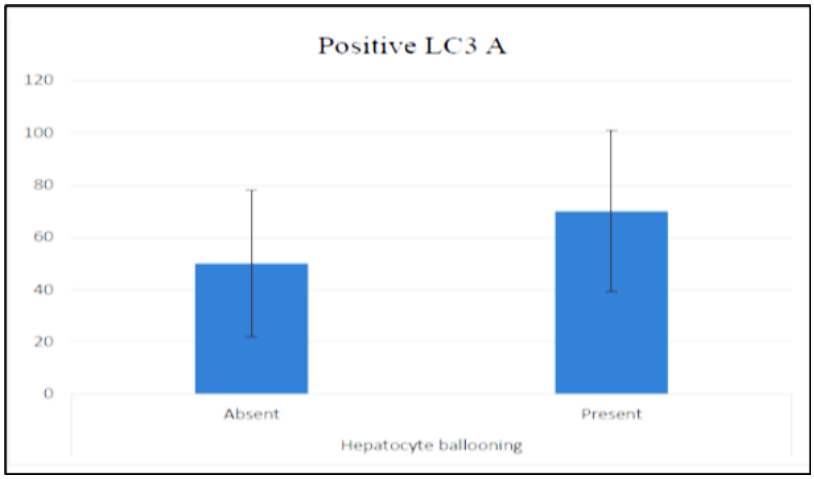

Figure 2. Association between Higher Percentage of *LC3A* Expression and Presence of Hepatocyte Ballooning in All Studied Cases of Pediatric Steatosis

Table 3. Association between *LC3A* Positivity and H.score of Expression and Clinicopathological Parameters in the Studied Inborn Error of Metabolism (IEM) Group (n = 36)

		LC3 A Positivity		
		Negative (n=17)		Positive (n = 19)
		No.	%	No.
Gender	Male	11	64.7	13
	Female	6	35.3	6
	Test of Sig. (p)	χ^2=0.056, p=0.813		
Liver enzymes	Normal	3	17.6	4
	High	14	82.4	15
	Test of Sig. (p)	χ^2=0.066, ^{FE}p=1.000		
Hepatotropic viruses	No	17	100	17
	Yes	0	0	2
	Test of Sig. (p)	χ^2=1.895, ^{FE}p=0.487		
Non Hepatotropic viruses	No	15	88.2	18
	Yes	2	11.8	1
	Test of Sig. (p)	χ^2=0.496, ^{FE}p=0.593		
Hepato-splenomegaly	Absent	2	11.8	4
	Present	15	88.2	15
	Test of Sig. (p)	χ^2=0.557, ^{FE}p=0.662		
Obesity	Absent	7	41.2	9
	Present	10	58.8	10
	Test of Sig. (p)	χ^2=0.139, p=0.709		
Degree of steatosis	1	15	88.2	15
	2	1	5.9	3
	3	1	5.9	1
	Test of Sig. (p)	χ^2=1.077, ^{MC}p=0.800		
Inflammation	Minimal	0	0	0
	Mild	16	94.1	17
	Moderate	1	5.9	2
	Test of Sig. (p)	χ^2=0.253, ^{FE}p=1.000		
Fibrosis	Absent	0	0	0
	Minimal + Mild	11	64.7	5
	Moderate	4	23.5	11
	Marked	2	11.8	3
	Test of Sig. (p)	χ^2=5.539, ^{MC}p=0.066		
	0, minimal, mild	11	64.7	5
	moderate, marked	6	35.3	14
	Test of Sig. (p)	χ^2=5.355*, p=0.021*		
Liver architecture	Preserved	11	64.7	5
	Distorted	6	35.3	14
	Test of Sig. (p)	χ^2=5.355*, p=0.021*		
Hepatocyte ballooning	Absent	1	5.9	5
	Present	16	94.1	14
	Test of Sig. (p)	χ^2=2.697, p=0.182		
Pigments	Absent	17	100	17
	Present	0	0	2
	Test of Sig. (p)	χ^2=1.895, ^{FE}p=0.487		

H, H for Kruskal Wallis test; U, Mann Whitney test; χ^2, Chi square test FE, Fisher Exact; MC, Monte Carlo; p, p value for comparing between the different categories; #, Excluded from the comparison due to small number of case (n = 1); *, Statistically significant at $p \leq 0.05$

Table 3. Continued

		LC3 A Positivity		
		Negative (n=17)		Positive (n = 19)
		No.	%	No.
Cholestasis	Absent	11	64.7	11
	Present	6	35.3	8
	Test of Sig. (p)	χ^2=0.175, p=0.676		
Neutrophils	Absent	11	64.7	12
	Present	6	35.3	7
	Test of Sig. (p)	χ^2=0.009, p=0.923		

H, H for Kruskal Wallis test; U, Mann Whitney test; χ^2, Chi square test FE, Fisher Exact; MC, Monte Carlo; p, p value for comparing between the different categories; #, Excluded from the comparison due to small number of case (n = 1); *, Statistically significant at $p \leq 0.05$

Table 4. Comparison between All Studied Groups Regarding *LC3A* Immunohistochemical Expression

LC3A		CLD (n = 45)		IEM (n = 36)
		No.	%	No.
Positivity	Negative	22	48.9	17
	Positive	23	51.1	19
	Sig. bet. grps.	p_1=0.881, p_2=0.021*, p_3=0.033*		
Intensity	Negative	22	48.9	17
	Mild	11	24.4	9
	Moderate	8	17.8	5
	Strong	4	8.9	5
	Sig. bet. grps.	p_1>0.05, p_2>0.05, p_3>0.05		
	Median	1		1
	Sig. bet. grps.	p_1>0.05, p_2>0.05, p_3>0.05		
Percentage	Median	10		10
	Sig. bet. grps.	p_1=0.498, p_2<0.001*, p_3=0.004*		
H score	Median	10		15
	Sig. bet. grps.	p_1=0.485, p_2=0.002*, p_3=0.017*		

χ^2, Chi square test; MC, Monte Carlo; H, H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test); p, p value for comparing between the three studied subgroups; p_1, p value for comparing between CLD and IEM; p_2, p value for comparing between CLD and Control; p_3, p value for comparing between IEM and Control; **, Statistically highy significant at $p \leq 0.01$

positively in the cytoplasm of 86.1% of IEM cells, 100% of CLD cells, and 100% of normal liver tissue. It's interesting to note that the Glycogen Storage Disease (GSD) etiology is responsible for the 13.9% of the IEM group who showed negative Beclin 1 expression. The vast spectrum of the illness and the existence of 15 distinct GSD disorders may help to explain why Beclin 1 expression is reduced in some but not all cases of GSD. These findings corroborated those of Gautam et al. [19] and Nascimbeni et al. [20], who noted decreased autophagy in specific glycogen storage disorders (GSD) subtypes known as GSD 1a and GSD II, respectively. In agreement with Farah et al. [21] who claimed that a number of glycogen storage disorders had aberrant autophagy, which can impair normal mitochondrial and/or cellular metabolism.

High Beclin 1 H-score of expression was shown to be substantially correlated with advanced fibrosis stage and deformed liver architecture in the chronic liver disease cases evaluated in the current investigation. These findings may be attributed to the hypothesis put up by Tan et al. [22] that Arrestin, beta 1 (-arr1), a scaffolding protein that controls the transmission of G protein-coupled receptor (GPCR) signaling, promotes liver fibrosis through autophagy-mediated Snail signaling. In addition, Ye et al. [23] have shown that ursodeoxycholic acid (UDCA) has an anti-fibrotic impact on rats with liver fibrosis through inhibiting autophagy. However, Ni et al. [24] discovered that the loss of autophagy in hepatocytes leads to proteotoxicity and disruption of pro- and anti-apoptotic protein homeostasis, which results in liver fibrosis.

The study's findings showed no evidence of a statistical relationship between Beclin 1 expression and steatosis. However, Korovila et al. [25] demonstrated that liver fat deposition brought on by a high-fat diet is linked to autophagy failure, which disturbs proteostasis. This might promote the accumulation and stability of lipid droplets even more. The authors of that study employed an animal model that was dependent on a high-fat diet, which is distinct from the numerous etiologies in our

study, therefore it does not match our investigated cases very well.

In terms of *LC3A* immunoexpression, our study's findings showed a statistically significant difference between the three groups: control, CLD, and IEM. In comparison to CLD and IEM groups, the control group had a higher percentage of *LC3A* immunohistochemical positive. Additionally, there was a notable difference between the three groups and the *LC3A* H-score. Compared to the normal control group, the IEM group showed lower autophagy activity by roughly 30%. The continued autophagy activity at later stages of the autophagy pathways is reflected in the expression of *LC3A*. Lower autophagosome content and lower *LC3* are caused by reduced autophagy initiation or impaired autophagosome production. The decreased Beclin 1 levels seen in the metabolic storage disorder in the current study series may help to explain this.

However, we found that *LC3A* degrees on intensity varied widely between different cases, typically increasing or decreasing. This supports data demonstrating that impairment of autophagic activity can happen at various stages of autophagy regulation throughout the onset of fatty liver disease. Many writers contend that ER stress, which is frequently seen in lipid overload/lipotoxicity models, is the upstream indicator of decreased autophagic flux. It's interesting to note that Mitofusin-2 (Mfn2), which was recently reported by Hernández-Alvarez et al. [26], binds to phosphatidylserine and is in charge of phospholipid transfer between the ER and mitochondria to create phosphatidylethanolamine and phosphatidylcholine. Reduced Mfn2 content and abnormal lipid metabolism are found in mice fed on MCD and HFD as well as human NASH patients. While lipid metabolism was not corrected by preventing ER stress, indicators of fibrosis and inflammation were [26]. Cellular lipid composition and mitochondrial phosphatidyl ethanolamine production are known to be needed for full autophagic activation, despite the fact that diminished Mfn2 has been strongly correlated with defective autophagic flux in numerous cell types and tissues [27-30]. Since ATG3 and ATG7, which stand for autophagy-related proteins, integrate phosphatidyl ethanolamine into *LC3-I*, decreased availability in NASH patients may also be a factor in impaired autophagic flux.

In the current investigation, the *LC3A* positive, advanced fibrosis, and deformed liver architecture in the inborn error of metabolism (IEM) group were statistically significantly correlated. The findings of Wan et al. [31] and Shu et al. [32] showing *LC3*-associated phagocytosis protects against hepatic fibrosis were in disagreement with this. Using multiple age groups and varying samples, this result can be explained. The findings of this study clearly showed that steatosis in CLD differs from steatosis in IEM in terms of the various mechanistic pathologic processes involved. This difference may be attributed to the complexity of the cellular makeup of the liver and the variety in how these cells react to pathological or physiological stimuli, necessitating a cell type-specific approach for modulating autophagy function to achieve the desired effect.

In Conclusion, Autophagy might be a double-edged sword in pediatric hepatic steatosis as Beclin 1 and *LC3A* immunohistochemistry revealed different intensity grades reflecting fluctuation of autophagy at different stages of the disease and different disease etiology.

Study Limitations

First, The CLD included 19 cases of chronic hepatitis of undetermined etiology as diagnosed at different years since 2015. Those cases would turn out to be steatohepatitis as the patients suffered from an underlying liver disease that revealed steatosis and inflammation in hepatic parenchyma. This will expand the number of cases of steatohepatitis that would help in exploring the role of autophagy in MAFLD. Second, the small number and diverse etiology of cases with steatosis in this study made it difficult to answer the question raised by this investigation. Therefore, it is recommended to design the same study using single etiology for steatosis.

Author Contribution Statement

Marwa Salah Gadallah and Shaymaa El-Gammal evaluated the slides and wrote the manuscript, Mona Kandil and Nanis Holah prepared the results and figures, Nermine Ehsan prepared the discussion, Gihan Sobhy helped in diagnosis of all cases in this study and Mohamed Mohamady helped in immunostaining process.

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Ethical Approval

This paper was approved by ethical committee of faculty of medicine, Menofia university

Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from all subjects.

Data Availability Statement

All data and results included in this paper are available with the corresponding author upon request

Competing interests

No conflict of interest regarding this manuscript

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