Effect of Ramadan fasting on hepatic steatosis as quantified by controlled attenuation parameter (CAP): a prospective observational study

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Abstract

Background: Although Ramadan fasting has a beneficial effect on health, the role of Ramadan fasting on patients with non-alcoholic fatty liver disease (NAFLD), as quantified by the controlled attenuation parameter (CAP), is not determined yet. So, this study aimed to determine the effect of Ramadan fasting on patients with NAFLD by assessing the controlled attenuation parameter (CAP) and biochemical parameters of the fatty liver.

Patients and methods: A prospective observational study was conducted on 40 NAFLD patients, who were diagnosed by ultrasonography and quantified with controlled attenuation parameter (CAP) in transient elastography (FibroScan) and fasted the month of Ramadan. Transient elastography for CAP and liver stiffness measurement (LSM) were performed. Fibrosis 4 score (FIB4) and NAFLD fibrosis score (NFS) were also calculated.

Results: There is a statistically significant change in body mass index, fasting blood glucose, HbA1c, triglycerides, LDL cholesterol, HDL cholesterol, total cholesterol, serum albumin, total protein, AST, ALT, and alkaline phosphatase after Ramadan fasting. There were significant clinical improvements after Ramadan fasting in FIB-4 (1.31 ± 0.26 and 1.24 ± 0.25 respectively, p < 0.001), CAP (318.52 ± 34.59 and 294.0 ± 20.34, respectively, p < 0.001), and LSM (6.95 ± 1.62 and 6.59 ± 1.49, respectively, p < 0.001).

Conclusion: Our study demonstrates that Ramadan fasting could improve liver steatosis in patients with NAFLD proved with a significant reduction in the CAP and LSM.

Keywords: NAFLD, CAP, Ramadan fasting, FibroScan

Introduction

Ramadan is a month for Muslims during which they withhold from drinking, eating, smoking, and sexual relationships between dawn and sunset, and they can eat during the remaining hours [1]. As expected, caloric intake is often reduced, and the quality of ingested nutrients can also differ during this month [2].

Non-alcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease [3]. NAFLD is defined as the accumulation of fat in the liver in the absence of excessive alcohol intake. It includes a wide range of liver damage extending from simple steatosis to steatohepatitis, fibrosis, and cirrhosis that can progress to liver failure and hepatocellular carcinoma [4].

Liver biopsy has still been considered as the gold standard in the diagnostic assessment of NAFLD. But it is an invasive method with the risk of hemorrhage and infection [5]. The non-invasive method by ultrasound is more suitable for evaluating hepatic steatosis, with a sensitivity
of 60–94% and a specificity of 66–97%. Ultrasound is advised as a first-line diagnostic test in most guidelines, rather than liver biopsy and other imaging tools [6].

Hepatic steatosis is measured non-invasively using vibration-controlled transient elastography (FibroScan, Echosens) with controlled attenuation parameter (CAP) [7]. The CAP value is expressed in decibel per meter (dB/m) and ranges from 100 to 400 dB/m. Higher values denote greater liver fat contents. CAP at a cutoff of 283 dB/m has 76% sensitivity and 79% specificity to detect steatosis [8].

Transient elastography also conducts liver stiffness measurement (LSM), based on the shear wave propagation speed; the results of which are reported in kilo Pascal (kPa) with a range of 2.5–75 kPa [9].

A study showed significant effects on NAFLD patient parameters during Ramadan fasting such as decreasing insulin, ALT enzyme, and systolic blood pressure (SBP) and diastolic blood pressure (DBP) and increasing HDL-C after an average of 27 days fasting [10]. But in other studies, there is no significant change in ALT or AST enzymes in NAFLD cases after Ramadan fasting [11].

A study by Farshidfar et al. described a significant increase in high-density lipoprotein cholesterol (HDL-C) and a decrease in low-density lipoprotein cholesterol (LDL-C) at day 28 of Ramadan [12].

The role of Ramadan fasting on patients with NAFLD, as quantified by controlled attenuation parameter (CAP), is not determined yet. So, this study aimed to determine the effect of Ramadan fasting on patients with NAFLD by assessing the improvement of controlled attenuation parameter (CAP) and biochemical parameters of the fatty liver.

Patients and methods

Study design
This was a prospective observational study that was conducted in the Internal Medicine Department in Zagazig University Hospitals during the month of Ramadan in 1442 higri in Sharqiyah Governorate, Egypt. This work had been carried out in collaboration between outpatient clinics and specific centers of Zagazig University Hospitals via poster advertisement or direct request. The duration of the study extended from April 2021 to May 2021. The Zagazig University institutional review board approved the study (ZU-IRB#6891-13-12-2020). Written informed consent was obtained from all individual participants included in the study.

Patient selection and data collection
To be eligible for this study, the patient had to fulfill the following inclusion criteria: patients who were male and female between 18 and 65 years old with NAFLD that diagnosed by ultrasonography and quantified with controlled attenuation parameter (CAP) in transient elastography (FibroScan) and fasted month of Ramadan. We excluded subjects who fasted for less than 20 days and had body mass index (BMI) < 25 kg/m², uncontrolled DM (HbA1c > 8), any definite or suspected alcohol consumption, other causes of chronic liver disease (e.g., hepatitis B and C, autoimmune hepatitis); patients with end-organ failure such as decompensated liver diseases, heart failure, and renal failure; patients who had taken medications that had an influence on ALT and AST; and pregnant and lactating women.

Clinical and laboratory assessments
The following data was collected for each patient eligible for this study at the baseline: age, gender, Body mass index (BMI), residency, smoking status, comorbidities, medical history, and general examination including blood pressure, with special consideration for signs of metabolic syndrome. Baseline laboratory tests including complete blood picture (by automated blood counter), viral markers (HCV by HCV, Abs by ELISA, HBV and HBeAg by ELISA), liver function tests (serum bilirubin (total and direct), serum albumin, serum ALT and AST measured by kinetic methods), coagulation profile (PT, PTT, and INR), alkaline phosphatase, lipid profile (triglycerides, cholesterol, LDL, HDL), renal function, and coagulation tests were performed. Besides, fasting blood glucose and HbA1c in diabetic cases and pregnancy test for females in the child-bearing period were measured for the study group within 7 days before and after Ramadan fasting.

Abdominal ultrasonography (US) was performed to assess the degree of fatty liver (steatosis). Patients with NAFLD included in the present study were classified to mild, moderate, and severe cases according to the grading of diffuse hepatic steatosis by abdominal ultrasonography [13].

Transient elastography (FibroScan) for measurement of controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) were performed. Fibrosis 4 score (FIB4) and NAFLD fibrosis score (NFS) were also calculated [14, 15].

Samples
In BD Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ), blood samples were obtained. At baseline, 6 tubes were collected including one citrate, two plain, and two EDTA tubes from each patient. One EDTA tube was used for the complete blood picture. The second EDTA tube was utilized to assess HbA1C. The plain vacutainer was allowed to clot for 30 min after collection. After this period, the sample was centrifuged at 1200×g
for 10 min to separate the serum. The citrate sample immediately was centrifuged at 2000×g for 15 min to assess coagulation tests.

Methods

The complete blood count was performed by the XS500i Hematology analyzer (Sysmex, Kobe, Japan). The differential cells were counted using the blood film. Coagulation tests were performed by Sysmex CS2100i (Siemens, Munich, Germany). All biochemical tests were quantified using the Cobas 8000 Modular Analyzer (Roche Diagnostics, Mannheim, Germany) except HbA1C was performed on Cobas 6000 Modular Analyzer (Roche Diagnostics, Mannheim, Germany).

Transient elastography (FibroScan) for measurement of controlled attenuation parameter (CAP) and liver stiffness measurements (LSM) were performed by an experienced physician who was blinded to the clinical data of the patients. The measurements were performed using a 3.5-MHz standard probe on the right hepatic lobe through the intercostal spaces with the patient lying supine. Measurements were considered valid if the following criteria were met: (I) there were at least 10 valid shots, (II) the success rate was at least 60%, and (III) the interquartile range was less than 30% of the median values of the CAP and LSM. The final CAP and LSM were recorded as the median values of all measurements, and they were expressed in dB/m and kPa, respectively.

Statistical analysis

Quantitative parameters were displayed as the mean and standard deviation (SD) while categorical ones were displayed as absolutes and percentages. The Student t-test and paired t-test were utilized for unrelated and related quantitative variables, respectively. The chi-squared test was used to compare the categorical ones. The primary endpoint was the evaluation of change in the FibroScan and biochemical parameters of patients with NAFLD after Ramadan fasting from the baseline. The outcome was the improvement of FibroScan and biochemical parameters of patients with NAFLD after Ramadan fasting. All statistical analysis was performed using the statistical software program, SPSS, for Windows version 25.0 (SPSS; Chicago, IL, USA). If the p-value is below 0.05, the test is statistically significant.

Results

Forty patients with non-alcoholic fatty liver disease (NAFLD) who had fasted Ramadan participated in the present study. The demographic and biochemical characteristics of the subjects of the study group before and after Ramadan fasting are presented in Table 1. There is a statistically significant change in body mass index, fasting blood glucose, HbA1c, triglycerides, LDL cholesterol, HDL cholesterol, total cholesterol, serum albumin, total protein, AST, ALT, and alkaline phosphatase after Ramadan fasting. However, there is a non-significant change in either hemoglobin, INR, serum total bilirubin, direct bilirubin, serum creatinine, BUN, or platelet count.

The comparison of liver parameters between before and after Ramadan fasting in the study group is presented in Table 2. There is a statistically significant change before and after Ramadan fasting in FIB-4 (1.31 ± 0.26 and 1.24 ± 0.25, respectively, p < 0.001) as presented in Table 2, CAP (318.52 ± 34.59 and 294.0 ± 20.34, respectively, p < 0.001) as presented in Fig. 1, and LSM (6.95 ± 1.62 and 6.59 ± 1.49, respectively, p < 0.001) as presented in Fig. 2. However, there is a non-significant change in NFS (1.1 ± 1.45 and 1.06 ± 1.3, respectively, p < 0.219) as presented in Table 2.

The comparison of the ultrasonographic grading of fatty liver before and after Ramadan fasting in the study group is presented in Table 3. There is a statistically non-significant change in ultrasonographic grading of fatty liver before and after Ramadan fasting (p = 0.059).

Discussion

NAFLD is a rapidly emerging epidemic, leading to the search for cost-effective routes to prevent metabolic syndrome and NAFLD as well as the progression into cirrhosis and hepatoma.

The initial diagnosis of NAFLD in clinical practice depends on the laboratory findings and radiological imaging techniques in the absence of other causes of fatty liver [16]. Recently, attention has been focused on transient elastography, which is a non-invasive ultrasound-based method that uses shear wave velocity to assess the stiffness of liver tissue. Depending on the physical characteristics such as the velocity and intensity attenuation of the shear wave, the acquired data are processed and presented as LSM and CAP.

On the other hand, simple blood-based scores can be easily obtained as NAFLD fibrosis score (NFS) [15], which has shown high sensitivity for detecting advanced fibrosis [17]. Additionally, FIB-4 is a simple, inexpensive, and non-invasive test that can be easily obtained to determine the degree of hepatic fibrosis [18]. In the present study, transient elastography for the measurement of CAP and LSM was performed. Also, FIB4 and NFS were also calculated.

Most of the studies evaluated the changes of some clinical, biological, and anthropometric factors among metabolic syndrome, diabetic, hyperlipidemia patients; cardiovascular patients; and/or healthy adults [19–22].
Our study demonstrates that there is a statistically significant decrease in body mass index, fasting blood glucose, and HbA1c; this finding does not agree with M’guil et al.’s results that investigate the lack of effect of Ramadan fasting on blood glucose in type 2 diabetes patients [23]. But our study agrees with the study of Ebrahimi et al. that revealed a significant improvement in anthropometric measures as well as fasting glucose, plasma insulin, and insulin resistance [24]. Also, it differs from a study by Nematy et al. showing that there is no difference in insulin and FBS after Ramadan fasting [19].

Our study illustrates there is a statistically significant decrease in triglycerides, LDL cholesterol, total

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**Table 1** Anthropometric and biochemical parameters of the study group before and after Ramadan fasting

| Variables (N = 40) | Before Ramadan fasting | After Ramadan fasting | p-value |
|--------------------|------------------------|-----------------------|---------|
| Age (years), mean ±SD (18–65) | 46 ± 9 | | |
| Sex, N (%) | Female | 27 (67.5%) | Male | 13 (32.5%) |
| Residence, N (%) | Rural | 23 (57.5%) | Urban | 17 (42.5%) |
| Comorbidities, N (%) | Diabetes mellitus | 10 (25%) | Diabetes/hypertension | 7 (17.5%) | Hypertension | 7 (17.5%) | Hypothyroidism | 2 (5%) |
| Smoking, N (%) | No | 34 (85%) | Yes | 6 (15%) |
| BMI (kg/m²), mean ±SD | 30.9 ± 2.42 | 29.4 ± 1.93 | < 0.001** |
| FBG (mg/dL), mean ±SD | 108.3 ± 11.68 | 107.2 ± 11.78 | 0.035* |
| HbA1C (%), mean ± SD | 7.33 ± 0.63 | 6.97 ± 0.65 | < 0.001** |
| White blood cells (10⁹/L) | 7.4 ± 1.9 | 6.8 ± 1.8 | < 0.001** |
| Hemoglobin (g/dL) | 11.99 ± 0.96 | 11.99 ± 0.95 | 0.323 |
| Platelets (10⁹/L) | 249.5 ± 36.57 | 248.9 ± 36.09 | 0.265 |
| Total bilirubin (mg/dL) | 0.852 ± 0.15 | 0.851 ± 0.11 | 0.035* |
| Direct bilirubin (mg/dL) | 0.248 ± 0.11 | 0.25 ± 0.1 | 0.515 |
| ALT (U/L) | 45.2 ± 7.66 | 38.75 ± 5.64 | < 0.001** |
| AST (U/L) | 35.93 ± 4.29 | 30.68 ± 3.83 | < 0.001** |
| Albumin (g/dL) | 4.39 ± 0.17 | 4.21 ± 0.16 | < 0.001** |
| Total plasma protein (g/dL) | 7.54 ± 0.29 | 7.39 ± 0.29 | < 0.001** |
| Alkaline phosphatase (U/L) | 91.55 ± 18.45 | 87.18 ± 24.6 | 0.01* |
| INR | 1.02 ± 0.096 | 1.009 ± 0.093 | 0.625 |
| Creatinine (mg/dL) | 0.772 ± 0.134 | 0.773 ± 0.136 | 0.225 |
| BUN (mg/dL) | 14.05 ± 2.46 | 13.93 ± 2.38 | 0.096 |
| Cholesterol (mg/dL) | 223.2 ± 34.6 | 200.5 ± 13.4 | < 0.001** |
| Triglyceride (mg/dL) | 167.1 ± 69.6 | 143.5 ± 23.7 | < 0.001** |
| LDL (mg/dL) | 167 ± 36 | 118.6 ± 27 | < 0.001** |
| HDL (mg/dL) | 44.6 ± 6.8 | 49.6 ± 8.2 | < 0.001** |

HCV: hepatitis C virus, HBV: hepatitis B virus, BMI: body mass index, HbA1C: hemoglobin A1C, FBG: fasting blood glucose, INR: international normalized ratio, BUN: blood urea nitrogen, ALT: alanine transferase, AST: aspartate transferase, LDL: low-density lipoprotein, HDL: high-density lipoprotein.

*p < 0.05 is statistically significant

**p < 0.001 is statistically highly significant

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**Table 2** Comparison of the liver parameters between before and after Ramadan fasting in the study group

| Parameters | NAFLD group (N = 40) | Before Ramadan fasting (mean ± SD) | After Ramadan fasting (mean ± SD) | p-value* |
|------------|----------------------|------------------------------------|----------------------------------|---------|
| FIB4       | 1.31 ± 0.26          | 1.24 ± 0.25                       | < 0.001**                        |
| NFS        | 1.1 ± 1.45           | 1.06 ± 1.3                        | 0.219                            |
| CAP (dB/m) | 318.52 ± 34.59       | 294.0 ± 20.34                     | < 0.001**                        |
| LSM (kPa)  | 6.95 ± 1.62          | 6.59 ± 1.49                       | < 0.001**                        |

FIB4: fibrosis 4 score, NFS: NAFLD fibrosis score, CAP: controlled attenuation parameter, LSM: liver stiffness measurement

* *p < 0.001 is statistically highly significant

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cholesterol, serum albumin, and total protein. But in some studies, an increase in plasma cholesterol and TG and a decrease in SBP were shown but in the healthy population [19, 25, 26].

Our study shows that there is a statistically significant increase in HDL cholesterol. The evidence that an obvious increase in plasma HDL-C occurs after Ramadan fasting is promising, as observed in some studies [26–28].

Our study demonstrates that there is a statistically significant decrease in AST, ALT, and alkaline phosphatase after Ramadan fasting. The ALT enzyme

**Table 3** Comparison of the ultrasonographic grading of fatty liver between before and after Ramadan fasting in the study group

| NAFLD group (N = 40) | Fatty liver (ultrasonographic grading)* | Before Ramadan fasting, N (%) | After Ramadan fasting, N (%) | p-value |
|----------------------|----------------------------------------|------------------------------|----------------------------|---------|
| Mild                 | 16 (40%)                               | 19 (47.5%)                   | 0.059                      |
| Moderate             | 21 (52.5%)                             | 20 (50%)                     |                            |
| Severe               | 3 (7.5%)                               | 1 (2.5%)                     |                            |

Grading of diffuse hepatic steatosis on liver ultrasonography has been used to know the extent of fatty changes in the liver [13]
decreases significantly after Ramadan in the present study that was along with Unalacak et al.'s findings [29], and Arabi et al.'s study on 50 NAFLD patients revealed that Ramadan fasting was associated with decreased serum insulin, ALT level, and systolic and diastolic blood pressure, with an increase in HDL-C after an average of 27 days of fasting [10]. Also, in a previous study, lifestyle change and losing at least 5% of body weight have a significant improvement on ALT enzyme in NAFLD patients [30].

However, in other studies, there is no significant change in ALT or AST enzymes [11, 31]. Also, Rahimi et al. reported an increase in ALT levels after Ramadan fasting in NAFLD patients [32].

Our study demonstrates that there is a statistically significant improvement after Ramadan fasting in FIB-4, CAP, and LSM. However, there is a non-significant change in NFS, so we suggest that Ramadan fasting could improve the liver condition in patients with NAFLD proved with a significant reduction in the CAP and LSM. This agrees with the dietary regimen used to reduce weight for both lean and obese NASH patients and found significant improvement in histopathology in both groups 1 year after weight reduction [33].

On the other hand, there is a statistically non-significant change in ultrasonographic grading of fatty liver before and after Ramadan fasting during the short duration of this study. This is not in agreement with that Ramadan fasting was found to improve liver steatosis as measured by ultrasound grading in NAFLD patients in the study of Aliasghari et al. [34].

Our study included 2 patients with hypothyroidism; this is not enough to relate between hypothyroidism and NAFLD. Although the meta-analysis of He et al. showed strong epidemiological evidence for the significant relationship between hypothyroidism and NAFLD, patients with hypothyroidism either subclinical or overt are at a higher risk for development of NAFLD than those with normal thyroid function [35].

The strength of our study is that this is the first prospective study for the effect of Ramadan fasting on hepatic steatosis as quantified by controlled attenuation parameter (CAP). Certain limitations of our study are that our study is observational and has a relatively small sample size, which might limit the generalizability of the result. Further studies are recommended to confirm this study's results after excluding other comorbidities, e.g., hypothyroidism.

In conclusion, our results showed an improvement of FibroScan and biochemical parameters of patients with NAFLD after Ramadan fasting, and this study suggested that Ramadan fasting may be effective in improving the liver steatosis in NAFLD patients. Further studies with a large sample are recommended to confirm our results and approve fasting as a potential treatment for NAFLD.

Abbreviations
CAP: Controlled attenuation parameter; DM: Diabetes mellitus; FBS: Fasting blood sugar; FIB-4: Fibrosis 4 score; LSM: Liver stiffness measurement; NAFLD: Non-alcoholic fatty liver disease; NFS: NAFLD fibrosis score; US: Ultrasonography.

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Authors' contributions
AG generated the research idea. AB and HA performed the clinical examination and followed up the patients. AB and AG collected the laboratory data. All authors shared in analyzing and interpreting the patient data and in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
The Zagazig institutional review board approved the study (ZU-IRB#6819-13-12-2020). Written informed consent was obtained from all individual participants in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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