Biochemical Parameters in Obese Egyptian Patients as a Non-Invasive Marker for Disease Screening in Early Diagnosis of Non-Alcoholic Fatty Liver Disease

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Abstract

BACKGROUND: Non-alcoholic fatty liver disease (NAFLD) has recently been considered as the most public liver problem worldwide and a major clinicopathologic health burden in the developed countries. Biochemical tests are important in verifying a better understanding of many diseases and hence help to have the right decisions for achieving better management.

AIM: This study was conducted to assess biochemical markers in NAFLD Egyptian patients.

METHODS: Forty obese subjects (32 females and 8 males, mean age was 42.32 ± 9.12 years) (20 with NAFLD and 20 without NAFLD**)** and 20 normal participants were selected.

RESULTS: Body mass index (BMI) was 40.86 ± 5.45 in obese FL versus 22.07 ± 2.10 in control, p < 0.001 and versus 35.83 ± 5.94 in obese non-FL, p = 0.003. Alanine aminotransferase (ALT) was 57.30 ± 46.24 in obese FL versus 25.45 ± 7.12 in control, p = 0.003 and versus 27.35 ± 11.09 in obese non-FL, p = 0.005. Aspartate aminotransferase (AST) (41.40 ± 36.09 in obese FL vs. 21.7 ± 3.81 in control, p = 0.015 and vs. 24.05 ± 7.50 in obese non-FL, p = 0.032). Total bilirubin (T.Bil) (0.62 ± 0.25 in obese FL vs. 0.47 ± 0.15 in control, p = 0.014). Prothrombin time (PT) (86.80 ± 11.32 in obese FL vs. 97.86 ± 4.31 in control, p < 0.001) and International Normalization Ratio (INR) (1.11 ± 0.13 in obese FL vs. 1.01 ± 0.02 in control, p = 0.002). Triglycerides (TGs) (128.20 ± 43.49 in obese FL vs. 88.35 ± 24.26 in control, p < 0.001 and vs. 94.50 ± 31.65 in obese non-FL, p = 0.003). Ferritin (88.21 ± 54.88 in obese FL vs. 47.65 ± 32.07 in obese non-FL, p = 0.006). Alpha-fetoprotein (AFP) (2.42 ± 1.67 in obese FL vs. 1.20 ± 0.75 in control, p = 0.001). Fasting blood sugar (FBS) (119.70 ± 49.11 in obese FL vs. 84.10 ± 7.19 in control, p < 0.001 and vs. 80.50 ± 8.84 in obese non-FL, p < 0.001) and postprandial (P.P) (152.80 ± 82.86 in obese FL vs. 94.35 ± 3.70 in control, p < 0.001 and vs. 93.35 ± 7.77 in obese non-FL, p < 0.001). Serum high-density lipoprotein (HDL) level was significantly lower in NAFLD patients compared to obese non-FL (40.05 ± 5.81 vs. 41.9 ± 4.85, p < 0.001).

CONCLUSION: NAFLD is associated with changes in biochemical parameters. Its early assessment can help in modifying the disease course and delaying complications.

Introduction

Obesity is a state of an excess body fat which causes higher risk of metabolic derangements [1]. In patients with severe obesity (usually with body mass index "BMI" >35 kg/m^2), the hepatic steatosis prevalence is over 90% from those patients undergoing bariatric surgery [2]. Obesity is considered as a tremendous threat for health in Egypt. More than one from each three Egyptians is obese which is considered among the highest rates in the world. According to a recent study, 19 million Egyptians are obese, representing 35% from the total adult population. Moreover, about 3.6 million children in Egypt are overweight and obese representing 10.2% of all Egyptian children [3]. Liver steatosis develops when the input rate of hepatic fatty acids (synthesis and uptake) is more than the rate of output of fatty acids (secretion and oxidation). Obesity is considered as one of the non-alcoholic fatty liver disease risk factors [4].

NAFLD is a growing and frequent cause for chronic liver diseases, influencing about 20–30% from the general population worldwide. NAFLD patients, particularly those with non-alcoholic steatohepatitis (NASH), are at higher risk for progression to cirrhosis and its consequent complications, representing a higher rate also for cardiovascular diseases and cancer compared to those without fatty liver [5]. NAFLD includes a broad spectrum of clinicopathologic events ranging from simple hepatic steatosis to NASH. Simple hepatic steatosis is relatively benign. It is characterized by hepatic steatosis in the absence of inflammation or fibrosis. The progression into NASH is characterized by ballooning of hepatocyte, inflammatory infiltration and cellular necrosis that may give rise to complications such as liver cirrhosis, hepatocellular carcinoma and hepatic failure [6].

Biochemical tests are very useful for achieving a better understanding of the disease and consequently allow good management decisions to be made. Liver ultrasound examination has relatively high specificity (88–95%) and sensitivity (60–95%) [7]. Hence, NAFLD diagnosis has been made depending on liver ultrasound examination as well as measuring various biochemical parameters manifesting hepatic damage or injury. Integrating the results into several scores may support the diagnosis [8]. Meanwhile, many investigators are still searching for simple tools for diagnosis with higher specificity and sensitivity that can help as screening markers for excessive accumulation of hepatic fat. The data elucidate that male prevalence may be recorded for NAFLD or an equivalent distribution for gender and can be even diagnosed in case of the absence of obesity and diabetes. NAFLD may exist at any age including the childhood, although the most prevalent is found in the age between 40 and 50 years. With some restrictions, both hospital-based and population studies from Western countries have reported that about 10–24% of global population in addition to 57–75% of obese persons might have NAFLD [9]. Thus, according to the mentioned contest, the current study aimed to access the association between NAFLD and different biochemical parameters, especially liver profile and lipid profile in Egyptian population.

Subjects and Methods

Subjects

This study was a case–control study, conducted on 40 obese adult subjects aged between 18 and 65 years, selected from the outpatient clinic attending gastrointestinal unit of Nasser Institute Hospital, Cairo, Egypt, from September 2016 to September 2017.

They were selected according to the following:

Inclusion criteria

- Obese subjects (BMI > 30 kg/m^2) and age 18–65 years were included in the study.

Exclusion criteria

- Presence of autoimmune hepatitis as detected by antinuclear antibody (ANA), presence of Wilson disease as detected by ceruloplasmin, presence of alpha-1-antitrypsin deficiency as detected by alpha-1-antitrypsin assay, presence of hepatitis C and B as achieved by hepatitis BsAg, hepatitis C antibody, presence of iron overload as detected by ferritin, presence of hepatocellular carcinoma (HCC)

as detected by alpha-fetoprotein (AFP), and ongoing drug abuse or alcohol abuse.

The 40 patients were divided into two groups based on abdominal ultrasound investigation as follows:

Group I: (n = 20) Obese with non-alcoholic fatty liver disease (obese FL).

Group II: (n = 20) Obese without non-alcoholic fatty liver disease (obese non-FL) with normal liver appearance by ultrasound.

Control: (n = 20) Normal weight adult subjects (BMI < 25 kg/m^2) with normal liver appearance by ultrasound.

The study protocol conformed to ethical guidelines of the 1975 Declaration of Helsinki, approved by the Institutional Review Board (IRB) for Human Subject Research at National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo, Egypt (serial: 11-2016). All patients included in this study signed an informed consent. All hematological and random blood sugar tests were done immediately after sample collection.

All patients were subjected to full medical history taking, abdominal ultrasound and thorough clinical examination including abdominal examination with focus on liver examination and anthropometric assessment (height [Ht], weight [Wt] and body mass index [BMI]).

The following biochemical investigations were performed:

Liver function was determined by BECKMAN with kits supplied from DADE BEHRING, Germany, including direct bilirubin [10], total bilirubin [11], alanine aminotransferase (ALT) [12], aspartate aminotransferase (AST) [13], alkaline phosphatase (ALP) [14], total proteins (TPs) [15], serum albumin (ALB) [16], and gamma-glutamyl transpeptidase (GGT) [17].

Prothrombin time (PT) and International Normalization Ratio (INR) were determined using the STAGO compact (USA). Complete blood count (CBC) was determined using the Sysmex SE-9000 (Germany). Blood glucose was determined according to the method of Kunst *et al.* [18] by the automated device, BECKMAN, using kit from DADE BEHRING (Germany).

Hepatitis markers were determined by enzyme-linked immunosorbent assay (ELISA) 3rd generation technique as follows; hepatitis C virus (HCV) by the method of Van der Poel *et al.,* [19] (Ortho-Clinical Diagnostics [USA]), while hepatitis BsAg was detected as described by the method of Thomas [20] (Axiom Gesellschaft für Diagnostica und Biochemica mbH Company [Germany]). Antinuclear antibody (ANA) was detected by enzyme immunoassay (EIA) technique according to the method of Tan *et al.* [21] (Binding site Company [UK]). Alpha-fetoprotein (AFP) was quantitatively assayed by enzyme immunoassay (EIA)

technique according to the method of Uotila *et al.* [22] (Axiom Gesellschaft für Diagnostica und Biochemica mbH Company [Germany]). Alpha-1-antitrypsin assay was determined by immunoturbidimetric assay technique according to the method of Miravitlles *et al.* [23] by cobas c 311/501 (Roche/Hitachi cobas c systems [Germany]). Ferritin was quantitatively assayed according to the method of White *et al.* [24] (Bios Company [USA]). Ceruloplasmin was determined by immunoturbidimetric assay with a kit supplied from Roche/Hitachi cobas c systems (Germany) according to the method of Kroll *et al.* [25] by cobas c 311/501.

Lipid profile was determined by Dimension with kits supplied from Dimension® clinical chemistry system, USA, including total cholesterol (TC) according to the method of Rautela *et al.* [26], triglycerides (TGs) according to the method of Rautela *et al.* [27] and high-density lipoprotein (HDL-C) according to the method of Moshides [28].

Kidney function which was determined by BECKMAN with kits supplied from DADE BEHRING, Germany, including creatinine according to the method of Taussky [29], blood urea nitrogen (BUN) according to the method of Talke and Schubert [30], and uric acid (UA) as described by Henry [31].

Statistical analysis

Data were statistically analyzed by the computerized program "Statistical Package for the Social Sciences (SPSS)" software, version "20" for Windows. "One-way ANOVA," "LSD," and "Duncan test" were used to compare between mean values of the analyzed parameters. Data were represented as Mean ± S.D. Values were considered significant at $p \leq 0.05$.

Results

Patients were suspected to have NAFLD (by abdominal ultrasound). In addition, 20 age-matched non-obese healthy subjects were taken as control group with a mean age of 30.15 ± 11.53 years.

According to the results of abdominal ultrasound, patients were divided into two subgroups (Table 1):
- Group 1: Obese with non-alcoholic fatty liver disease (NAFLD); they were 20 patients (14 females and 6 males) with mean age 44.96 ± 8.71 years.
- Group 2: Obese without NAFLD; they were 20 patients (18 females and 2 males) with mean age 39.15 ± 8.27 years (Table 1).

There was a highly significant difference when compared BMI of the obese FL group with the control group with $p < 0.001$. Furthermore, there was a highly significant difference between the obese non-FL group and the control group with $p < 0.001$ and a significant difference between the obese FL group and obese non-FL group with $p = 0.003$ (Table 1).

Table 1: Sex and BMI of control, obese FL group, and obese non-FL group

Parameter	Control	Obese FL	Obese non-FL
Sex	M=12, F=8	M=6, F=14	M=2, F=18
BMI (kg/m^2)	22.07 ± 2.10[a]	40.86 ± 5.45[b]	35.83 ± 5.94[c]

One-way ANOVA was used. LSD and Duncan test were used. Data are represented as mean±S.D. Values that share different letters in the same row are significant, otherwise are non-significant.

Table 2 shows liver function and coagulation profile of all studied groups. A significant difference between the control group and the obese FL group for the total bilirubin was recorded, while no significant differences was noticed among all groups in case of direct bilirubin. In respect to liver enzymes ALT and AST, there was a significant difference for each of the obese FL group compared to the control group ($p = 0.003$ and $p = 0.015$, respectively) and the obese FL group compared to the obese non-FL group ($p = 0.005$ and $p = 0.032$, respectively). On the other hand, ALP showed no significant difference among all studied groups. The albumin showed a significant difference when comparing the obese FL group with the control group and the obese non-FL group with the control group ($p = 0.046$ and $p = 0.029$, respectively), while no significant difference was noticed for the total protein among all studied groups. Data obtained for PT showed a significant difference when comparing the obese FL with the control group and the obese non-FL group with the control group ($p < 0.001$ and $p = 0.004$, respectively), while there was a significant difference only for the INR between the obese FL and control $p = 0.002$, but no significance between the obese non-FL and the control group was noticed.

Table 2: Liver profile and coagulation profile of control, obese FL group, and obese non-FL group

Parameter	Control	Obese FL	Obese non-FL
T.Bil (mg/dl)	0.47 ± 0.15[a]	0.62 ± 0.25[b]	0.55 ± 0.16[ab]
D.Bil (mg/dl)	0.11 ± 0.04	0.14 ± 0.06	0.13 ± 0.04
ALT (IU/L)	25.45 ± 7.12[a]	57.30 ± 46.24[b]	27.35 ± 11.09[a]
AST (IU/L)	21.70 ± 3.81[a]	41.40 ± 36.09[b]	24.05 ± 7.50[a]
ALP (IU/L)	81.30 ± 20.38	66.65 ± 21.01	83.65 ± 36.41
TP (gm/dl)	7.53 ± 0.59	7.49 ± 0.50	7.33 ± 0.47
ALB (gm/dl)	4.17 ± 0.32[a]	3.96 ± 0.31[b]	3.94 ± 0.30[b]
PT % activity	97.86 ± 4.31[a]	86.80 ± 11.32[b]	89.58 ± 6.23[b]
INR	1.01 ± 0.02[a]	1.11 ± 0.13[b]	1.06 ± 0.06[ab]

One-way ANOVA was used. LSD and Duncan test were used. Data are represented as mean±S.D. Values that share different letters in the same row are significant, otherwise are non-significant.

Kidney profile and blood sugar of all studied groups are illustrated in Table 3. There was a significant reduction in BUN for the obese FL group compared to the control group ($p = 0.028$), also there was a highly significant reduction for the obese non-FL group compared to the control group ($p = 0.001$). The creatinine showed a significant increase when comparing the obese FL group with the control group or with the obese non-FL group ($p = 0.028$ and $p = 0.046$, respectively), while there was a significant increase only for the UA between the obese FL and control $p = 0.007$, but no

significance between the obese non-FL and the control group was noticed. In respect to FBS and P.P, there was a highly significant increase for each of the obese FL group compared to the control group (p < 0.001 and p < 0.001, respectively) and the obese FL group compared to the obese non-FL group (p < 0.001 and p < 0.001, respectively).

Table 3: Kidney profile and blood sugar of control, obese FL group, and obese non-FL group

Parameter	Control	Obese FL	Obese non-FL
BUN (mg/dl)	13.95 ± 4.02[a]	11.72 ± 2.07[b]	10.55 ± 2.98[b]
Creat (mg/dl)	0.68 ± 0.15[a]	0.80 ± 0.22[b]	0.69 ± 0.16[a]
UA (mg/dl)	3.59 ± 1.33[a]	4.73 ± 1.27[b]	4.15 ± 1.26[ab]
FBS (mg/dl)	84.10 ± 7.19[a]	119.70 ± 49.11[b]	80.50 ± 8.84[a]
P.P (mg/dl)	94.35 ± 3.70[a]	152.80 ± 82.86[b]	93.35 ± 7.77[a]

One-way ANOVA was used. LSD and Duncan test were used. Data are represented as mean±S.D. Values that share different letters in the same row are significant, otherwise are non-significant.

Table 4 shows lipid profile of all studied groups. The TG showed a highly significant increase when comparing the obese FL group with the control group or with the obese non-FL group (p < 0.001 and p = 0.003, respectively), while there was a significant increase only for the HDL-C between the obese FL and obese non-FL groups (p = 0.022), but no significance between the obese non-FL and the control group was noticed. On the other hand, total cholesterol (TC) showed no significant difference among all studied groups.

Table 4: Lipid profile of control, obese FL group, and obese non-FL group

Parameter	Control	Obese FL	Obese non-FL
TC (mg/dl)	178.85 ± 27.90	176.90 ± 41.79	170.90 ± 49.16
TG (mg/dl)	88.35 ± 24.26[a]	128.20 ± 43.49[b]	94.50 ± 31.65[a]
HDL-C (mg/dl)	41.90 ± 4.85[ab]	40.05 ± 5.81[a]	45.16 ± 9.15[b]

One-way ANOVA was used. LSD and Duncan test were used. Data are represented as mean±S.D. Values that share different letters in the same row are significant, otherwise are non-significant.

Table 5 shows blood picture of all studied groups. There was a significant difference in RBCs for the obese non-FL group compared to the control group (p = 0.012), while no significant difference was noticed for the RBCs when comparing obese FL group to the control group. On the other hand, WBCs, Hb, and Plt showed no significant difference among all studied groups.

Table 5: Blood picture of control, obese FL group, and obese non-FL group

Parameter	Control	Obese FL	Obese non-FL
WBCs (10³/μl)	6.53 ± 1.82	7.62 ± 2.07	6.45 ± 2.38
RBCs (10⁶/μl)	5.07 ± 0.53[a]	4.84 ± 0.65[ab]	4.64 ± 0.38[b]
Hb g/dl	13.36 ± 1.94	13.01 ± 1.55	12.59 ± 0.99
Plt (10³/μl)	259.25 ± 51.66	289.65 ± 67.90	274.40 ± 62.56

WBCs: White blood cells, RBCs: Red blood cells, Hb: Hemoglobin. One-way ANOVA was used. LSD and Duncan test were used. Data are represented as mean ± S.D. Values that share different letters in the same row are significant, otherwise are non-significant.

AFP, ceruloplasmin, alpha-1-antitrypsin, and ferritin of all studied groups are illustrated in Table 6. There was a highly significant difference in AFP for the obese FL group compared to the control group (p = 0.001), while no significant difference was noticed for the AFP when compared obese non-FL group to the control group. The ceruloplasmin showed a significant

Table 6: AFP, ceruloplasmin, alpha-1-antitrypsin, and ferritin of control, obese FL group, and obese non-FL group

Parameter	Control	Obese FL	Obese non-FL
AFP (ng/ml)	1.20 ± 0.75[a]	2.42 ± 1.67[b]	1.77 ± 0.69[ab]
Ceruloplasmin (mg/dl)	33.60 ± 6.99[a]	42.35 ± 9.98[b]	34.70 ± 9.83[a]
Alpha-1-antitrypsin (mg/dl)	131.20 ± 15.25	134.25 ± 19.06	134.00 ± 25.31
Ferritin (ng/dl)	67.14 ± 45.32[ab]	88.21 ± 54.88[a]	47.65 ± 32.07[b]

AFP: Alpha-fetoprotein. One-way ANOVA was used. LSD and Duncan test were used. Data are represented as mean ± S.D. Values that share different letters in the same row are significant, otherwise are non-significant

increase when comparing the obese FL group with the control group or with the obese non-FL group (p = 0.003 and p = 0.010, respectively), while there was a significant increase only for the ferritin between the obese FL and obese non-FL group (p = 0.006), but no significance between the obese FL and the control group was noticed. On the other hand, alpha-1-antitrypsin showed no significant difference among all studied groups.

Discussion

The present study was conducted to investigate the relationship between NAFLD and different biochemical parameters, especially liver profile and lipid profile in Egyptian population.

The body mass index (BMI) was significantly higher in NAFLD cases than in normal control participants (p < 0.001). Moreover, BMI was significantly lower in obese non-FL group compared to obese FL group (p = 0.003). This finding agrees with the results obtained by other authors who reported that NAFLD has been detected as a complication for the majority (>95%) of patients having severe obesity (BMI = 35 or more) [32], [33]. Yang *et al*. [34] found that BMI was confirmed as the most useful predictive factor for NAFLD onset in both sexes in a community-based retrospective longitudinal cohort study. On the other hand, the previous studies concluded that NAFLD can occur in non-obese subjects who are physically inactive "Metabolic obesity" [35], [36].

Approximately 80% of patients with NAFLD have liver function tests in normal ranges; only a small proportion exhibits mild elevation of aminotransferases [37]. In the present study, serum ALT level was significantly higher in NAFLD cases than in normal control participants (p = 0.003) and in obese FL group compared to obese non-FL group (p = 0.005). This agrees with the previous studies which concluded that ALT is more predictive for accumulation of liver fat among the liver enzymes and correlates with liver fat independent of obesity and can be an independent predictor of the inflammation degree [38], [39], [40]. Elevation of liver enzymes, particularly ALT, is often the first sign for NAFLD; about 1–3 times increase of its normal level being reported [41].

Serum AST level was significantly higher in obese FL group compared to normal control

participants (p = 0.015) and in obese FL group compared to obese non-FL group (p = 0.032). This agrees with the previous studies which found that AST was independently associated with NAFLD and can be considered as an independent marker for hepatic fibrosis severity [40], [42], [43].

This study showed that serum T.Bil level was significantly higher in obese FL group than in normal control participants (p = 0.014), while there was no significant change in serum T.Bil level between obese FL group and obese non-FL group (p = 0.226). Data obtained in the present study can be interpreted either as an undiagnosed Gilbert's disease or as a response to combat NASH by the protective antioxidant role of bilirubin [44]. Tian *et al.* [45] reported that elevated levels of serum bilirubin are inversely associated with NAFLD which was explained on the basis of the antioxidant effect of bilirubin. Serum bilirubin, the end product of haem metabolism, has been found to possess potential antagonizing oxidative stress and inflammatory properties by acting as antioxidant and cytoprotectant *in vitro* and *in vivo*. Meanwhile, NAFLD is frequently demonstrated to strikingly associate with the risk of metabolic syndrome, type 2 diabetes, and cardiovascular diseases independent of other classical risk factors [20], [21], [22]. Therefore, a straightforward hypothesis has been proposed that bilirubin may contribute to protection against NAFLD risk, probably based on the antioxidant effects of bilirubin.

The serum albumin in this study although in the normal range, it was significantly lower in NAFLD cases when being compared to normal control (p = 0.046). It seems that the obtained results for albumin are in accordance with Hadizadeh *et al.* [44] who reported that albumin was said to be a factor related to steatohepatitis and as a predictor for hepatic-related mortality.

In the present study, plasma PT level was significantly lower in obese FL group compared to normal control participants (p < 0.001) and INR ratio was significantly higher in obese FL group compared to normal control participants (p = 0.002), but they are still in the normal range. This agrees with Saremi *et al.* [46] who found that PT and PTT (partial thromboplastin time) have a negative association with NAFLD.

Serum FBS level was significantly higher when either comparing obese FL group to normal control participants (p < 0.001) or to obese non-FL group (p < 0.001). In addition, serum P.P glucose level was significantly higher in obese FL group when compared to either normal control participants (p < 0.001) or to obese non-FL group (p < 0.001). Macherla [47] reported similar results. Furthermore, Saini *et al.* [48] found that HbA1C and FBS levels in NAFLD patients were found to be significantly higher compared to the control group. It has been shown that excess deposition of fat in liver (NAFLD) has powerful cross-sectional associations with insulin resistance, obesity, and T2DM. Pang *et al.* [49] found that there is a higher NAFLD prevalence in pre-diabetic individuals as well as overt T2DM.

The level of serum TG was significantly higher in obese FL group compared to normal control participants (p < 0.001) or to obese non-FL group (p = 0.003), while serum HDL-C level was significantly lower in obese FL group compared to obese non-FL group (p = 0.022). It was reported that low levels of HDL-C and high levels of TGs are among the most important criteria of NAFLD patients [34]. The obtained results for TGs and HDL-C in the present study are in agreement with other studies [50], [51].

Serum creatinine and UA levels were significantly higher in obese FL group compared to normal control participants (p = 0.028 and 0.007, respectively), even they are in the normal range. Only serum creatinine level was significantly higher in obese FL group compared to obese non-FL group (p = 0.046). On the other hand, there was a significant decrease in serum BUN level when comparing obese FL group to the control group (p = 0.028). This agrees with Darmawan *et al.* [52] who stated that higher levels of serum UA are independently and positively associated with the presence of hepatic steatosis. Moreover, he added that serum UA within the normal range correlated positively with tumor necrosis factor-α (TNF-α), interleukin-18 (IL-18), and IL-6. It also induced oxidative stress in vascular cells and adipocytes. UA increases the lipogenic effects of fructose by increasing the expression of ketohexokinase (KHK) that resulted in accumulation of TGs in hepatocytes. Furthermore, Feng *et al.* [53] mentioned that NAFLD patients had elevated levels of renal function parameters (BUN, creat, and UA) compared to control participants, even if they are within the normal ranges.

The obtained results of serum AFP level were significantly higher in NAFLD cases compared to normal control participants (p = 0.001), even if it is still in the normal range. These results were similar to other studies Babali *et al.* [54], Xu *et al.* [55] who observed that serum AFP levels are significantly raised in NAFLD patients and that levels of AFP are significantly associated with metabolic parameters. Univariate logistic analysis showed that increased levels of serum AFP are associated with an increased NAFLD risk. However, multivariate logistic regression analysis revealed that AFP is not associated independently with the NAFLD risk factors. The study suggested a significant association between NAFLD and AFP. They finally said that AFP acts as a cofactor for NAFLD, but not as an independent factor. The mechanism by which AFP increases may be as a result of ongoing inflammation, most probably secondary to cellular destruction or stimulation of AFP production by cytokines. Elevated serum AFP levels may also be due to altered hepatocyte-hepatocyte interaction and the loss of normal architectural arrangements.

Serum ceruloplasmin (CP) level was significantly higher in obese FL group compared to

normal control participants (p = 0.003), although it was still in the normal range. Furthermore, serum ceruloplasmin level was significantly higher in obese FL group compared to obese non-FL group (p = 0.010). This finding is in agreement with the study of Xu *et al*. [56]. In addition, it is well known that CP is an acute-phase reactant, and CP levels in the blood plasma increase when the immune system responds to infection and inflammation. Inflammatory responses are largely mediated by cytokines. Patients with acute, subacute, and chronic liver failure had the lowest mean serum CP. However, data obtained in the present study can be interpreted on the basis that the studied patients were still in a degree of liver inflammation and did not reach the degree of liver failure.

Serum ferritin level was non-significantly higher in obese FL group compared to normal control participants. However, serum ferritin level was significantly higher in obese FL group compared to obese non-FL group (p = 0.006). This agrees with Du *et al*. [57]. Furthermore, Barros *et al.* [58] added that serum ferritin can be considered as a non-invasive prognostic marker for NAFLD patients.

Conclusion

The present study revealed an altered liver function and lipid profile in cases of NAFLD. Altered values for the above biochemical parameters may play a crucial role in monitoring the disease progression and severity. Early detection could support not only in modifying the course of the disease but delaying any further complications as well. Recently, various serum biomarkers and laboratory tests have been proposed as surrogates of liver histology. Notably, non-invasive serum biomarkers, when combined, may reduce the number of liver biopsies needed for correctly classifying hepatic steatosis.

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