

# Investigation of the effects of wet cupping therapy on some inflammatory factors in patients affected by non-alcoholic fatty liver disease (NAFLD): A quasi-experimental trial study with self-controls

Nooshin Abbasi<sup>1</sup>, Mahdi Biglarkhani<sup>2</sup>, Azam Meyari<sup>2</sup>, Razieh Amini<sup>2</sup>, Marco Fiaschi<sup>3</sup>, Rezvan Najafi<sup>2</sup>

<sup>1</sup> University of Padua

<sup>2</sup> Hamadan University of Medical Sciences

<sup>3</sup> MFC Elettronica

**Funding:** This study was funded by Iran's National Elites Foundation (INEF), an Iranian cooperation postdoctoral program for overseas specialists under Grant number 140007205787.

**Potential competing interests:** The author(s) declared that no potential competing interests exist.

## Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. In this study, we assess the effects of wet cupping therapy, an ancient therapeutic method, on some inflammatory cytokines believed to impact the inflammatory state of the liver of patients affected by NAFLD. A total of sixteen NAFLD grade II and III patients, diagnosed by abdominal ultrasound, participated in this quasi-experimental study with self-control. The subjects received wet cupping therapy three times over six weeks. We assessed anthropometric and biochemical parameters pre- and post-interventions. Results showed that wet cupping therapy reduced C-reactive protein (CRP) by an average of 50% in 67% of NAFLD patients. The first two wet cupping treatments reduced interleukin-1 $\beta$ , especially the second one for an average of 29% in 67% of patients. The efficacy of wet cupping to modify interleukin-6 was seen in the first wet cupping treatment with an average increase of 182% in 41% of patients. This treatment showed an average reduction of 16% of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in 59% of patients. Wet cupping therapy may be seen as a treatment to reduce the inflammatory state in patients affected by NAFLD when given at proper intervals.

Nooshin Abbasi<sup>1</sup>, Mahdi Biglarkhani<sup>2</sup>, Azam Meyari<sup>2</sup>, Razieh Amini<sup>1</sup>, Marco Fiaschi<sup>3</sup>, Rezvan Najafi<sup>1</sup>

<sup>1</sup> Research center for molecular medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

<sup>2</sup> Department of Persian Medicine, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

<sup>3</sup> MFC Elettronica, Padua, Italy

**Keywords:** Non-Alcoholic Fatty Liver Disease (NAFLD); Wet Cupping Therapy; Tumor Necrosis Factor- $\alpha$ ; Interleukin-1 $\beta$ ; Interleukin-6; High-Sensitivity C-Reactive Protein.

## 1. Introduction

Non-alcoholic fatty liver disease (NAFLD), recently re-named metabolic dysfunction associated with fatty liver disease (MAFLD), is the most common chronic liver disease. It affects more than a quarter of adults and children worldwide. NAFLD is defined as fat deposition or the presence of hepatic steatosis (HS) in  $\geq 5\%$  of hepatocytes by histology or the presence of intrahepatic triglyceride content  $\geq 5.5\%$  by imaging in hepatic parenchyma of individuals with little or no alcohol consumption<sup>[1][2][3]</sup>. It ranges from hepatic steatosis to non-alcoholic steatohepatitis (NASH) both are asymptomatic and may lead to the development of cirrhosis and liver cancer. NAFLD is also associated with hypertension, cardiovascular diseases, dyslipidemia, type 2 diabetes mellitus, and obesity<sup>[4]</sup>. However, studies suggest that metabolically unhealthy individuals with metabolic comorbidities have a higher risk of fatty liver disease compared with healthy individuals, irrespective of body mass index (BMI) as NAFLD with its heterogeneous pathogenesis occurs in both obese and lean phenotypes<sup>[5]</sup>. It seems that metabolic health is influenced by 1. genetics, epigenetics, diet, lifestyle, enterohepatic circulation, and gut microbiota<sup>[6]</sup>.

The molecular pathways involved in pathogenesis and progression of NAFLD although not fully studied it is believed that inflammatory molecules including C-reactive protein (CRP), TNF, IL-6 and IL-8, IL-1 receptor antagonist protein (IL-1RA), and CXCL10 (CXCL10) may have a key role in hepatic injury and NAFLD progression<sup>[3]</sup>.

Currently, no effective therapy to treat NAFLD or to fully target inflammation status in this disorder has been approved. No pharmacological therapy is available. The only successful therapeutic strategy is lifestyle modification including diet modification, weight loss, and exercise<sup>[7][8]</sup>. These observations postulate that targeting inflammation in subjects affected by NAFLD may be seen as a novel therapeutic strategy to postpone the disease progression.

Cupping therapy, an ancient medical therapy practiced mainly in the Middle East and China, is believed to improve quality of life through muscle stimulation and to treat pain in the head, neck, and back areas (From National Center for Complementary and Integrative Health). There have been few studies that assessed the effects of cupping therapy on inflammatory cytokines involved in the inflammatory state of the liver<sup>[9]</sup>. Although the anti-inflammatory effects of cupping have been proven to different pathological conditions including rheumatoid arthritis and autoimmune diseases.<sup>[10][11][12][13]</sup>

In this study, the effects of wet cupping therapy on some inflammatory cytokines believed to take part in the inflammatory cascade derived from increased visceral adipose tissue in the liver of patients affected by NAFLD were accessed.

## 2. Materials and Methods

### 2.1. Subjects

This study is a quasi-experimental trial study with self-controls designed to assess the anti-inflammatory effects of wet cupping therapy on inflammatory cytokines believed to be produced in the liver of patients affected by non-alcoholic fatty liver disease. The study took place at the Omid Clinic in Hamadan, Iran between July 2021 and November 2021. The twenty participants, ranging in age from 20-60 (mean age 45.5) were diagnosed with non-alcoholic fatty liver disease grade II and III with or without alteration in their liver enzymes after medical examination and ultrasonography.

Patients who were affected by other chronic liver diseases or hepatitis and anemia; also heavy smokers and alcohol drinkers with intake greater than 2 alcohol units (20 g/day) for women and greater than three alcohol units (30 g/day) for men and patients with prolonged intake of drugs induced fatty liver diseases<sup>[14]</sup> were excluded. Four of our patients have left the study. Sixteen patients have finished all the follow-ups.

In this study, all methods were carried out under relevant guidelines and regulations and conducted according to the principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the Faculty of Medicine, Hamadan University of Medical Sciences with identity number IR.UMSHA.REC.1400.291 and was registered at the Iranian Registry of Clinical Trials (IRCT) and approved on 07/07/2022 with Trial Id 63179. Written informed consents were obtained from all patients and are available.

## 2.2. Clinical measurements

Ambulatory blood pressure (ABP) measurements were obtained before and after treatments. Body mass index (BMI), weight (kg) divided by height squared ( $m^2$ ), before and after treatment were calculated and waist circumferences, using a tape measurer, were measured as well. Fatty liver index (FLI), as a simple algorithm based on BMI, waist circumference, triglycerides and GGT with a ROC-AUC of 0.84 (95%CI 0.81-0.87) to detect FL<sup>[15]</sup>, was calculated for patients before and after treatment to confirm the likelihood of the disease using the formula:

$$FLI = \frac{(e^{0.953 \cdot \log_e(\text{triglycerides}) + 0.139 \cdot BMI + 0.718 \cdot \log_e(GGT) + 0.053 \cdot \text{waistcircumference} - 15.745})}{(1 + e^{0.953 \cdot \log_e(\text{triglycerides}) + 0.139 \cdot BMI + 0.718 \cdot \log_e(GGT) + 0.053 \cdot \text{waistcircumference} - 15.745})} \times 100$$

## 2.3. Laboratory analyses

Auto Hematology Analyzer BC-5150 was used to measure red blood cell count (RBC), hemoglobin concentration (HGB), platelet count (PLT), and white blood cell count (WBC). High sensitivity C-reactive protein (hs-CRP) was measured by CRP detection kit (Nephelometry) from Genrui Biotech Inc. Alkaline phosphatase was measured by Alkaline Phosphatase DEA Kit from ST diagnostic company. Triglycerides (GPO-PAP) kit from Parsazmun company was used to measure serum triglycerides through a photometric method. Alanine transaminase (ALT) by ALT (GPT) kit from Sarantashkhis co., aspartate aminotransferase (AST) by AST (GOT) activity assay kit from Paadco, serum gamma-glutamyl transferase (GGT) by GGT quantitative kit through photometric method from Parsazmun co., serum cholesterol by CHOD quantitative kit from Parsazmun co. and serum glucose by GOD quantitative kit from Parsazmun co. were measured. Serum low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol were measured using LDL and HDL-C quantitative kits from Parsazmun co. through the photometric method.

**Inflammatory and proinflammatory cytokines analyses.** To measure serum Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), Interleukin-1 $\beta$  (IL-1 $\beta$ ), and Interleukin-6 (IL-6) we used ZellBio GmbH enzyme-linked immunosorbent quantitative assay (ELISA) kits based on the biotin double antibody sandwich technology.

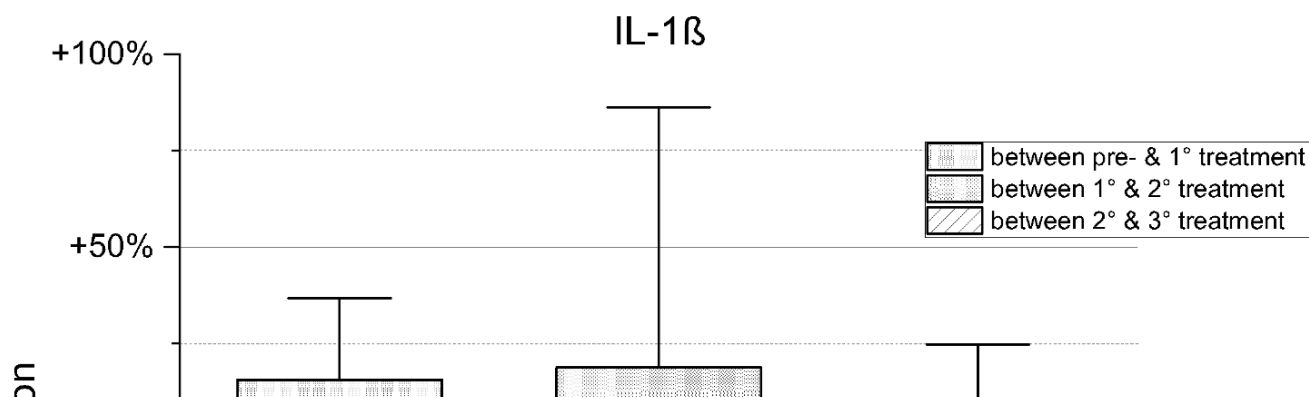
## 2.4. Wet cupping procedure

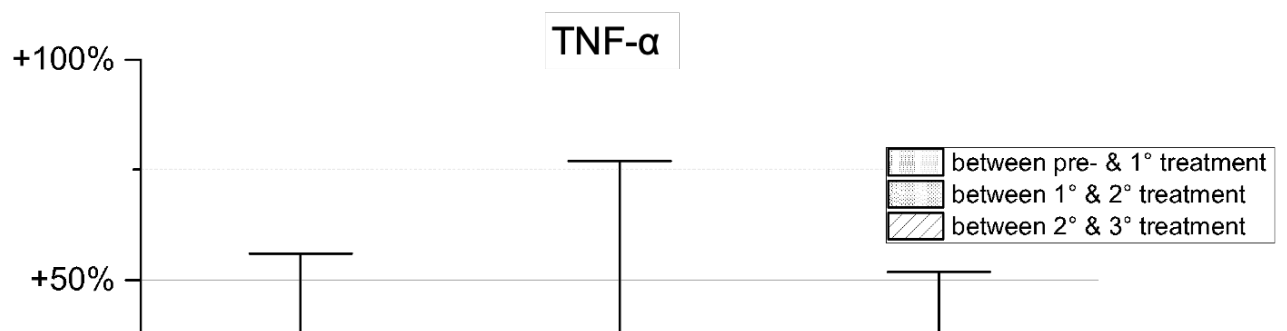
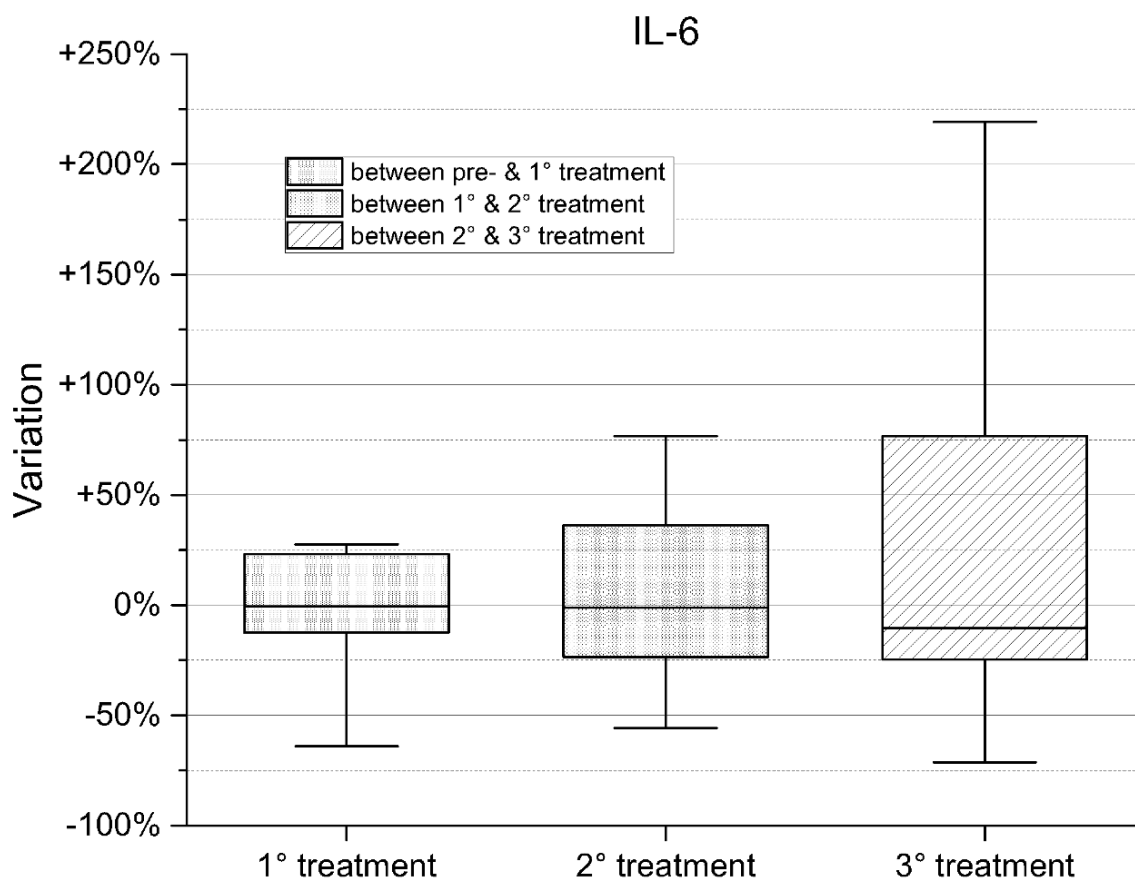
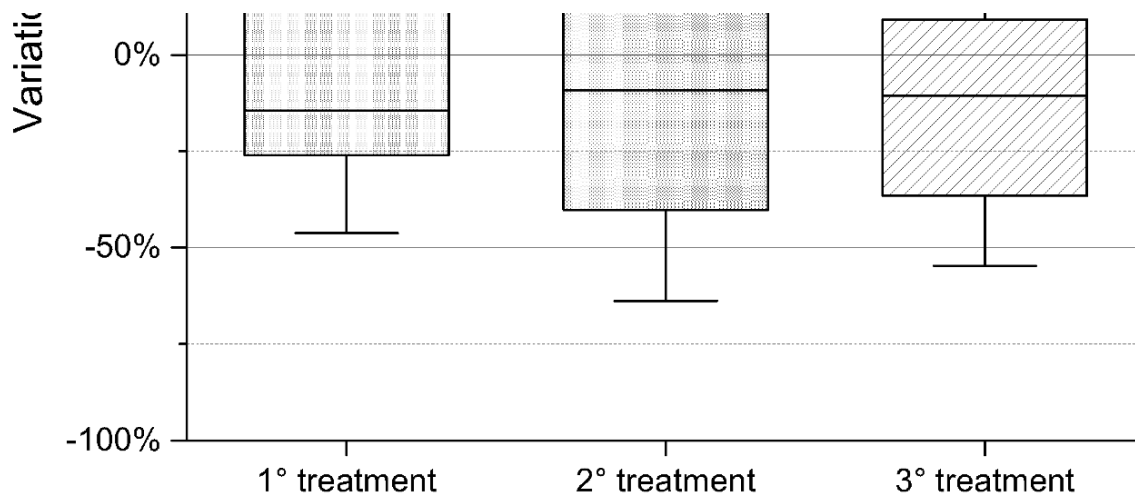
Participants' blood samples on day 0 were collected as our controls to measure all complete blood count (CBC) parameters, ALP, hs-CRP, triglycerides, LDL-C, HDL-C, ALT, AST, and GGT. 2 ml serums were centrifugated and separated on day 0 and kept in -20°C to use later for ELISA assays. Afterward, participants were invited to come to the clinic to do wet cupping therapy once every 15 days for a total of three times. In the first wet cupping therapy, patients were asked to eat some snacks before coming and to sit down on the bed. The interscapular area, brown adipose tissue, was disinfected and suctioned through a negative pressure created by a vacuumed cup connected to a suction device. Then the skin under the cup area was scarified with multiple superficial oblique scratches, and blood flow into the cup following the second suction. The cup was removed, and the extracted blood was gently cleaned from the skin surface. The suction and extracting of capillary blood were repeated three times on the same site. Finally, the location of the cupping was cleaned and covered by a layer of honey and sterile gauze was fixed on it with surgical tapes.

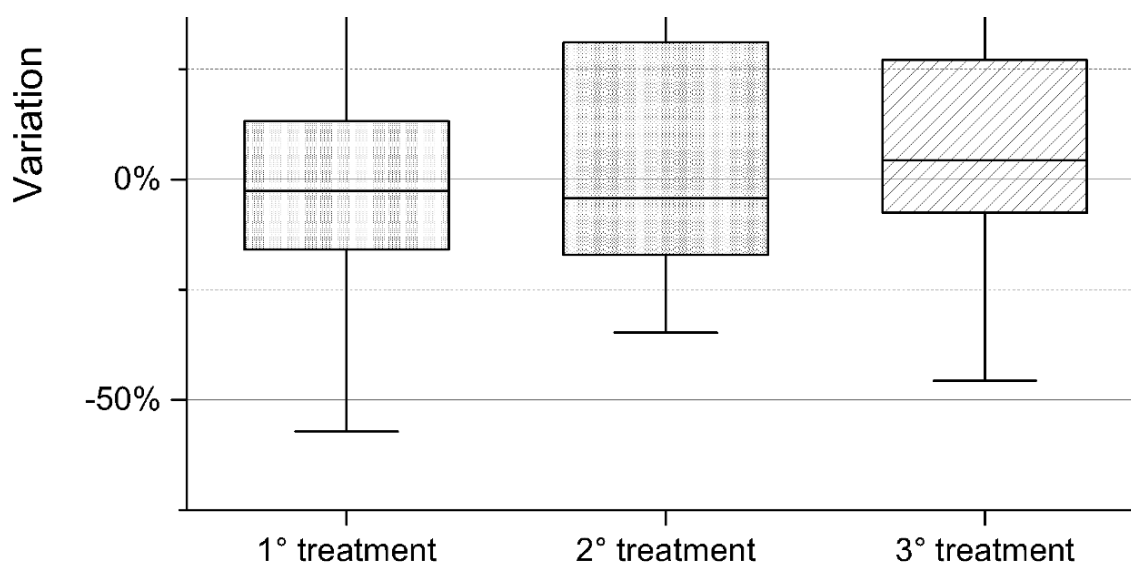
## 2.5. Statistical Analysis

Since we had a small sample who underwent cupping therapy, it was not possible to have a control group and apply Kruskal-Wallis' tests. Thus, we applied standard statistical analyses and verified whether the data distribution model was normal. We calculated statistically significant differences through the Student T test. Statistical significance was set at  $\alpha = 0.05$ .

Figures 1 and 2 show the four boxplots, which describe the variations of inflammatory cytokines pre-and post-treatment. The lowest point on the box plot is the minimum value of the data set and the highest point is the maximum value of the data set (excluding any outliers). The box is drawn from the First quartile ( $Q_1$ ) to the Third quartile ( $Q_3$ ) with a horizontal line drawn in the middle to denote the median.





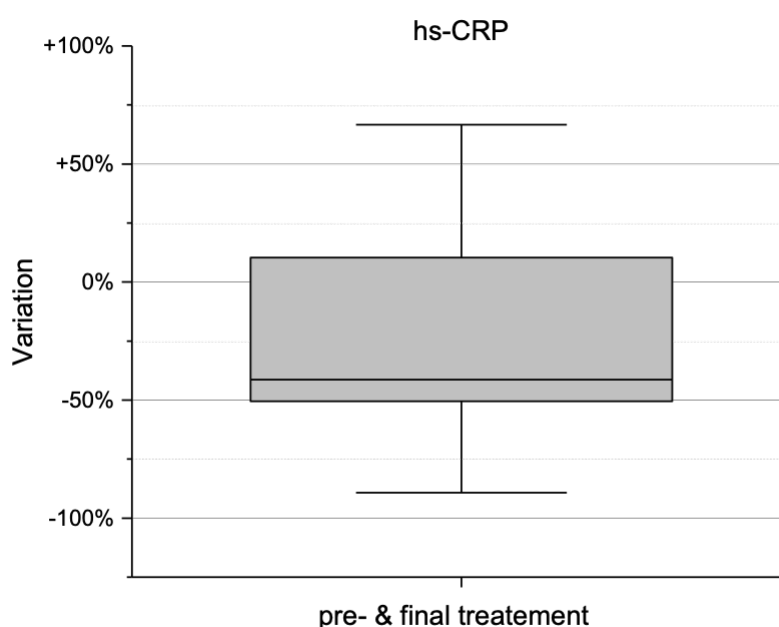


**Figure 1. The statistical variation of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  in different intervals of wet cupping therapy.** The figure 1 includes three boxplots, which describes the variations of inflammatory cytokines pre- and post-treatments. We have done wet cupping 3 times with our patients, with an interval of 15 days each.

The IL-1 $\beta$ 's boxplots illustrate a reduction of IL-1 $\beta$  in the first, second and third wet cupplings for an average of 24% in 69% of patients, of 29% in 67% and of 28% in 64% respectively. However, the p value in our complete IL-1 $\beta$  dataset showed no significant statistical reduction.

The IL-6's boxplots illustrate an increase of IL-6 in the first wet cupping with a bimodal distribution in 61% of our patients: an average increase of 17% and the other one with an average of 403%. The second and third wet cupplings could increase IL-6 for an average of 20% in 64% of patients and of 95% in 40% respectively. However, the p value in our complete IL-6 dataset showed no significant statistical reduction.

The TNF- $\alpha$ 's boxplots illustrate a reduction of TNF- $\alpha$  in the first, second and third wet cupplings for an average of 16% in 59% of patients, of 15% in 63% and of 12% in 47% respectively. However, the p value in our complete TNF- $\alpha$  dataset showed no significant statistical reduction.



**Figure 2. C-reactive protein (CRP) statistical variation pre- and post-wet cupping therapy.** The figure 2 shows the one boxplot of high sensitivity-C reactive protein (hs-CRP), as a sensitive systemic marker of inflammation pre- and final treatment with a reduction of 50% in 67% of our population. The lowest point on the box-plot is the minimum value of hs-CRP and the highest point is the maximum value of hs-CRP (excluding any outliers). The box is drawn from First quartile (Q<sub>1</sub>) to Third quartile (Q<sub>3</sub>) with a horizontal line drawn in the middle to denote the median. The hs-CRP reduction is confirmed by p value of 0.018 in our dataset.

### 3. Results

#### 3.1. Anthropometric Parameters, Hematological and Biochemical Analyses Pre- and Post-Treatment.

73% of our patients had a greater FLI index than 60%, which had been first proved by liver ultrasonography. As table 1 demonstrates, statistical analyses on RBC pre- and post-treatment did not show any negative effect and no patient showed any reduction in RBC from the normal range after the treatment; in 40% of patients, we saw an improvement of 4% in the RBC.

HGB remained almost constant pre-and post-treatment in all patients at the normal range. PLT did not show any significant variation pre-and post-treatment in 80% of patients. Analyses of WBC showed that wet cupping therapy increases the WBC by 33% in 20% of patients who were under the normal range; the remaining patients were in a normal range. The p-value in our complete WBC dataset showed no significant statistical variation.

FBS remained almost constant pre-and post-treatment in patients within the normal range; in one case with 307 mg/dl pre-treatment, the cupping therapy could reduce it by 40% (to 183 mg/dl). The p value in our complete FBS dataset showed no significant statistical variation.

ALP remained almost constant pre-and post-treatment in patients within the normal range. However, the p value of 0.014 in our complete ALP dataset showed a significant statistical increase but within the normal range of ALP (80-306 U/l).

ALT levels in 13% of patients showed a reduction of 30% after treatment, while other patients were in the normal range and remained constant. The p value in our complete ALT dataset showed no significant statistical variation.

AST in all patients but one remained constant in a normal range. One patient with a high AST showed an improvement of 38% after treatment. The p value in our complete AST dataset showed no significant statistical variation considering these results, wet cupping therapy could reduce the high-level AST and ALT in NAFLD patients.

Pre-treatment GGT of all patients was in a normal range. And still, in the normal range, cupping therapy could reduce GGT for 33% in 70% of patients. One patient with 48 U/l had an increase of 6% post-treatment (51 U/l). The p value in our complete GGT dataset showed no significant statistical variation.

Analyses of lipid markers showed that pre-treatment TG in 53% of patients was in a normal range and 47% of patients was higher than 150 mg/dl. 50% of patients with higher TG had an increase of 16% and the other 50% had a reduction of 28% post-treatment. The p value in our complete TG dataset showed no significant statistical variation.

LDL-C almost in all patients was upper than optimal range (<129 mg/dl) pre-treatment and wet cupping therapy could reduce LDL level by 22%. The p value of 0.012 in our complete LDL-C dataset showed a significant statistical decrease.

HDL-C in 33% of patients was under the optimal range pre-treatment, wet cupping therapy could increase the HDL level for 27% in 80% of them. The p value in our complete HDL-C dataset showed no significant statistical variation.

Total cholesterol after treatment was decreased by 14% in 80% of our patients. The p value of 0.015 in our complete cholesterol dataset showed a significant statistical decrease.

Table 1. Anthropometric and Biochemical Parameters Pre- and Post-Treatment

Table 1. Baseline Characteristics and Outcomes in Patients Who Did Not Receive Treatment																					
Age	Sex	Pre-treatment Blood Pressure	Post-treatment Blood Pressure	Pre-treatment BMI	Post-treatment BMI	Pre-treatment WBC	Post-treatment WBC	Pre-treatment FBS	Post-treatment FBS	Pre-treatment Alkaline Phosphatase	Post-treatment Alkaline Phosphatase	Pre-treatment ALT	Post-treatment ALT	Pre-treatment AST	Post-treatment AST	Pre-treatment GGT	Post-treatment GGT	Pre-treatment TG	Post-treatment TG	Pre-treatment Total Cholesterol	Post-treatment Total Cholesterol
52	F	110/80	110/68	32.0	32.6	3.60	4.00	90	90	100	120	32.0	21.0	31	27	30	47	191	260	261	210
45	M	110/70	120/80	29.9	29.7	5.70	5.90	70	90	114	110	35.0	31.0	33	22	22	25	100	111	197	141
38	M	112/70	105/70	27.5	26.8	6.67	5.05	92	76	165	161	34.0	34.0	25	28	47	41	167	183	142	150
47	M	110/70	100/65	34.0	34.0	3.54	5.79	80	83	88	121	61.0	34.0	45	28	34	23	60	92	55	102
45	F	121/79	108/79	31.2	31.2	5.99	4.27	307	183	169	158	37.0	32.0	26	29	16	21	80	91	188	171
50	F	135/82	142/88	42.2	43.8	7.01	6.85	88	70	135	126	20.0	10.0	16	14	25	11	85	102	193	174
51	F	120/75	125/80	25.5	25.5	8.27	8.08	156	177	186	209	37.0	44.0	29	30	48	51	238	196	112	97
50	F	115/70	110/72	32.0	31.4	6.80	7.17	89	85	53	96	11.0	13.0	16	16	11	17	203	100	172	169
48	F	115/80	120/90	28.9	28.9	6.29	7.83	97	91	261	283	46.0	38.3	29	27	34	30	182	146	228	199
32	F	106/65	128/95	31.5	31.5	8.14	7.80	105	101	161	177	22.0	29.4	19	22	25	43	88	162	180	193
48	F	116/90	130/80	33.2	34.5	8.31	8.39	102	91	146	140	22.0	16.4	23	23	24	11	152	173	252	204
49	M	110/75	120/70	26.5	27.5	4.32	5.37	90	98	143	134	34.0	24.0	25	20	15	14	151	159	214	201
41	M	116/90	125/90	32.0	32.7	5.31	5.72	98	94	159	162	39.5	30.0	29	24	18	18	135	118	171	139
36	M	100/75	125/60	26.6	26.6	6.28	7.97	84	83	98	120	16.0	16.0	17	17	21	24	218	169	192	175
52	M	148/105	130/70	25.5	24.9	5.53	7.09	127	134	138	166	29.0	36.0	22	25	47	24	72	86	99	84
p-values		>0.05		>0.05		>0.05		>0.05		0.014[1]		>0.05		>0.05		>0.05		>0.05		0.015[2]	

<sup>1</sup> The p value of 0.014 in our complete ALP dataset showed a significant statistical increase but within the normal range of ALP (80-306 U/l)

<sup>2</sup> The p value of 0.015 in our complete cholesterol dataset showed a significant statistical decrease

<sup>3</sup> The p value of 0.012 in our complete LDL-C dataset showed a significant statistical decrease.

**BMI:** Body Mass Index

**WBC:** White Blood Cell

**FBS:** Fasting Blood Sugar

**ALT:** Alanine Transaminase

**AST:** Aspartate Aminotransferase

**GGT:** Gamma Glutamyl Transferase

**TG:** Triglycerides

**HDL:** High-Density Lipoprotein

**LDL:** Low-Density Lipoprotein

### 3.2. Analyses of inflammatory factors Pre- and Post-Treatment

In Figures 1 and 2 statistical variations of some inflammatory factors are illustrated. Statistical analyses of inflammation state showed an average reduction of hs-CRP for about 50% in 67% of patients while 20% of patients remained in the normal range,  $\text{hs-CRP} \leq 3\text{mg/l}$ . The remaining 13% of patients showed an average increase of about 22.6%. The p-value of 0.018 in our complete hs-CRP dataset showed a significant statistical reduction.

The first wet cupping showed an average reduction of 24% of IL-1 $\beta$  in 69% of patients. The 31% of remaining patients showed an average increase of 46%.

The second wet cupping in 67% could reduce IL-1 $\beta$  by an average of 29%. And in 33% with an average reduction of 37%.

The third wet cupping in 64% of patients showed an average reduction of 28%. The 36% of remaining patients showed an average increase of 15%. We noticed that the second cupping was the most effective to reduce IL-1 $\beta$ . We may assume that to reduce IL-1 $\beta$ , wet cupping may not be repeated in a short interval. The P value in our complete IL-1 $\beta$  dataset showed no significant statistical reduction.

IL-6 in the first wet cupping was reduced by an average of 25% in 59% of patients. The remaining 61% of patients demonstrated an increase of IL-6 in a bimodal distribution which demonstrated some patients with an average increase of 17% and others with an average of 403%.

In the second wet cupping, 56% of patients had an average reduction of 20% while the remaining 64% of patients showed an average increase of 84% with a bimodal distribution.

Third wet cupping in 60% of patients showed an average reduction of 28% while 40% of patients showed an average increase of 95%. The P value in our complete IL-6 dataset showed no significant statistical variation.

In the first wet cupping, TNF- $\alpha$  was reduced by an average of 16% in 59% of patients and increased by an average of 22% in 41% of patients.

In the second cupping, 63% of patients had an average reduction of 15% while in 37% of patients was seen an average increase of 46%.

The third cupping in 47% could show an average reduction of 12%. And in the 53%, the remaining patients saw an average reduction of 30%. The P value in our complete TNF- $\alpha$  dataset showed no significant statistical reduction.

## 4. Discussion

No effective therapy to ameliorate inflammation status in NAFLD has been approved yet. We hypothesized that targeting inflammation in subjects affected by NAFLD may be seen as a novel therapeutic strategy to postpone the disease progression.

C-reactive protein (CRP) the first described acute-phase protein and a plasma protein synthesized by hepatocytes in the liver, is proved to be a sensitive systemic marker of inflammation and tissue damage<sup>[16]</sup>. Healthy young adults show a median concentration of 0.8mg/l, in 90% of this population the median concentration is 3.0mg/l and in 99% is 10mg/l<sup>[17]</sup>. Khadiga S. Abdulaziz and colleagues in a randomized control trial showed that cupping therapy could make a significant reduction in hs-CRP<sup>[18]</sup>. Our results also show that wet cupping therapy could modify inflammatory states in particularly reduced hs-CRP in 67% of participants significantly.

Tumor necrosis factor alpha (TNF- $\alpha$ ), an important adipocytokine synthesized in adipose tissues by a variety of immune cells, is induced by free fatty acids seen in hepatic steatosis through the activation of NF- $\kappa$ B. TNF- $\alpha$  progresses inflammation and may develop NAFLD through triggering multiple signaling pathways involved in inflammation, proliferation, and apoptosis<sup>[19]</sup>. Qi Zehang and colleagues established a cupping mice model and assess the effects of cupping therapy on polyunsaturated fatty acid (PUFA) metabolome through ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). They demonstrated that cupping therapy reduced the IL-6 and TNF- $\alpha$  production induced by lipopolysaccharide (LPS) in vivo<sup>[20]</sup>. Our study reveals that the first cupping reduced the TNF- $\alpha$  by an average of 16% in 59% of patients while the second one showed an average reduction of 15% in 63% and the final cupping showed an average reduction of 12% in 47%.

Interleukin-6 (IL-6) is a pro-inflammatory factor in human inflammatory and immune diseases such as rheumatoid arthritis and cytokine storm<sup>[21]</sup>. However, IL-6 inhibition is approved to treat inflammatory conditions but studies show that in some diseases such as ankylosing spondylitis the inhibition is not effective<sup>[21]</sup>. In the liver, IL-6 is produced in acute phase responses together with CRP secretion, haptoglobin, and other factors from hepatocytes<sup>[22]</sup>. IL-6 activity promotes cytoprotection and regeneration of hepatocytes. Inhibition of IL-6 or Interleukin-6 receptor (IL-6R) may cause hepatocellular injury thus its inhibition may not be indicated to treat the inflammatory conditions in the liver<sup>[23]</sup>. Our analyses showed that first cupping could increase the IL-6 in 61% of patients with a bimodal distribution: one with an average of 17% and the other with an average of 403%. The second cupping could increase the IL-6 by an average of 20% in 64% of patients while the third cupping showed an average increase of 95% in 40% of patients.

Interleukin-1 family (IL-1F) cytokines and their receptor as proinflammatory mediators induce many inflammatory processes in autoinflammatory, sterile inflammation, autoimmune and infectious diseases. IL-1 $\beta$ , a proinflammatory mediator, binds to the IL-1R and regulates T-helper 17<sup>[24]</sup>. IL-1 $\beta$  is produced following inflammasome activation, a strong inflammatory response in the liver due to toxic and metabolic stress, sepsis, and ischemia.

IL-1 family induces many NAFLD's features including insulin resistance<sup>[25]</sup>, adipose tissue inflammation, and atherosclerosis<sup>[26]</sup> with their pleiotropic effects on hepatocytes, stellate cells, and gut integrity. Anti-IL-1 therapies may have positive effects on diseases characterized by sterile inflammation<sup>[27]</sup>. Another randomized control trial assessed the efficacy of Acupuncture combined with cupping therapy for knee osteoarthritis. Their results revealed that treatment could decrease the levels of IL-1, IL-6, and TNF- $\alpha$  in joint fluid<sup>[28]</sup>. Our data showed that the first cupping could reduce the IL-1 $\beta$  by an average of 24% in 69% of patients and the second one reduced it by an average of 29% in 67% and the third cupping showed an average reduction of 28% in 64%.

A randomized controlled trial assessed the effects of wet cupping therapy on serum lipid concentrations. They found that wet cupping could reduce LDL cholesterol but with no statistically significant variation in total cholesterol; they believe that it may prevent atherosclerosis<sup>[29]</sup>. Our data showed that wet cupping therapy could reduce LDL and total cholesterol for p values of 0.012 and 0.015 in our complete dataset respectively.

To the best of our knowledge, this study is the first study to assess the effect of wet cupping therapy on inflammatory cytokines in patients affected by NAFLD. The P value of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in our complete dataset showed no significant statistical variation but we showed that the percentage variance calculated in different wet cupping therapy intervals for each inflammatory factor may be clinically important. However, we still do not know how many cupping procedures, and with what intervals, can reduce the inflammatory condition in our patients. The study limitation includes our small sample size. More studies should assess the molecular mechanism underlying the effects of wet cupping therapy in the future.

In conclusion, wet cupping therapy may be seen as a novel adjuvant therapeutic strategy along with other therapeutic strategies to ameliorate the inflammatory state in NAFLD patients.

## Declarations

### Ethics approval and consent to participate

In this study all methods were carried out per relevant guidelines and regulations and conducted according to the principles of the Declaration of Helsinki. The ethics committee of Hamadan University of medical sciences approved all the experimental steps (IR.UMSHA.REC.1400.291) and informed consent was obtained from all subjects.

### Clinical trial registration numbers and date of registration

The Iranian Registry of Clinical Trials (IRCT) approved this study with Trial Id 63179 on 07/07/2022

### Consent for publication

All the authors approved the version to be published and agreed to be accountable for all aspects of the work.

### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

### Competing interests

The authors declare that they have no competing interests.

### Funding

This study was funded by Iran's National Elites Foundation (INEF), an Iranian cooperation postdoctoral program for overseas specialists under Grant number 140007205787.

### Authors' contributions

All authors contributed to the study's conception and design. Nooshin Abbasi and Rezvan Najafi are responsible for the conception and design of the work. Nooshin Abbasi, Mahdi Biglarkhani, Azam Meyari, Razieh Amini, and Marco Fiaschi conducted the literature search, and data analysis, and drafted the article. All authors reviewed this draft, contributed, and approved the final manuscript.

## References

- <sup>1</sup>. <sup>a</sup>Institute NC. Nonalcoholic Fatty Liver Disease 2021 [updated 30/11/2021]. Available from: [https://ncit.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI\\_Thesaurus&ns=ncit&code=C84444](https://ncit.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI_Thesaurus&ns=ncit&code=C84444)
- <sup>2</sup>. <sup>a</sup>Eslam M, Sanyal AJ, George J. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology*. 2020 May;158(7):1999-2014.e1.
- <sup>3</sup>. <sup>a,b</sup>Wong VW-S, Adams LA, de Lédinghen V, et al. Noninvasive biomarkers in NAFLD and NASH — current progress and future promise. *Nature Reviews Gastroenterology & Hepatology*. 2018 2018/08/01;15(8):461-478.
- <sup>4</sup>. <sup>a</sup>Khan R, Quaiser S, Haque SF, et al. TNF-  $\alpha$  is An Inflammatory Marker of Cardiovascular Risks in Non-Alcoholic Fatty Liver Disease. *jf*.5(6):1237.
- <sup>5</sup>. <sup>a</sup>Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors, and prevention. *Nat Rev Gastroenterol Hepatol*. 2018 Jan;15(1):11-20.
- <sup>6</sup>. <sup>a</sup>Eslam M, George J. Genetic contributions to NAFLD: leveraging shared genetics to uncover systems biology. *Nature Reviews Gastroenterology & Hepatology*. 2020 2020/01/01;17(1):40-52.
- <sup>7</sup>. <sup>a</sup>Nonalcoholic fatty liver disease. *Nature Reviews Disease Primers*. 2015 2015/12/17;1(1):15081.
- <sup>8</sup>. <sup>a</sup>EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol*. 2016 Jun;64(6):1388-402.
- <sup>9</sup>. <sup>a</sup>Bashiri H, Bozorgomid A, Shojaeimotlagh V. Efficacy of Hijamat (wet cupping therapy) in Iranian patients with nonalcoholic fatty liver disease: a controlled clinical trial. *Turk J Med Sci*. 2020;50(2):354-359.
- <sup>10</sup>. <sup>a</sup>Baghdadi H, Abdel-Aziz N, Ahmed NS, et al. Ameliorating Role Exerted by Al-Hijamah in Autoimmune Diseases: Effect on Serum Autoantibodies and Inflammatory Mediators. *Int J Health Sci (Qassim)*. 2015 Apr;9(2):207-32.
- <sup>11</sup>. <sup>a</sup>Ahmed SM, Madbouly NH, Maklad SS, et al. Immunomodulatory effects of blood letting cupping therapy in patients with rheumatoid arthritis. *Egypt J Immunol*. 2005;12(2):39-51.
- <sup>12</sup>. <sup>a</sup>El Sayed SM, Baghdadi H, Abou-Taleb A, et al. Al-hijamah and oral honey for treating thalassemia, conditions of iron overload, and hyperferremia: toward improving the therapeutic outcomes. *J Blood Med*. 2014;5:219-37.
- <sup>13</sup>. <sup>a</sup>El-Shanshory M, Hablas NM, Shebl Y, et al. Al-hijamah (wet cupping therapy of prophetic medicine) significantly and safely reduces iron overload and oxidative stress in thalassemic children: a novel pilot study. *J Blood Med*. 2018;9:241-251.
- <sup>14</sup>. <sup>a</sup>Satapathy SK, Kuwajima V, Nadelson J, et al. Drug-induced fatty liver disease: An overview of pathogenesis and management. *Annals of Hepatology*. 2015 2015/11/01;14(6):789-806.
- <sup>15</sup>. <sup>a</sup>Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol*. 2006 Nov 2;6:33.
- <sup>16</sup>. <sup>a</sup>Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest*. 2003 Jun;111(12):1805-12.
- <sup>17</sup>. <sup>a</sup>Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. *Clin Chim Acta*. 1981 Nov 25;117(1):13-23.
- <sup>18</sup>. <sup>a</sup>Abdulaziz KS, Tareq Mohamad R, Saad El-Din Mahmoud L, et al. Effect of neurogenic acupoint cupping on high sensitive C-reactive protein and pain perception in female chronic pelvic pain: A randomized controlled trial. *J Musculoskelet Neuronal Interact*. 2021;21(1):121-129.
- <sup>19</sup>. <sup>a</sup>Weisberg SP, McCann D, Desai M, et al. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003 Dec;112(12):1796-808.
- <sup>20</sup>. <sup>a</sup>Zhang Q, Wang X, Yan G, et al. Anti- Versus Pro-Inflammatory Metabololipidome Upon Cupping Treatment. *Cell Physiol Biochem*. 2018;45(4):1377-1389.
- <sup>21</sup>. <sup>a,b</sup>Kang S, Tanaka T, Narazaki M, et al. Targeting Interleukin-6 Signaling in Clinic. *Immunity*. 2019 Apr 16;50(4):1007-1023.
- <sup>22</sup>. <sup>a</sup>Widjaja AA, Chothani SP, Cook SA. Different roles of interleukin 6 and interleukin 11 in the liver: implications for therapy. *Hum Vaccin Immunother*. 2020 Oct 2;16(10):2357-2362.
- <sup>23</sup>. <sup>a</sup>Matthews VB, Allen TL, Risis S, et al. Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance. *Diabetologia*. 2010 Nov;53(11):2431-41.

24. <sup>^</sup> Tilg H, Moschen AR, Szabo G. Interleukin-1 and inflammasomes in alcoholic liver disease/acute alcoholic hepatitis and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatology*. 2016 Sep;64(3):955-65.
25. <sup>^</sup> Tilg H, Moschen AR. Inflammatory mechanisms in the regulation of insulin resistance. *Mol Med*. 2008 Mar-Apr;14(3-4):222-31.
26. <sup>^</sup> Freigang S, Ampenberger F, Weiss A, et al. Fatty acid-induced mitochondrial uncoupling elicits inflammasome-independent IL-1 $\alpha$  and sterile vascular inflammation in atherosclerosis. *Nat Immunol*. 2013 Oct;14(10):1045-53.
27. <sup>^</sup> Dinarello CA, van der Meer JW. Treating inflammation by blocking interleukin-1 in humans. *Semin Immunol*. 2013 Dec 15;25(6):469-84.
28. <sup>^</sup> Qiu JQ, Liu SR, Lin QL, et al. [Acupuncture combined with cinesiotherapy cupping for knee osteoarthritis with qi stagnation and blood stasis syndrome: a randomized controlled trial]. *Zhongguo Zhen Jiu*. 2019 May 12;39(5):462-6.
29. <sup>^</sup> Niasari M, Kosari F, Ahmadi A. The effect of wet cupping on serum lipid concentrations of clinically healthy young men: a randomized controlled trial. *J Altern Complement Med*. 2007 Jan-Feb;13(1):79-82.