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Insulin Resistance in Egyptian Nonalcoholic Fatty Liver Disease patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAM designed the study, wrote the protocol. Authors EMAI and OS wrote the first draft of the manuscript. Authors WGS and AAH managed the literature searches. Authors MESM and HAAA analyses of the study and authors AAM and MA performed the analysis. Author AES revised manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: The liver has been recognized as a major target of injury in patients with insulin resistance or the metabolic syndrome. Insulin resistance is associated with fat accumulation in the

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liver, a condition called nonalcoholic fatty liver disease (NAFLD). NAFLD is a clinicopathologic entity that includes a spectrum of liver damage ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and rarely, progression to cirrhosis. Recent studies emphasize the role of insulin resistance, oxidative stress and subsequent lipid peroxidation, proinflammatory cytokines, adipokines and mitochondrial dysfunction in the development and progression of NAFLD. About 20% all adults have NAFLD and 2% to 3% of adults have NASH. A strong correlation exists between overweight, in particular visceral fat accumulation, and prevalence of NASH.

Aim: "This study aimed at assessing the effect of insulin resistance in a sample of Egyptian patients with non-Alcoholic fatty liver".

Methods: This study was conducted on 2 groups 104 NAFLD as diagnosed by ultrasound examination and 21 healthy participants as control group. All the participants were subjected to an abdominal ultrasonography, liver enzymes, lipid profile (triglycerides, HDL, LDL cholesterol), glucose and fasting insulin.

Results: The blood sugar and fasting insulin levels were significantly higher in NAFLD patients than control group (172.81±35.47 mg/ml vs 101.33±11.95 mg/ml and11.72±4.7 U/ml vs 5.93±4.68) respectively. 88.5% of NAFLD patients were obese (BMI ≥ 30) and 11.5% were over weight (BMI < 30) while 23.8% were obese and 76.2% were overweight for control group. HOMA-IR was significantly higher in NAFLD patients than in healthy controls (5.02±2.39 vs. 1.41±1.20; P<0.001). We found 81.7% of the studied patients fulfilled the metabolic syndrome criteria while 9.5% for controls. HOMA-IR ROC curve showed 94.23% sensitivity and 85.71 specificity in NAFLD group. Fasting Insulin ROC curve showed 91.35% sensitivity and 80.95% specificity in NAFLD group.

Conclusion: Patients with NAFLD have higher insulin resistance and have higher lipid profile, ALT & AST levels compared with their control group. Also the Ratio of the metabolic syndrome was higher in the NAFLD patients (81.7%).

Keywords: Non alcoholic fatty liver disease; non alcoholic steatohepatitis; insulin resistance and metabolic syndrome.

1. INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) is the most common liver disease since its prevalence is estimated to be 20-30% in general population of Western countries [1]. It has no age or sex predilection and about 2.6% of children are affected and this figure increases up to 22.5% in obese children [2]. However, other studies have shown that the prevalence of NAFLD increases with age [3]. NAFLD is characterized by significant lipid disposition in the hepatocytes of liver parenchyma [2].

The definition of NAFLD requires that there is evidence of hepatic steatosis, either by imaging or histology and there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders [4]. Histologically, NAFLD comprises a wide spectrum of liver damage ranging from simple benign non alcoholic steatosis to non alcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis [2]. NASH should be considered the most severe form of NAFLD spectrum with a histological findings ranging from hepatic steatosis and inflammation to steatosis plus hepatocyte injury with or without fibrosis [4,5]. NAFLD is generally benign whereas NASH can progress to cirrhosis in about 15-25% of cases, liver failure and hepatocellular carcinoma [4,6].

Metabolic syndrome (MS), also known as syndrome X or insulin resistance syndrome is currently defined as a clustering of risk factors combining central obesity plus at least two additional factors including: blood pressure ≥130/80, fasting blood glucose ≥100 mg/dl, triglycerides ≥ 150 mg/dl and HDL cholesterol <40 mg/dl in males and <50 mg/dl in females. MS predicts the development of diabetes and cardiovascular disease [5,7]. NAFLD is now recognized to be the hepatic component of the metabolic syndrome which along with its individual component, particularly diabetes and elevated triglycerides, are the major risk factors for the development of NASH [5,8].

NAFLD is strongly associated with both hepatic and adipose tissue insulin resistance as well as reduced whole-body insulin sensitivity [8,9]. Additionally, subjects with NAFLD exhibit a defect in insulin suppression of free fatty acids

(FFA), in keeping with insulin resistance at the level of the adipocyte [10]. Compared with control subjects, subjects with NAFLD also demonstrate a blunted inhibition of fatty acid oxidation, reflecting the decreased uptake and use of glucose as a source of fuel [9]. These findings suggest the possibility that insulin resistance may be an intrinsic defect in NAFLD, and that diminished insulin responsiveness at the level of the adipocyte may contribute to hepatic steatosis by excess FFA flux to the liver.

Insulin resistance (IR) is defined as decreased sensitivity or responsiveness to metabolic actions of insulin such as insulin-mediated glucose disposal [11] and inhibition of hepatic glucose production [11,12]. IR is a key pathogenic factor in both NAFLD and MS. IR and compensatory hyperinsulinemia have central etiologic roles in the development of MS [13]. Homeostasis model assessment of insulin resistance (HOMA-IR) is a simple and useful method for evaluating insulin resistance (or sensitivity) and β cell function, that is calculated from fasting plasma glucose and immunoreactive insulin and correlate with the results of glucose clamp test (the gold standard of evaluation of IR) in mild diabetic patients without significant hyperglycemia [14].

The incidence of NAFLD increases with increase in body mass index (BMI), [15] with a prevalence up to 60–70% in obese patients [16]. However, liver histology analysis showed that up to 15% of non-obese patients also have NAFLD and that 3% have steatohepatitis [17]. NAFLD is closely associated with central adiposity, type 2 diabetes mellitus, dyslipidemia, and IR, all of which are components of MS [18]. Thus the aim of this study was to evaluate the effect of insulin resistance in the development of nonalcoholic fatty liver disease in Egypt.

2. SUBJECTS AND METHODS

This study was conducted on 104 NAFLD patients (43 males and 61 females, aged 54.46±10.14years) and 21 healthy volunteers (12 males and 9 females, aged 40.90±15.5) of matched BMI as a control. In total, 161 adult patients with NAFLD were admitted to our gastroenterology out-patients clinic, El Sahel Teaching Hospital in the duration of January 2011 to May 2012. 19 patients with missing data 13 pregnant women and 25 patients with other liver diseases were excluded and only 104 patients were included in this study. All the participants involved in this study were subjected to ultrasonography. Patients were consecutively enrolled in the study if the ultrasonography showed fatty liver appearance with or without increased liver transaminases. The viral hepatitis (hepatitis B virus-HBV, hepatitis C virus-HCV, cytomegalo virus-CMV), toxic, autoimmune (AIH) and metabolic liver diseases (Wilson disease, α-1 antitrypsin deficiency, cystic fibrosis) were excluded by ELISA technique. Both HBs (HBV) Ag and HCV Ab were done using (Axiom GmbH Germany) and for CMVIgG using (Orgenium Laboratories Helsinki FINLAND) for both cases and controls. The diagnosis of NAFLD was based on the standard criteria accepted by the American Gastroenterology Association (AGA) by ultrasonographic finding of bright liver [19] that is defined as abnormally intense, high level echoes arising from the hepatic parenchyma, with amplitude similar to that of echoes arising from the diaphragm and according to Saverymuttu et al. [20], who reported that ultrasound examination can accurately identify steatosis with sensitivity of 94% and a specificity of 84%. Although liver biopsy remains the gold standard for characterizing liver histology in patients with NAFLD yet none of our patients was subjected to liver biopsy as both the stage & the grade were not at our consideration in the study, in addition to the several limitations of liver biopsy including the potential complications, the associated sampling error [21] and the high cost. We excluded patients with viral hepatitis, autoimmune diseases (Autoimmune liver diseases excluded by absence of auto-antibodies which indicative of autoimmune hepatitis), Wilson's disease, hemochromatosis, diabetes (Glycated hemoglobin (Hb A1C) ≥6.5% is diabetic), [22] malignancy, hypothyroidism, coronary artery disease, pregnancy, cigarette smoking, and oral contraceptives. 19 patients were highly educated, 33 medium education, 24 low education and 28 were illiterate. Informed consent was obtained from all participants before enrollment in the study. The study was carried out in accordance with the principles of the Declaration of Helsinki, and its appendices, and local and national laws.

All the participants were subjected to detailed clinical history taking, complete clinical examination, weight and height measurements and BMI was defined as the ratio of body weight to body height and expressed in kg/m². Both patients and control groups were further subdivided according to their BMI into overweight and obese with BMI between 25 and 30 kg/m2 and ≥30 kg/m2, respectively (The healthy volunteers were selected according to BMI

either overweight (BMI 25-30 kg/m2) or obese (BMI >30 kg/m2) to be matched with patients) [23]. In this study 87 patients have blood pressure ≥130/90. For laboratory investigations, 5ml of venous blood (5 ml) was collected after an overnight fasting in vacutainers without additive, allowed to clot for 30 min at room temperature and centrifuged at 5000 rpm for five minutes. The separated serum was stored into aliquots at-20ºC until biochemical analysis including liver enzymes (ALT & AST), fasting blood glucose, total serum cholesterol, triglyceride and HDL cholesterol (were analyzed enzymatically using kit obtained from Randox Laboratories Limited, Crumlin, UK), and serum insulin (detected by ELISA using commercial Human kit). The insulin cut off value in this study was >5.25 µU/ml.

Serum LDL cholesterol was determined from the values of total cholesterol and HDL-cholesterol using friedewalds formula [24,25].

LDL-cholesterol = TC – (TG/5) – HDL-cholesterol (mg/dl)

HOMA-IR was calculated using the following formula [12]:

HOMA-IR=fasting serum glucose (mg/dl) × fasting insulin (µU/ml)/405. The cut off value for IR for NAFLD patients in this study was >1.5.

2.1 Statistical Analysis

In this study, data analyzed using Statistical Package for Social (SPSS) version 19. Excel computer program was used to tabulate the results, and represent it graphically. For the quantitative variables which are normally distributed, independent t-test used to declare the significant difference between groups at $p<0.05$. Pearson's correlation coefficient used to declare the significant correlation between the quantitative parameters within each group at $p<0.05$. MedCalc creates a list of sensitivity, specificity, likelihood ratios, and positive and negative predictive values for all possible threshold values. MedCalc allows to perform Receiver operating characteristic curve ROC curve analysis easily and accurately, the Area under the curve (AUC) with standard error (SE) and 95% confidence interval (CI), with automatic calculation of corresponding sensitivity and specificity. The statistical power was 80%.

3. RESULTS

Demographic, clinical and laboratory characteristics of both patients and controls included in the study were shown in Table 1. Patients with NAFLD were significantly older, had higher values for BMI, FBS, fasting insulin, AST, ALT, total cholesterol triglycerides, LDL levels and significantly lower serum HDL levels compared to the control group. But serum AST/ALT showed no significant difference between patients and controls. HOMA-IR was significantly higher in NAFLD patients than in healthy controls (5.02±2.39 vs. 1.41±1.20; $P<0.001$) (Table 1). 88.5% of NAFLD patients were obese (BMI ≥ 30) and 11.5% were overweight (BMI < 30) while 23.8% were obese and 76.2% were overweight for control group (Table 2). In Table 3, comparison between NAFLD patients and control subgroups: the over weight with BMI < 30 and the obese with BMI ≥ 30, showed no significant difference in HOMA-IR between the two patients subgroups ($P =0.229$), however there was a significant difference between the two control subgroups ($P =0.041$), meanwhile, there was no significant difference in serum ALT, and AST levels between the two subgroups in both patients and control. In Table 4, HOMA-IR showed a weak positive correlation with triglycerides ($r=0.250$, $p= 0.01$), cholesterol ($r=0.232$, $p= 0.018$) and LDL level ($r=0.205$, $P=0.037$).We found 81.7% of the studied NAFLD patients fulfilled the metabolic syndrome criteria compared to 9.5% of the control (Table 5). A HOMA-IR value higher than 1.5 which represents the cut-off value associated with NAFLD in this study based on the ROC curve constructed for HOMA-IR with AUC 0.933, 94.23% sensitivity and 85.71% specificity. According to fasting insulin a value higher than 5.25 was the cut-off value associated with the NAFLD patients with AUC 0.818 91.35% sensitivity and 80.95% specificity (Table 6), Figs. 1 and 2.

According to the International Diabetes Federation (IDF, 2005) [26]: a person is defined as having the metabolic syndrome if he has central obesity (defined as waist circumference with ethnicity-specific for other groups plus two of the following four factors:

Raised triacylglycerol level: >150 mg/dl (1.7 mmol/L) or specific treatment for this lipid abnormality.

Reduced high density lipoprotein cholesterol < 40 mg/dl (0.9 mmol/L) in males and <50 mg/dl (1.1 mmol/L) in females or specific treatment for this lipid abnormality. Raised blood pressure: systolic blood pressure >

130 or diastolic blood pressure > 85 mmHg or treatment of previously diagnosed hypertension. Raised fasting plasma glucose > 100 mg/dl (5.6 mmol/L) or previously diagnosed type 2 diabetes. If above 5.6 mmol/L or 100 mg/dl, oral glucose tolerance test is strongly recommended but is not necessary to define the presence of this syndrome.

If BMI is >30 kg/m², central obesity can be assumed and waist circumference does not need to be measured.

Table 1. Characteristics of patients and control groups

Variables	Controls (n=21)	Patients (n=104)	p- value
Gender M/ F	12/9	43/61	NS
Age (Years)	40.90±15.52	54.46±10.14	0.000*
BMI (kg/m2)	25.95±4.28	36.03±5.39	0.000*
FBS (mg/dl)	101.33±11.95	172.81±35.47	0.000*
F-Insulin µU/ml	5.93±4.68	11.72±4.7	0.000*
HOMA-IR	1.41±1.20	5.02±2.39	0.000*
AST (U/L)	33.86±9.64	58.76±18.98	0.000*
ALT (U/L)	31.43±6.95	66.41±36.57	0.000*
AST/ALT	1.08±0.22	1.04±0.44	0.708
Total cholesterol (mg/dl)	168.14±25.39	202.51±27.81	0.000*
Triglycerides (mg/dl)	156.67±31.4	197.24±34.04	0.000*
LDL (mg/dl)	108.67±12.5	129.62±21.98	0.000*
HDL (mg/dl)	45.24±7.98	41.05±8.92	0.048*

*All data expressed as mean ± SD, * There is a significant difference between control group and patient group by using independent t-test at p<0.05, NS= Not Significant, BMI = Body mass index, FBS = Fasting Blood Sugar, HOMA-IR = Homeostasis model assessment – insulin resistance, AST = Aspartate Transaminase, ALT = Alanine Transaminase, LDL= Low Density Lipoprotein, HDL=High Density Lipoprotein*

Table 2. Comparison between NAFLD patients and control groups regarding to BMI

BMI		Group	
		Controls	Patients
<30	Count	16	12
	%	76.20%	11.50%
≥30	Count	5	92
	%	23.80%	88.50%
Total	Count	21	104
	%	100.00%	100.00%

BMI = Body Mass Index

Table 3. Comparison between NAFLD patients and control groups regarding to laboratory findings

Parameter	NAFLD Patients			Control		
	BMI <30 (n =12)	BMI > = 30 (n= 92)	p-value	BMI <30 (n =16)	BMI> = 30 (n= 5)	p-value
HOMA-IR	4.24±1.99	5.13±2.43	0.229	1.12±0.59	2.36±2.12	0.041*
ALT (U/L)	56.92±20.65	67.65±38.07	0.341	30.81±6.42	33.4±8.99	0.482
AST (U/L)	53.83±14.04	59.4±19.5	0.341	33.31±9.39	35.6±11.35	0.655

BMI = Body Mass Index, HOMA-IR = Homeostasis Model Assessment – Insulin Resistance, AST = Aspartate Transaminase, ALT = Alanine Transaminase.

*All values are represented as Mean ± Standard Deviation, *= There is a significant difference between groups by using independent t-test at p<0.05*

Table 4. Correlation coefficient of IR with lipid profile and liver enzymes in the NAFLD patients

Correlation IR vs.	TG	Cholesterol	HDL	LDL	ALT	AST
r- value	0.250*	0.232*	0.178	.205*	-0.017	-0.096

*HOMA-IR = Homeostasis Model Assessment – Insulin Resistance, TG Triglycerides, HDL=High Density Lipoprotein, LDL= Low Density Lipoprotein, ALT = Alanine Transaminase, AST = Aspartate Transaminase. *Correlation is significant at the 0.05 level*

Table 5. Comparison between NAFLD patients and control groups regarding to metabolic syndrome

Metabolic syndrome	Controls		Patients	
	Count	%	Count	%
Present	2	9.5	85	81.7
Absent	19	90.5	19	18.3
Total	21	100	104	100

Table 6. Evaluation of optimal cut-off values of HOMA-IR and F-insulin in NFALD

Variable	Cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HOMA-IR	>1.5	94.23	85.71	97.0	75.0
F-insulin	>5.25	91.35	80.95	96.0	65.4

HOMA-IR = Homeostasis Model Assessment – Insulin Resistance, PPV (Positive Predictive Value), NPV (Negative Predictive Value)

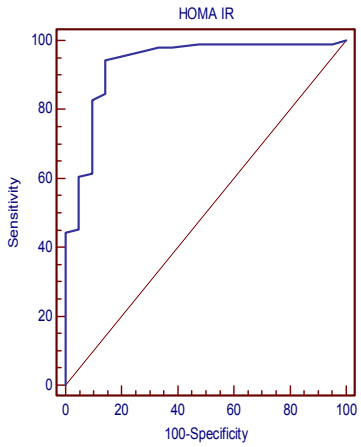

Fig. 1. Receiver operating characteristic curve for HOMA –IR (AUC= 0.933, p= 0.0001)

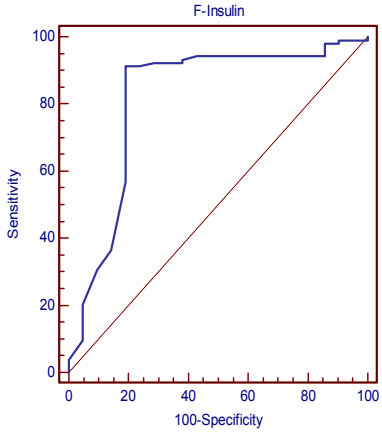

Fig. 2. Receiver operating characteristic curve for F- insulin (AUC= 0.818, p= 0.0001)

4. DISCUSSION

Obesity is a growing problem in the Middle East [27] and internationally, [28] which requires government action on the primary prevention of obesity. High rates of overweight and obesity increased in both men and women in most countries [27]. In this study we evaluated BMI, HOMA-IR and laboratory parameters and compared data to find associations between these parameters and the development of NAFLD in Egyptian Patients.

In the present study, the mean age of the cases was 54.46±10.14 years while that of controls was 40.90±15.52 years, and the ratio of male to female cases was 41.30% to 58.70%. In other study NAFLD was associated with increasing age, [29] and a significant relationship was found between the presence of NAFLD and the female sex in the cases [30].

The incidence of obesity has increased dramatically during recent decades. Obesity will cause a decline in life expectancy for the first time in recent history due to numerous co-morbid

disorders. Adipocyte and adipose tissue dysfunction belong to the primary defects in obesity and may link obesity to several health problems including increased risk of insulin resistance, type 2 diabetes, fatty liver disease, hypertension, and dyslipidemia [31].

In our study NAFLD patients had significantly higher BMI compared to their controls (36.03±5.39 vs. 25.95±4.28).These findings are in accordance with other studies [32,33]. NAFLD is associated with higher weight and higher body mass index [29]. Marchesani and his colleagues showed that 80% of patients with NAFLD were obese [8].

In a previous study only 21% of the population with normal BMI while the remaining 79% were either overweight or obese [34]. In our study 88.50% of NAFLD patients were obese (BMI ≥ 30 kg/m^2) and 11.5% were overweight. Goland et al. [35] proved patients with NAFLD had a significantly higher BMI. By dividing NAFLD patients and controls to subgroups (according to BMI, <30 or ≥ 30 (kg/m2) there was no significant difference between the two subgroups according to ALT and AST except to HOMA-IR in the control group.

Our current study showed that the degree of insulin resistance in NAFLD patients was more than twice of the normal subjects (5.02±2.39 vs. 1.41±1.20 in control group, p<0.000). Previously, it has shown that HOMA-IR was nearly doubled in NAFLD cases compared to matched controls as a result of increased insulin concentration and normal glucose level [36]. Also Babalı et al. [32] observed higher HOMA-IR levels in NAFLD patients compared to controls and incidence of IR increases with age, [37] and the optimal cut off value as assessed by ROC curve was higher than 1.5.

The prevalence of the disease has increased dramatically during the previous decade probably because of both, the changes of life-style (decreased physical activity and alterations in dietary habits) and the increased detection rate [38]. Insulin resistant subjects with NAFLD show reduced insulin sensitivity not only at the level of the muscle but also at the level of the liver and adipose tissue [39].

Insulin resistance was assessed by fasting serum insulin (the optimal cut off value was >5.25) and glucose and quantified by the HOMA index, a validated method of assessing IR [40,41]. The strong association between insulin resistance and NAFLD has been extensively demonstrated, and several authors have proposed to include NAFLD in the complex picture of the metabolic syndrome [42]. The hallmark feature of the pathogenesis of NAFLD, both histologically and metabolically, is the accumulation of triacylglycerol (TAG) in the liver. An increased intake of dietary fat has been suggested to lead to increased accumulation of lipids in the liver, thus leading to hepatic steatosis [43]. Available evidence suggests that insulin resistance affects hepatic fat accumulation by increasing release of free fatty acids from adipose tissue, increasing fatty acid and triglycerides synthesis in the liver, reducing fatty acid oxidation and reducing very low-density lipoprotein (VLDL) production. Insulin resistance and hyperinsulinemia are also associated with the inflammatory and fibrotic reaction that complicates advanced stages of the disease [44]. HOMA-IR showed a weak positive correlation with triglycerides (r=0.250, p= 0.01), cholesterol (r=0.232, p= 0.018) and LDL level (r=0.205, P=0.037) and also with ALT and AST (Table 4).

It is not surprising that many studies have highlighted the association between NAFLD and several factors of metabolic syndrome, especially abdominal obesity, insulin resistance, increased serum triglycerides and small dense LDL and low HDL [45]. NAFLD is associated with metabolic syndrome, as well as higher levels blood glucose [29]. It has also been proposed that NAFLD could be considered the hepatic manifestation of metabolic syndrome [46]. Prevalence of the metabolic syndrome in NAFLD has been estimated to vary from 18% in normal-weight to 67% in obese subjects [47]. Nigam et al. [48] reported that prevalence of the metabolic syndrome was higher in cases as compared to controls.

Ninety percent of individuals with NAFLD have at least one risk factor of MS, and 33% have all the features of MS. A study concluded that liver fat content is significantly increased in subjects with the MS as compared with those without the syndrome, independently of age, gender, and body mass index [49]. In our study 81.7% NAFLD patients have metabolic syndrome.

Obesity and overweight have been found to be important risk factors for elevated serum alanine aminotransferase (ALT) [15] and related to non-alcoholic fatty liver [50]. The levels of ALT was significantly higher in the cases as compared to

those in the controls [30]. Also NAFLD patients in our study have significantly higher ALT and AST compared to controls. Some studies suggest the association between insulin resistance and ALT value [51]. In subjects with NAFLD, the prevalence of elevated ALT in the presence of each metabolic risk factor, such as obesity, fasting plasma glucose, total cholesterol, TG, and hyperuricemia, did not differ from that of subjects with normal ALT levels [52]. It has been suggested that elevated liver enzymes does not strongly correlate with the level of liver injury, fibrosis or inflammation; [53] however, it is a diagnostic evaluation hint.

Regarding to socioeconomic status of our patients we found in our study 19 patients were highly educated, 33 medium education, 24 low education and 28 were illiterate. Santos and colleagues, [54] in a community-based study showed that the prevalence of metabolic syndrome is significantly higher in women of lower socioeconomical classes as defined by income and education.

5. CONCLUSION

Our study reported a higher insulin resistance, higher fasting insulin levels and HOMA index in NAFLD patients. These data support a role for insulin and put it to be suitable serum marker in predicting liver steatosis in patients with NAFLD but these findings need to be confirmed in larger studies. We recommend a large-scale screening in the high-risk population and it is crucial to treat this condition as soon as possible in order to avoid the progression to end stage liver disease.

ETHICAL APPROVAL

Informed consent was obtained from all participants before enrollment in the study. The study was carried out in accordance with the principles of the Declaration of Helsinki, and its appendices, and local and national laws.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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