Insulin Resistance and Related Factors in Non-Alcoholic Fatty Liver Disease (NAFLD): An Analytic Cross-Sectional Study

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is characterized by fatty change of liver without inflammation. The aim of this study was to evaluate presence of clinical and metabolic components in non-diabetic patients with NAFLD and to assess the relationship between insulin resistance and these factors.

Materials and Methods: In this study, a group of 50 sonographically confirmed patients with NAFLD was studied. Following an overnight fasting, blood samples were obtained to measure serum levels of Triglyceride, Cholesterol, Low Density Lipoprotein (LDL-C), High Density Lipoprotein (HDL-C), SGOT and SGPT, haemoglobin A_1C, Fasting Blood Sugar (FBS) and peripheral blood insulin level. Based on homeostatic model assessment (HOMA) score, patients were divided into four quartiles. Other variables including BMI, waist and hip circumference were also measured.

Results: The mean age was 42 ± 10.3 years (range, 22-65), 33 cases (66%) were men, and 17 cases (34%) were women. Mean insulin level was higher in females (female=15.3 ± 6.7, males=12.9 ± 5.7). Variables including waist (P=0.38) and LDL-C (P =0.49) were significantly different among defined study groups. The higher the HOMA index, the lower the HDL-C level (P <0.05).

Conclusion: Patients with insulin resistance showed significant higher values of LDL and Waist circumference. Values of HDL were significantly lower in these patients. Body mass index, Weight, Triglyceride, Cholesterol, AST and ALT values showed no relation with insulin resistance.

Keywords: Non-alcoholic fatty liver, Insulin resistance, Liver

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by fatty change of the liver with and without inflammation, similar to those of alcoholic liver disease but in the absence of significant alcohol intake.(1), NAFLD is one of the most common causes of elevated liver enzymes among adults.(2), and encompasses a spectrum of clinicopathologic entities, all of which include an accumulation of fat in the hepatic parenchyma ranging from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, and may lead to hepatocellular carcinoma.(3), The epidemiology of NAFLD has been a subject of great interest among clinical investigators. With the current epidemic of obesity and dia-

betes worldwide, it is expected that the prevalence of NAFLD likewise will increase.(4), In unselected populations, the prevalence of NAFLD has been estimated to be between 9% and 36.9%. (5-11), The prevalence of NAFLD also has been evaluated in selected populations. In patients with diabetes mellitus, the prevalence of NAFLD can be as high as 63%. (2),The main risk factors associated with NAFLD are obesity, diabetes mellitus, hyperlipidemia, and the metabolic syndrome. In many case series of patients with NAFLD, obesity, diabetes mellitus, and hyperlipidemia were observed in up to 93%, 55%, and 92% of patients, respectively.(12-20), Obesity, diabetes mellitus, and hyperlipidemia are components of the metabolic syndrome. Many experts believe that NAFLD is the hepatic manifestation of the metabolic syndrome. Most patients who have NAFLD have underlying insulin resistance, and many patients who have NAFLD fulfil criteria for the metabolic syndrome. (14,18,21,22), Despite the number of studies connecting fatty liver to insulin resistance, it is still unclear whether a diagnosis of NAFLD can help identify apparently healthy individuals with an increased risk of further complications of metabolic syndrome such as diabetes. Evaluation of insulin resistance in non-diabetic NAFLD patients could potentially lead to the better understanding of the possible risk of NAFLD itself to induce more serious metabolic disorders. Moreover, to the best of our knowledge the presence of insulin resistance and its relationship with other associated risk factors of NAFLD in non-diabetic patients is not well understood. The aim of this study was to evaluate whether the clinical and metabolic components were also present in non-diabetic patients with NAFLD and also to evaluate the relationship between insulin resistance and these factors.

MATERIALS AND METHODS

This analytic cross-sectional study was performed at Tehran University of Medical Sciences. A group of 50 patients with the diagnosis of NAFLD were included in this study. Diagnosis of the NAFLD in patients was based on sonographic finding that was performed by a single radiologist in all patients. Fasting plasma glucose was checked for patients. Subjects who had a positive history of diabetes mellitus were excluded from the study. Other criteria for exclusion were high blood pressure (in history or above 140/90 mm Hg in our measurements), long starvation, pregnancy, severe infection, uraemia, alcohol consumption, corticosteroid consumption and positive markers for viral hepatitis. After describing the study process in detail for all patients, written informed consents were obtained before entering the study. All patients were referred to another physician to perform the required measurements.

All subjects' weight, height, waist and hip circumferences were measured and body mass index (BMI) was calculated as weight/height.(2) Waist to Hip ratio was simply calculated by dividing Waist to Hip circumference. All patients were told to fast overnight and attend the hospital laboratory next morning. Blood samples were obtained to measure serum levels of Triglyceride, Cholesterol, Low Density Lipoprotein (LDL-C), High Density Lipoprotein (HDL-C), SGOT (AST), SGPT (ALT), haemoglobin A_1C, Fasting Blood Sugar (FBS) and Peripheral blood insulin level. Insulin resistance was calculated by the homeostatic model assessment (HOMA) index method as follows: HOMA= FBS x Insulin level / 405, where insulin level is expressed in mIU/mL and FBS in mg/dl. (23), Insulin resistance as determined by this method correlates closely with more complex techniques, such as the euglycemic clamp method. (24)

Based on HOMA score, patients were categorized in four quartiles (groups A, B, C, and D). In each quartile, descriptive data of the above-mentioned variables were calculated. Correlation of HOMA with other variables, were assessed in two higher quartiles (A+B) and then, in two

lower quartiles (C+D). Moreover, descriptive information of patients with HOMA score of higher than 1.64 were also analyzed. (25), This study was approved by the ethics committee of Tehran University of Medical Sciences. Statistical analysis was performed using SPSS software version 15.0 (SPSS Inc. Chicago, IL). All data are expressed as mean ± SD. Correlation was used for comparing quantitative data. Multiple group comparisons were performed using ANOVA. When statistical significance was seen on ANOVA, the Tukey honestly significant difference test was applied. Statistical significance was established at a p-value less than 0.05 (p<0.05).

RESULTS

According to the findings in ultrasonography, 50 subjects with fatty infiltration of liver were involved in the study. Values of age, weight, height, waist and hip circumference were obtained by a physician. After acquiring written informed consents, blood samples were obtained for the required biochemistry tests. The mean age for the study population was 42 ± 10.3 years (range, 22-65). Thirty-three (66%) subjects were men, and 17 (34%) were women. The mean BMI was 28.2 ± 3.8 Kg/m^2 with a range of 21 up to 36.3 Kg/m2. Demographic information of the study population is shown in table 1 according to gender. The mean fasting plasma glucose for the study population was 96.6±9.5 mg/dl (range 79-114). As shown in table 1, the mean age was 40.9 ± 9.9 years and 44 ± 10.9 years for males and females, respectively. Females had higher mean BMI (29.3 ± 4.2 Kg/m^2) while men comprised lower (27.6 ± 3.6 Kg/m^2) but this difference could not reach to a significant level. Values of variables including weight (P <0.001) and height (P =0.002) were significantly different between males and females. Other factors including age, BMI and Waist to Hip ratio were similar among the two genders.

Table 1: Demographic characteristics of the patients according to gender (all the values are presented as mean±SD)

Variable	Males	Females	Total	P-value
Age (year)	41±10	44±11	42±10	0.325
Weight (Kg)	83.6±12.7	73±9.8	79.7±12.7	<0.05*
Height (cm)	173.7±7	158±5	168±10	<0.001*
Body Mass Index (BMI) (Kg/m^2)	27.6±3.6	29.3±4.2	28.2±3	0.15
Waist to hip ratio	0.91±0.06	0.88±0.09	0.90±0.07	0.08

* Statistically significant difference

Obtained laboratory data are shown in table 2. Mean insulin level for males was 12.9 ± 5.7 mIU/mL, which was higher in female patients (15.3 ± 6.7 mIU/mL), without any noticeable difference (P=0.191). The study population had a mean computed homeostasis model assessment index of 3.30 ± 1.57 (range, 1.43-7.98) and statistical analysis did not show any difference between males and females. Among obtained laboratory data, HbA$_1$C (P=0.029), SGOT (P<0.001) and SGPT (P<0.001) were diverse between males and females.

Table 2: Laboratory characteristics of the patients according to gender (all the values are presented as mean±SD)

Variable	Males	Females	Total	P-value
Insulin level (μu/mL)	12.9±5.7	15.3±6.7	13.7±6.1	0.191
Fasting blood glucose (mg/dl)	95±8.6	99.5±10.7	96.6±9.5	0.119
HOMA	3±1.3	3.7±1.8	3.30±1.57	0.106
HbA1C(%)	5±1.1	5.8±1	5.3±1.2	0.029*
Triglyceride (mg/dl)	166.4±57.6	182.5±75	172.2±64.1	0.398
Cholesterol (mg/dl)	205.3±36.2	196.6±25.2	202.2±32.7	0.375
LDL-C (mg/dl)	125.6±30.3	115±15.8	122.7±27.3	0.39
HDL-C (mg/dl)	40.4±8.5	40±7.7	40.3±8.4	0.913
SGOT (IU/l)	42.2±21	23.5±8.1	35.4±19.6	<0.001*
SGPT (IU/l)	74.4±41.3	41.2±24.1	62.5±39.2	<0.001*

* Statistically significant difference

All patients were divided into four groups (quartiles), according to the HOMA index. Table 3 demonstrates the demographic and laboratory data of theses groups and also the rela-

tionship of different factors among groups.

Table 3: Analysis of the patients' characteristics according to the HOMA level (all the values are presented as mean±SD).

Variable	A (n=12)	B (n=13)	C (n=13)	D (n=12)	P-value
Age (year)	43.4±13.6	40.2±11.2	44.6±6	39.8±9.6	0.817
Waist circumference (cm)	92.5±10.7	93.4±9.8	100.6±9.5	98.4±9.1	0.038*
Weight (Kg)	77.3±11.8	78.3±13.9	83.1±12.5	80±13.2	0.138
Waist to Hip ratio	0.88±0.09	0.89±0.08	0.92±0.07	0.91±0.06	0.095
Body Mass Index (BMI) (Kg/m^2)	27±3.0	27.6±3.7	29.8±4.7	28.3±3.6	0.151
Triglyceride (mg/dl)	166.2±58.7	182.3±76	150.7±22	198.3±76	0.791
Cholesterol (mg/dl)	182.9±48.9	233.8±38.4	192.2±26.4	198.3±32.2	0.239
LDL-C (mg/dl)	104.6±39.4	128.2±37	121±29.7	122.2±23.8	0.049*
HDL-C (mg/dl)	56±26.9	45±9.3	34.6±6.4	34.7±5.1	0.032*
SGOT (IU/l)	35.7±18.8	44.6±27.1	32.1±19	32.6±13.7	0.367
SGPT (IU/l)	64.7±47.3	79.3±46.7	39.7±15.1	62.5±33.5	0.572
HOMA	1.8±0.2	2.4±0.2	3.2±0.2	5.6±1.3	-

* Statistically significant difference

Table 4. Laboratory and demographic characteristics of the patients with insulin resistance (HOMA values above 1.64) (all the values are presented as mean±SD).

Variable	A (n=47)
Age (year)	42.8±(10.1)
Waist circumference (cm)	96.7±(10.2)
Weight (Kg)	79.7±(13.0)
Waist to Hip ratio	0.90±(0.08)
Body Mass Index (BMI) (Kg/m2)	28.3±(3.9)
Triglyceride (mg/dl)	174±(61.2)
Cholesterol (mg/dl)	203±(33.2)
LDL-C (mg/dl)	124±(27)
HDL-C (mg/dl)	40±(8)
SGOT (IU/l)	35.6±(20)
SGPT (IU/l)	61.1±(38)
HOMA	3.4±(1.5)

Waist, LDL-C and HDL-C were significantly different among patient when we divided them according to the HOMA level.

As mentioned above, according to previous studies 1.64 was considered as cut off for insulin resistance, though 47 patients out of 50 were considered as insulin resistant whose demographic and laboratory data are shown in table 4. Analysing the correlation between HOMA index and other variables demonstrated significant relationship of HOMA level with waist, BMI, cholesterol and LDL-C in lower two quartiles (groups A and B) but such relationship was not observed in upper quartiles (groups C and D).The result of the analysis is shown in table 5. Moreover, after dividing the patients into two groups (regarding the quartile 50 as cut off point value), the mean waist value was significantly lower in patients of groups A and B [93±(10)] in comparison with groups C and D [99.5 ±(9.2)] (P-value=0.020, Table 5). In addition, the patients of two lower quartiles [49.7±(18.9)] had a significantly higher HDL than the ones in two upper quartiles [34.6 ±(5.4)] (P-value=0.014, Table 5).

DISCUSSION

As one of the most common causes of abnormal liver enzyme tests, NAFLD affects about 25% of general population. (26), It is defined as accumulation of fat in the liver exceeding 5-10% by weight in the absence of alcohol abuse, contributing medications and viral hepatitis. (22,27) Most patients are asymptomatic and on physical examination, hepatomegaly is a common finding. The histological abnormality of NAFLD is within a range including simple steatosis, steatohepatitis (NASH), fatty infiltration with ballooning degeneration, fibrosis and cirrhosis. Approximately 24-30% of patients with fibrosing steatohepatitis may progress to cirrhosis and

Table 5: Correlation of the patients' characteristics with HOMA level in upper and lower quartiles (all the values are presented as mean±SD)

Variable	A+B (n=25)	Within group P-value	C+D (n=25)	Within group P-value	Between groups P-value
Age (year)	41±(12.3)	0.35	42±(8.1)	0.857	0.84
Waist (cm)	93±(10)	0.041*	99.5±(9.2)	0.501	0.020*
Weight (Kg)	77.8±(12.6)	0.09	81.6±(12.7)	0.843	0.29
Waist to Hip ratio	0.88±(0.08)	0.127	0.92±(0.06)	0.532	0.121
Body Mass Index (BMI) (Kg/m²)	27±(3.3)	0.042*	29±(4.2)	0.566	0.111
Triglyceride (mg/dl)	174±(67)	0.547	173±(59)	0.161	0.956
Cholesterol (mg/dl)	204±(47)	0.002*	195±(29)	0.746	0.421
LDL-C (mg/dl)	118±(38)	0.003*	121±(25)	0.907	0.784
HDL-C (mg/dl)	49±(19)	0.107	34±(5)	0.996	0.014*
SGOT (IU/l)	40.3±(23)	0.754	32.4±(16)	0.715	0.171
SGPT (IU/l)	72.3±(3)	0.273	50.6±(27)	0.342	0.053
HOMA	2.1±(0.4)	-	4.4±(1.4)	-	-

* Statistically significant difference

liver-related death. (26-30), Numerous studies have been undertaken to assess and evaluate the pathogenesis of NAFLD. Central to the pathogenesis of NAFLD is insulin resistance. Insulin resistance is demonstrated almost universally in patients who have NAFLD. (14,22), In one study, patients who had simple steatosis and those who had NASH, insulin resistance was associated with NAFLD, independent of BMI or glucose tolerance. (31), The same results were noted in another study, and in addition, the degree of insulin resistance appeared more pronounced in patients who had NASH. (22)

Chitturi and colleagues noted that 98% of patients who had NASH in their series had insulin resistance. (14), Because insulin resistance can be demonstrated in patients who have simple steatosis and NASH, other events are believed to be operative in the progression from simple steatosis to steatohepatitis to cirrhosis. These findings have given rise to the multi-hit hypothesis.(1)

The first hit is fat accumulation in the hepatocyte. This is believed to be caused by insulin re-sistance by means of increased lipolysis and increased delivery of free fatty acids to the liver. (32)

Other abnormalities that contribute to fat accumulation include decreased synthesis of apolipoproteins and microsomal transfer protein gene polymorphism, both conditions potentially lead to decreased export of triglycerides out of the liver. (33,34)

The presence of hepatic steatosis is thought to then, set the stage for the development of inflammation and liver cell injury that is characteristic of NASH. There are several factors or second hit that have been proposed. They include oxidative stress from reactive oxygen species produced in mitochondria. (22)

and by cytochrome P-450 enzymes. (22,35,36) The contribution of iron to oxidative stress in NASH is controversial. (36,37), Cytokines, in particular tumor necrosis factor a (TNF-α), have been implicated as second hits. (38)

Insulin resistance and obesity, especially central obesity, contribute to the hepatocyte injury in NASH by means of free fatty acids, the levels of which are increased in NASH. (22,31), Increased levels of free fatty acids can lead to increased reactive oxygen species production through increased mitochondrial and peroxisomal free fatty acids oxidation. (39)

Insulin resistance also can lead to upregulation of CYP2E1, which contributes to oxidative stress. (40), Fibrogenesis in NASH occurs by means of hepatic stellate cell (HSC) activation by oxidative stress and cytokines. Adipokines such as leptin and resistin, which are elevated in NASH, may enhance fibrogenesis in NASH through its direct effect on HSC or its indirect effects on production of TGF-ß in sinusoidal and Kupffer cells. Adiponectin seems to have a protective effect in patients with NAFLD. (41)

The main risk factors associated with NAFLD are obesity, diabetes mellitus, hyperlipidemia, and the metabolic syndrome. In many case series of patients with NAFLD, obesity, diabetes mel-

litus, and hyperlipidemia were observed in up to 93%, 55%, and 92% of patients, respectively. (12-17,19,20,31), Obesity, diabetes mellitus, and hyperlipidemia are components of the metabolic syndrome. In one study, patients who had NASH were more likely than patients who had simple steatosis to fulfil criteria for the metabolic syndrome (88% versus 53%). (31)

In another study, 87% of patients who had NASH fulfilled criteria for the metabolic syndrome. (14), There are other causes of NAFLD, such as medications, jejunoileal bypass, and prolonged total parenteral nutrition. NAFLD caused by these conditions has been called secondary NAFLD to differentiate them from NAFLD associated primarily with insulin resistance. (42)

To our knowledge, only a few studies have reported the relevance of insulin resistance with obesity, lipid profile and liver enzyme tests in non-diabetic NAFLD patients. (43)

Chitturi et al reported most patients with NASH have insulin resistance and there is a near-universal association between NASH and IR irrespective of obesity (14). Another study reported high rate of hyperinsulinemia and insulin resistance in patients with NASH. (44)

Our study compared to previous studies, revealed a high rate of insulin resistance among non-diabetic NAFLD patients. Also, patients with higher levels of HOMA index had higher waist and LDL-C levels and comprised lower HDL-C levels.Evaluating the HOMA index in 197 non-diabetic NAFLD patients by Musso et al in 2008 showed that HOMA index >2 is correlated with age, ALT, HDL Cholesterol, Triglyceride-to-HDL cholesterol ratio, Waist and. (43), Similar to our study, the patients evaluated in the study of Musso et al. (43) were all non-obese non-diabetic subjects. They showed that in non-diabetic NAFLD patients, HDL Cholesterol was significantly higher in patients with HOMA index ≤2 [57.3±10.5 vs. 48.3±10.5 mg/dl, P=0.0002]. Also, a smaller waist [88±8 vs. 92±8 cm, P=0.005] and lower BMI [25±2 vs. 25.8±2.2 kg/m2, P=0.030] were seen in these patients. (43), Although the cut point for HOMA index was different in our study [the value of quartile 50 (HOMA>2.93)] in comparison with the study of Musso et al. (43), almost the similar results were shown. Impaired lipoprotein metabolism and oxidized LDL accumulation are potential candidates for mechanism(s) linking to these results. (45-48)

The presence of NAFLD in non-diabetic insulin-resistant subjects, may therefore indicate a host of unsuspected derangements in oxidative balance that contribute to increased cardiovascular disease risk. Based on our findings, waist, LDL and HDL values were showed significant difference in four quartiles (Table 1).

Also, the differences in waist and HDL were significant after dividing the patients into two groups (regarding quartile 50, Table 5). In lower quartiles (A+B), mean HOMA value was 2.16±3.9 that showed significant correlation with cholesterol, LDL and waist. In higher quartiles (C+D), mean HOMA value was 4.44±1.47, but it did not show any correlation with other variables. However, the calculated power showed that this lack of significances may be due to the low sample size (n=25) of this group to show the correlations between HOMA index and other variables.

CONCLSUION

In conclusion, non-diabetic patients with insulin resistance showed significant higher values of LDL and waist circumference.

HDL-C levels were also lower in insulin resistant patients. BMI, Waist to Hip ratio, Triglyceride, Cholesterol, AST and ALT values showed no relation with insulin resistance. Only at lower quartiles (A+B) of insulin resistance (1.43 <HOMA index<2.81) a relationship could be demonstrated.

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