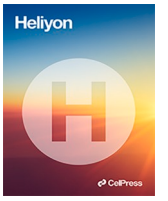
Heliyon 10 (2024) e27557

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Imbalance of mitochondrial fusion in peripheral blood mononuclear cells is associated with liver fibrosis in patients with metabolic dysfunction-associated steatohepatitis

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ARTICLE INFO

Keywords:
Non-alcoholic fatty liver disease
Liver cirrhosis
Mitochondria

ABSTRACT

Mitochondrial dysfunction and inflammation contribute to the pathophysiology of metabolic dysfunction-associated steatohepatitis (MASH). This study aims to evaluate the potential association between mitochondrial dynamics and cell death markers from peripheral blood mononuclear cells (PBMCs) and the presence of MASH with significant liver fibrosis among metabolic dysfunction-associated steatotic liver disease (MASLD) patients. Consecutive patients undergoing bariatric surgery from January to December 2022 were included. Patients with histologic steatosis were classified into MASH with significant fibrosis (F2-4) group or MASLD/MASH without significant fibrosis group (F0-1). Mitochondrial dynamic proteins and cell death markers were extracted from PBMCs. A total of 23 MASLD/MASH patients were included (significant fibrosis group, n = 7; without significant fibrosis group, n = 16). Of the mitochondrial dynamics and cell death markers evaluated, OPA1 protein, a marker of mitochondrial fusion is higher in MASH patients with significant fibrosis compared to those without (0.861 ± 0.100 vs. 0.560 ± 0.260 proportional to total protein, p = 0.001). Mitochondrial fusion/fission (OPA1/DRP1) ratio is significantly higher in MASH patients with significant fibrosis (1.072 ± 0.307 vs. 0.634 ± 0.313, p = 0.009). OPA1 (per 0.01 proportional to total protein) was associated with the presence of significant liver fibrosis with an OR of 1.08 (95%CI, 1.01–1.15, p = 0.035), and adjusted OR of 1.10 (95%CI, 1.00–1.21, p = 0.042). OPA1 from PBMCs is associated with MASH and substantial fibrosis. Future studies should explore if OPA1 could serve as a novel non-invasive liver fibrosis marker.

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https://doi.org/10.1016/j.heliyon.2024.e27557
Received 5 January 2024; Received in revised form 27 February 2024; Accepted 1 March 2024
Available online 8 March 2024

1. Introduction

The prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) has increased in recent years [1]. Metabolic dysfunction-associated steatohepatitis (MASH) is prevalent in individuals with metabolic syndrome and obesity [2,3]. MASH is an independent risk for cardiovascular, liver-related, and overall mortality [3–5]. Liver fibrosis is currently considered as the best predictor of disease severity and patient outcomes. Long-term overall mortality, liver transplantation, and liver-related events are all correlated with the grade of liver fibrosis in MASH patients [6]. The gold standard for diagnosis of MASH and liver fibrosis involves a liver biopsy. However, liver biopsy is an invasive procedure with potentially fatal complications. Thus, non-invasive methods to evaluate liver fibrosis in these patients are being intensively investigated.

Previous data from both *in vivo* and human studies showed that hepatic mitochondrial dysfunction is one of the significant pathogenic findings in MASLD and MASH. In animal models with MASH, abnormal mitochondrial dynamic function (fission and fusion) [7–9], oxidative stress [10], necroptosis, apoptosis [11,12], and mitophagy were significantly associated with the severity of the disease. A comparison between patients with MASLD and healthy controls found changes in liver oxidation [13], fatty oxidation [14], levels of reactive oxygen species (ROS) [15,16], and mitochondrial activity [17–22]. Furthermore, correlations were found between these parameters and disease severity [23]. Recent evidence showed that whole fatty acid oxidation, mitochondrial biogenesis markers, hepatic mitophagy, and hepatic mitochondrial dynamic markers from the liver tissue were associated with MASH [24]. Mitochondria are also known to regulate apoptosis and necroptosis, which are a part of key regulatory processes in human metabolic liver disease [25]. As a result, apoptosis and necroptosis were found to be linked to MASH pathogenesis [26].

Inflammation is essential in the development and progression of MASH, involving several immune cell-mediated inflammatory processes [27]. In a mouse model of obesity-related MASH, the progression of the disease induced characteristic transcriptional alterations of an inflammatory immune response in myeloid cells in both the liver and bone marrow, indicating the systemic effect of MASH on monocytes [28]. It is currently unknown if the mitochondrial dysfunction that was demonstrated in the liver of patients with MASH also occurs in the patient's peripheral blood mononuclear cells (PBMCs). Furthermore, an association between mitochondrial dynamics and cell death markers and disease severity has not yet been evaluated. Therefore, our research aimed to investigate the association between mitochondrial dynamics and cell death markers from PBMCs and MASH with and without significant liver fibrosis.

2. Methods

2.1. Study design and patient population

This study was a cross-sectional study. Twenty-nine consecutive MASLD patients were prospectively recruited from the bariatric surgery unit at Maharaj Nakorn Chiang Mai, Thailand, from January 2022 to December 2022. The inclusion criteria were as follows: (i) aged more than 18 years; (ii) a diagnosis with metabolic syndrome according to NCEP III criteria; (iii) scheduled for bariatric surgery and (iv) a history of alcohol drinking less than 10 g/day in females and 20 g/day in males in the past 12 months. The exclusion criteria were as follows: (i) presence of other conditions that may be associated with hepatic steatosis identified by history, physical examination, and laboratory investigation; (ii) the presence of viral hepatitis or autoimmune liver disease; (iii) having renal dysfunction (estimated glomerular infiltration rate by Chronic Kidney Disease Epidemiology Collaboration method $<$60 mL/min/1.72 m^2); (iv) pregnancy or breastfeeding and (v) patients unable to provide informed consent. The study was approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University (MED-2564-08711).

2.2. Liver biopsy and histology

Participants provided written informed consent for an intraoperative liver biopsy during bariatric surgery. Liver biopsies were performed with 16-gauge biopsy needles (Hunter^{tm} biopsy; DKSH) or wedge liver resection by surgeon. The adequacy of the liver biopsy samples was determined by a length $>$1.5 cm and containing at least 11 portal tracts [29]. Liver histology was interpreted by an experienced pathologist. Patients were included in the analysis if they had histologic diagnosis of MASLD defined as histologically confirmed steatosis of $\geq$5%. MASLD patients were then assigned into groups based on the histopathological findings; MASH with significant liver fibrosis and MASLD/MASH with no significant liver fibrosis. Histologic MASH was defined by the presence of steatosis with lobular and/or portal inflammation, and/or hepatocellular ballooning [30]. Mallory staining was used for fibrosis estimation. The biopsy specimens were then graded and staged into stage 0, 1, 2, 3, 4 according to Metavir scoring system [31]. Significant liver fibrosis was defined by those who had stage 2–4 fibrosis and no significant liver fibrosis was defined by those who had stage 0–1 fibrosis.

2.3. Data collection and definitions

Medical history, clinical assessment, and laboratory tests were evaluated at baseline. Waist circumferences were measured by the investigator in accordance with the WHO criteria. All blood testing was performed on the morning of surgery after an overnight fast of 8–12 h. Serum biochemistry included fasting glucose, complete blood count, creatinine, liver biochemistry, gamma GT, prothrombin time, HBsAg, Anti-HCV, ANA-titer, IgG, lipid profile, and PBMCs.

Comorbidities, including diabetes mellitus, hypertension, and hyperlipidemia, were defined as follows. Diabetes was defined as the

patients who had a clinical history of diabetes, current use of antidiabetic medications, and/or fasting plasma glucose ≥126 mg/dL. Hypertension was defined as patients who had a clinical history of hypertension, current use of antihypertensive medications, and/or documented high blood pressure (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg). Hyperlipidemia was defined as patients who had a clinical history of hyperlipidemia, current use of antilipemic medications, and/or serum triglycerides ≥150 mg/dL, serum cholesterol ≥240 mg/dL, serum low-density lipoprotein cholesterol ≥190 mg/dL, or high low-density lipoprotein cholesterol indicated for lipid-lowering treatment (16).

2.4. Mitochondrial dynamics and cell death markers from PBMCs

PBMCs were isolated by Ficoll gradient technique as previously described [32]. The protein was extracted from PBMCs using a radioimmunoprecipitation assay (RIPA) buffer. The total protein (1 mg/mL) was mixed with loading buffer, loaded onto 10% SDS-acrylamide gels, and then transferred to nitrocellulose membranes in a glycine/methanol-transfer buffer using a Wet/Tank blotting system (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were blocked in 5% bovine serum albumin in tris-buffered saline and tween buffer for 1 h, and the membranes were incubated with anti-RIPK1, RIPK3, MLKL, Caspase 3, Caspase 8, *p*-Drp1ser616, Drp1, Mfn1, Mfn2, OPA1, PINK1, and Parkin overnight at 4 °C. Total protein was used as a loading control. Bound antibodies were detected using horseradish peroxidase-conjugated with anti-mouse or rat IgG (1:200 dilution; Cell Signaling technology, Danvers, MA, USA). The membranes were exposed to an ECL Western blotting substrate (Bio-Rad Laboratories), and then densitometric analysis was carried out using a ChemiDoc Touch Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). The proteins were normalized to the total protein (Supplementary materials Figure 1A) using stain-free staining and the results are reported as the ratio of the proteins of interest to total protein.

2.5. Statistical analyses

Patient characteristics are presented as mean and standard deviation, median and interquartile range (IQR) or frequencies as appropriate. The differences between groups were compared using an independent *t*-test for parametric data, Wilcoxon rank sum test for non-parametric data, or chi-square test, or fisher exact test for categorical data. Univariate and multivariate analyses of markers associated with MASH and significant liver fibrosis were analyzed using logistic regression analysis. All statistical analyses were performed using Stata Version 15.1 (Stata Corp LCC, Texas) software, and a p-value < 0.05 was considered statistically significant.

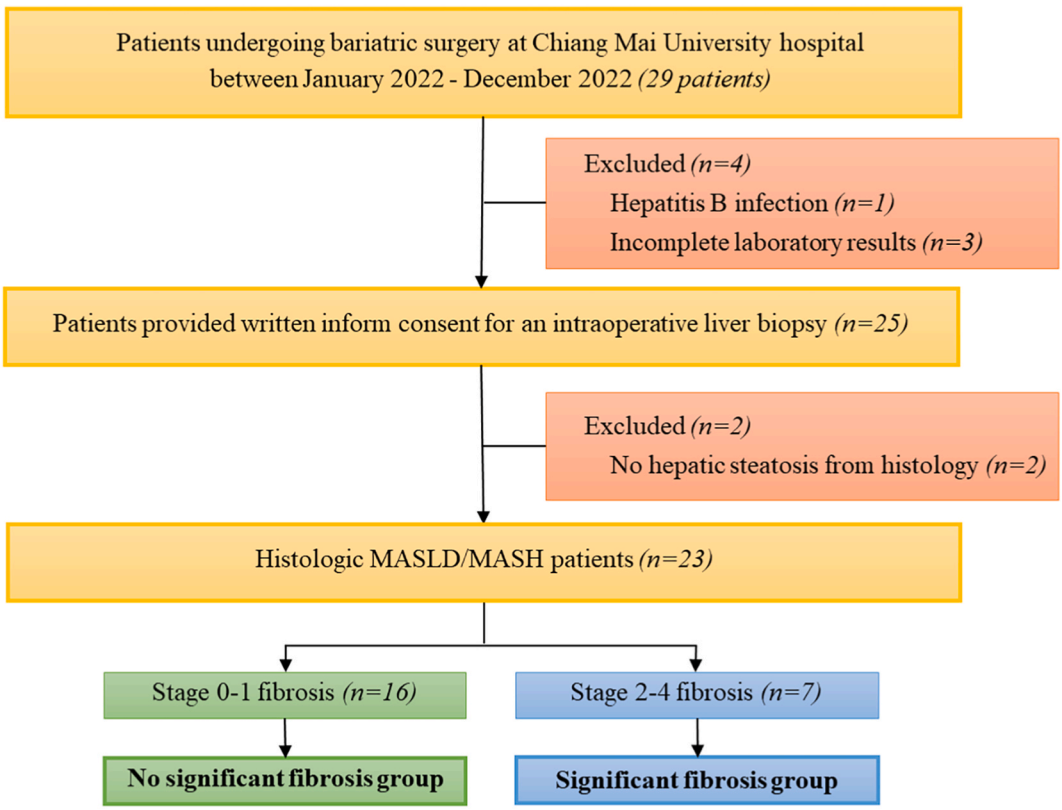

Fig. 1. Flow diagram of patient inclusion.

3. Results

A total of twenty-nine patients were enrolled. After exclusion of 4 patients due to the exclusion criteria and 2 patients whose liver histology showed no steatosis, 23 MASLD patients were included in the current study (Fig. 1).

3.1. Baseline characteristics

Baseline characteristics of all patients, both with and without significant fibrosis, are shown in Table 1. The majority of participants were female (60.9%) with a mean BMI of 43.7 ± 8.4 kg/m^2 and waist circumference of 124.5 ± 16.1 cm. The median age was 30.2 (IQR 28,41) years. There were 7 patients in the MASH with significant fibrosis group (5 patients with F2, 1 patient with F3, and 1 patient with F4) and 16 patients in the non-significant fibrosis group. Representative histological images from patients with each stage of liver fibrosis are shown in Fig. 2(A–E).

There were no significant differences in age, sex, waist circumference, BMI, diabetes mellitus, hypertension, medication used, and total NAFLD activity scores (NAS) between the two groups. Compared to patients without significant fibrosis, patients with liver fibrosis had a higher proportion of hyperlipidemia (100% vs. 68.9%, p = 0.020), and a lower level of high-density lipoprotein cholesterol (HDL-C) (33.4 ± 5.8 vs. 42.9 ± 10.5 mg/dL, p = 0.038). AST level was slightly higher in the patients with liver fibrosis group (31 ± 9.1 vs. 23.1 ± 10.3 U/L, p = 0.093).

3.2. Mitochondrial dynamics and cell death markers in peripheral blood mononuclear cells

Details of densitometric quantification of mitochondrial dynamics and cell death markers in PBMCs are shown in Table 2. Full Western blot images are shown in Supplementary materials Figs. 1(A–G) and 2(A–G). The level of Optic atrophy 1 (OPA1), a marker of mitochondrial fusion, was higher in patients with significant liver fibrosis compared to those without liver fibrosis (0.861 ± 0.100 vs. 0.560 ± 0.260 proportional to total protein, p-value = 0.001) (Fig. 3(A)). The mean ratio of OPA1 to Dynamin-related protein 1 (DRP1), indicating mitochondrial fusion to fission, is higher in the significant fibrosis group (1.072 ± 0.307 vs. 0.634 ± 0.313, p-value

Table 1
Patient demographics and clinical characteristics in MASLD populations and differentiated by group of patients with significant fibrosis and patients without significant fibrosis.

Characteristics	Total (N = 23)	Significant fibrosis (n = 7)	No significant fibrosis (n = 16)	p-value
Male sex, n (%)	9 (39.1)	2 (28.6)	7 (43.8)	0.512
Age, years, median (IQR)	30.2 (28, 41)	35.5 (28, 50.5)	29.4 (27.8, 31.8)	0.127
Body weight, kg, mean ± SD	121.1 ± 26.4	119.2 ± 29.8	122.0 ± 25.7	0.826
Body mass index, kg/m^2, mean ± SD	43.7 ± 8.4	42.1 ± 6.9	44.5 ± 9.1	0.538
Waist circumference, centimeters, mean ± SD	124.5 ± 16.1	126.1 ± 16.9	124.8 ± 16.3	0.755
Diabetes mellitus, n (%)	12 (52.2%)	5 (71.4%)	7 (43.8%)	0.241
Hypertension, n (%)	16 (69.5%)	6 (85.7%)	10 (62.5%)	0.240
Hyperlipidemia, n (%)	18 (78%)	7 (100%)	11 (68.9%)	0.020
Medication use, n (%)				
Metformin	8 (34.8%)	4 (57.1%)	4 (25%)	0.182
SGLT2 inhibitor	2 (8.7%)	2 (28.6%)	0 (0%)	0.083
GLP-1 receptor agonist	5 (21.8%)	2 (28.6%)	3 (18.8%)	0.621
Insulin	2 (8.7%)	1 (14.3%)	1 (6.3%)	0.526
Aspirin	1 (4.4%)	0 (0%)	1 (6.3%)	1.000
Statin	8 (34.8%)	4 (57.1%)	4 (25%)	0.182
Albumin, mg/dL, mean ± SD	4.2 ± 0.3	4.1 ± 0.3	4.2 ± 0.3	0.485
AST, U/L, mean ± SD	25.5 ± 10.4	31 ± 9.1	23.1 ± 10.3	0.093
ALT, U/L, median (IQR)	29 (20, 57)	35 (21, 61)	28 (17.5, 52)	0.483
Total bilirubin, mg/dL, mean ± SD	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.409
Platelet count, k/mcL, mean ± SD	303.0 ± 64.7	299.1 ± 60.7	304.6 ± 68.3	0.857
INR, mean ± SD	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.708
Gamma-GT, mg/dL, median (IQR)	31 (25, 42)	28 (22, 42)	33 (28.5, 43.5)	0.639
Fasting plasma glucose, mg/dL, mean ± SD	106.6 ± 19.9	115 ± 22.4	102.9 ± 18.3	0.237
Cholesterol, mg/dL, mean ± SD	175.6 ± 37.5	159.3 ± 44.5	182.8 ± 33.1	0.173
Triglyceride, mg/dL, median (IQR)	141 (117, 180)	153 (106, 265)	138.5 (117.5, 179)	0.769
HDL-C, mg/dL, mean ± SD	40 ± 10.2	33.4 ± 5.8	42.9 ± 10.5	0.038
LDL-C, mg/dL, mean ± SD	110.3 ± 27.7	100.4 ± 33.4	104.6 ± 24.7	0.267
Fibrosis stage, n (%)				
F0	11 (47.8)	0	11 (67.8)	–
F1	5 (21.7)	0	5 (31.2)	–
F2	5 (21.7)	5 (71.4)	0	–
F3	1 (4.3)	1 (14.3)	0	–
F4	1 (4.3)	1 (14.3)	0	–
Total NAFLD activity score	3.304 ± 1.579	3.188 ± 1.721	3.571 ± 1.272	0.561

Abbreviations: AST: Aspartate transferase; ALT: Alanine aminotransferase; GLP-1: glucagon-like peptide 1; HDL-C: high-density lipoprotein cholesterol; LDL-C: lower density lipoprotein cholesterol; SGLT2: Sodium-glucose transport protein 2.

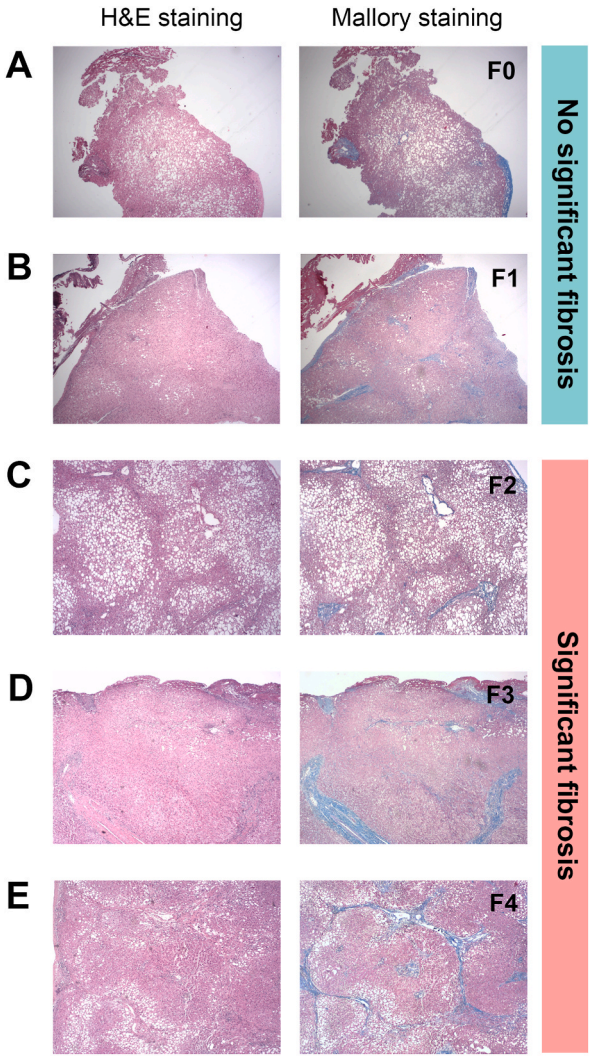

Fig. 2. Representative histological images from patients with each stage of liver fibrosis according to Metavir scoring system. (A) specimen assessed as fibrosis stage 0, (B) specimen assessed as fibrosis stage 1, (C) specimen assessed as fibrosis stage 2, (D) specimen assessed as fibrosis stage 3, (E) specimen assessed as fibrosis stage 4, (Left, H&E stain; Right, Mallory stain; 40x original magnification).

= 0.009) (Fig. 3(B)). Further analysis showed that patients with liver fibrosis had significantly higher levels of OPA1 as demonstrated by univariate analysis (OR 1.08 per 0.01 proportional to total protein; 95%CI, 1.01–1.15, p = 0.035), and by multivariable analysis adjusted for age, sex, and diabetes mellitus (adjusted OR 1.10 per 0.01 proportional to total protein; 95%CI, 1.00–1.21, p = 0.042) **(Supplementary materials Table 1).**

There were no significant differences between groups regarding other mitochondrial dynamics and cell death markers in PBMCs, including DRP1, MFN1, MFN2, PINK1, Parkin, RIPK1, RIPK3, MLKL, activated Caspase3, and Caspase 8.

4. Discussion

The main finding of our study is that a higher level of OPA1 from PBMCs is associated with MASH and liver fibrosis in patients with MASH/MASLD. OPA1 is a significant protein controlling mitochondrial fusion. It is also involved in the biogenesis [33] and respiration [34] of cristae. OPA1 has a vital role in the immune system, involving both lymphoid and myeloid series. OPA1 works together with the transcription factor, the nuclear factor kappa B (NF-κB), in monocytes to regulate the inflammatory response [35,36]. NF-κB plays a vital role in regulating innate and adaptive immune functions and is a key mediator of inflammatory responses [35]. A preclinical study demonstrated that OPA1 deletion results in defective activation of NF-κB signaling and dysfunctional macrophage functioning [36]. Despite many studies having described the detrimental effect of the loss of OPA1 function in various diseases, the overexpression of OPA1 can also disrupt mitochondrial morphology [37]. Hence, the appropriate level of OPA1 expression is considered critical for normal mitochondrial function.

Table 2
Densitometric quantification of mitochondrial dynamics and cell death markers in peripheral blood mononuclear cells differentiated by group of patients with significant fibrosis and patients without significant fibrosis.

Tests	Significant fibrosis (n = 7)	No significant fibrosis (n = 16)	p-value
Mitochondrial dynamics			
Mitochondrial fission			
DRP1/total protein, mean ± SD	1.251 ± 0.186	1.261 ± 0.168	0.907
Mitochondrial fusion			
MFN1/total protein, mean ± SD	0.822 ± 0.249	0.750 ± 0.191	0.452
MFN2/total protein, mean ± SD	0.850 ± 0.200	0.903 ± 0.166	0.509
OPA1/total protein, mean ± SD	0.861 ± 0.100	0.560 ± 0.260	0.001
Mitochondrial fusion/fission ratio			
MFN1/DRP1, mean ± SD	0.649 ± 0.139	0.602 ± 0.167	0.493
MFN2/DRP1, mean ± SD	1.092 ± 0.349	1.265 ± 0.330	0.289
OPA1/DRP1, mean ± SD	1.072 ± 0.307	0.634 ± 0.313	0.009
Mitophagy			
PINK1/total protein, mean ± SD	0.690 ± 0.183	0.685 ± 0.139	0.944
Parkin/total protein, mean ± SD	0.775 ± 0.248	0.787 ± 0.187	0.904
Necroptosis markers			
RIPK1/total protein, mean ± SD	0.852 ± 0.252	0.820 ± 0.145	0.703
RIPK3/total protein, mean ± SD	0.445 ± 0.251	0.418 ± 0.155	0.756
MLKL/total protein, mean ± SD	0.873 ± 0.268	0.854 ± 0.199	0.851
Apoptosis markers			
Cleaved Caspase3/Procaspase3, mean ± SD	1.257 ± 0.325	1.755 ± 0.625	0.062
Caspase 8/total protein, mean ± SD	0.837 ± 0.264	0.839 ± 0.198	0.982

Abbreviations: DRP1: dynamin-related protein 1; MFN1: Mitofusin-1; MFN2: Mitofusin-2; OPA1: optic atrophy-1; PINK1: PTEN-induced kinase 1; RIPK1: Receptor-interacting serine/threonine-protein kinase 1; RIPK3: Receptor-interacting protein kinase-3; MLKL: Mixed lineage kinase domain like pseudokinase.

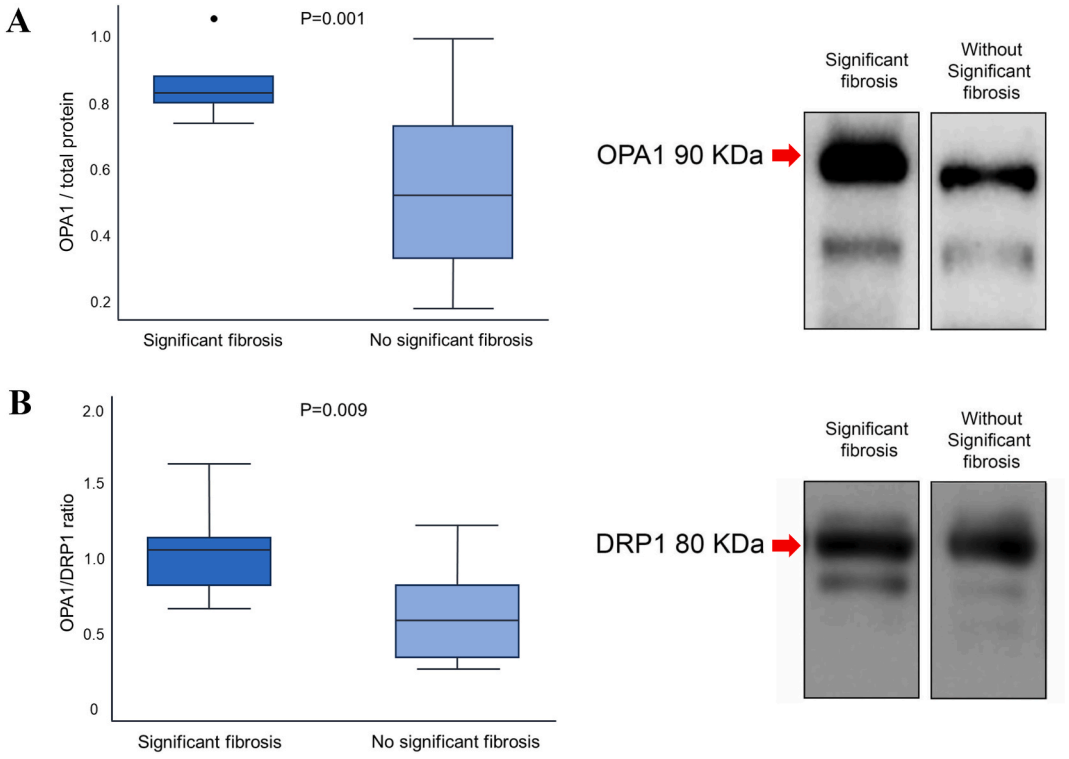

Fig. 3. Densitometric quantification graphs and representative Western blot images of (A) OPA1/total protein in patients with and without significant liver fibrosis, and (B) OPA1/DRP1 ratio in patients with and without significant liver fibrosis. For full, non-adjusted images of Western blots see Supplementary materials Figure 4A,G.

There are few studies exploring the alterations of mitochondrial dynamics in the context of MASLD/MASH. One recent study showed that liver tissue with MASH had lower levels of OPA1 compared to patients without MASLD [24]. Another fusion marker, MFN2, was also significantly reduced in the liver of those with MASLD and MASH [24,38]. While there is evidence of alteration in mitochondrial fusion in patients with MASLD/MASH, studies of the association between mitochondrial dynamics and the degree of liver fibrosis are scarce. There was a trend towards lower hepatic OPA1 level in the patients with definite MASH, a group with a higher prevalence of liver fibrosis, than those with borderline MASH [24]. However, to our knowledge, there is no data comparing the changes of liver mitochondrial fusion markers in MASH patients with and without significant liver fibrosis.

Changes in peripheral monocytes have been demonstrated to be linked to MASLD-related liver fibrosis. Circulating monocytes isolated from patients with fibrosis have been found to express elevated levels of the inflammatory cytokines [39]. Our findings from PBMCs show higher level of the fusion marker OPA1 in MASH patients with liver fibrosis. While our findings contradict the results of a prior study in liver tissue [24], We hypothesize that the changes in the PBMCs could be the result of a compensatory mechanism responding to the low levels of expression of OPA1 in the liver tissue. Alternatively, MASH may have different effects on different tissues. Future research is needed to determine the mechanisms associated with this finding.

Mitophagy markers from the liver tissue including PINK1 and Parkin were previously reported to be unchanged across MASLD/MASH patients with varying degree of MASH severity and fibrosis [24]. This is in line with our study results showing no difference in these markers from PBMCs between patients with significant and non-significant liver fibrosis. Furthermore, in the current study no differences were found in the cell death markers in PBMCs of patients with and without liver fibrosis. Prior studies demonstrated an increase in apoptotic cells in liver tissue from an animal model of MASLD [40] and patients with MASH [41]. Our results can be interpreted to postulate that systemic apoptotic activity does not show a correlation with the severity of liver fibrosis in patients with MASLD. This could possibly be explained by the fact that cell death mainly occurs in the liver but that it does not occur systemically.

Currently, there are no approved pharmacological treatments for MASH or MASLD. Drugs approved for the treatment of comorbidities, e.g., obesity or diabetes, may have potential benefits for MASLD and may be considered for use with selected patients [42]. To our knowledge, it is presently unknown whether current pharmacological treatments, including GLP-1 receptor agonists and SGLT2 inhibitors can potentially affect mitochondrial dynamics. Further research in this area is needed. Furthermore, bariatric surgery may possibly have an effect on mitochondrial dynamics. Roux-en-Y gastric bypass surgery has been shown to improve markers of mitochondrial dynamics in a rat model of obesity [43]. A study in humans demonstrated an increase in hepatic mitochondrial respiratory capacities and mitochondrial biogenesis after bariatric surgery [44]. This issue is not addressed in the current study as our study is cross-sectional in design. The effect on the changes of mitochondrial dynamic in PBMCs resulting from bariatric surgery remains to be elucidated. Lastly, our research focused on the study of mononuclear cells and the granulocytes were discarded. A previous study suggested that neutrophils, the most abundant subset of granulocytes, also play an important role in regulating inflammation in MASH and can be used to predict MASLD progression [45]. However, the relationship between mitochondrial dynamics alteration in granulocytes, particularly neutrophils, in MASLD patients still needs further investigation.

Our study has several limitations to be addressed. First, the mitochondrial dynamics and cell death markers were not tested in the liver. Thus, we cannot directly evaluate the correlation between these parameters from hepatic tissue and PBMCs. Secondly, the protein extraction in our study was performed using a semi-quantitative method, therefore the cut-off for determining liver fibrosis cannot be assessed with precision. Consequently, additional research is required to measure the levels of OPA1 using a quantitative method. Finally, as our research aimed to differentiate the patients with significant liver fibrosis from MASLD patients without significant fibrosis and due to ethical consideration, liver tissue and PBMCs from healthy individuals to serve as a control group were not collected. Future research should include such a control group to validate the changes across the groups. The strength of our study includes the histologic diagnosis of MASH and liver fibrosis at the time of blood sample collection for PBMCs. The study also demonstrated the potential for a novel non-invasive marker from PBMCs to identify significant fibrosis in MASH patients.

In conclusion, our study showed that a marker for mitochondrial fusion, OPA1, from PBMCs was associated with MASH and significant liver fibrosis. Future studies should investigate further the potential for OPA1 to serve as a novel non-invasive marker for liver fibrosis in patients with MASH.

Ethics declarations

This study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University (IRB-No MED-2564-08711). All participants provided written informed consent to participate in the study. All experiments were performed in accordance with relevant named guidelines and regulations.

Funding

This study was funded by the Faculty of Medicine Research Fund, Chiang Mai University, Thailand (Grant No. MED-2564-08711), the NSTDA Research Chair Grant from the National Science and Technology Development Agency Thailand (NC), the Distinguished Research Professor Grant from the National Research Council of Thailand (SCC), and Chiang Mai University Center of Excellence Award (NC).

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Thanaput Kunlayawutipong anlayawutipong: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Nattayaporn Apaijai:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Kanokkan Tepmalai:** Writing – review & editing, Data curation, Conceptualization. **Sarawut Kongkarnka:** Writing – review & editing, Data curation, Conceptualization. **Apinya Leerapun:** Writing – review & editing, Conceptualization. **Kanokporn Pinyopornpanish:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Atiwat Soontornpun:** Writing – review & editing, Methodology, Conceptualization. **Siriporn C. Chattipakorn:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Nipon Chattipakorn:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Kanokwan Pinyopornpanish:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank all the participants in the study and Mrs. Antika Wongthanee, M. Sc. for statistical consultation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27557.

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