Prevalence of Non-Alcoholic Fatty Liver Disease and Its Predictors in North of Iran

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is one of the aspects of metabolic syndrome (MetS). Due to the increase of MetS in Iran, this study was conducted to determine the prevalence of NAFLD, its potential predictors and their sex distribution in north of Iran, Amol.

Methods: In 2008 this population based cross-sectional study included 5023 adult individuals who were randomly selected from Amol healthcare centers. Blood analysis and hepatic sonography was performed for each individual and Clinical histories were reviewed. MetS was defined according to the National Cholesterol Education Program Adult Treatment Panel III. Chi-square test, univariate and multivariate logistic regression were used to analyze data.

Results: The prevalence of NAFLD and metabolic syndrome was 43.8% and 29.6% respectively. Both NAFLD and metabolic syndrome were significantly more prevalent in women. There was a stronger association between these two factors in women which may indicate MetS has a much more potency to result in NAFLD in women. The strongest predictors of NAFLD in men were waist circumference>102 cm, serum ALT ≥40 (U/L) and the age group of 40-60 years. The strongest predictors of NAFLD in women were waist circumference>88 cm, the age groups of 40-60 and >60 years.

Conclusions: The observed prevalence is alarming because almost 7 out of 10 subjects with MetS had NAFLD. As high waist circumference was an important predictor of NAFLD in both sexes, health care policies to reduce the incidence of obesity in the country will have an important impact on the occurrence of NAFLD.

Keywords: Non-Alcoholic fatty liver disease (NAFLD), Metabolic syndrome, Risk factors

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent types of liver diseases in western countries. It is defined as storage of triglycerides in hepatocytes more than 5% of liver weight, less than 20 g/d of alcohol consumption and exclusion of any other causes of chronic liver diseases (1-3). This disease has a wide spectrum of manifestations from simple hepatic steatosis to steatohepatitis with different grades of fibrosis to cirrhosis and in rare cases hepatocellular carcinoma (4). It is usually associated with visceral obesity, type 2 diabetes, dyslipidemia and other components of metabolic syndrome so it is considered to be one of the aspects of metabolic syndrome (1-3, 5-8). The prevalence of NAFLD in adults is 20-30% in western countries (9-13). In eastern
In countries the disease did not used to be frequent but recent studies have shown an increasing rise in the prevalence of NAFLD and it has been associated with the change of life style (diet, physical activity) and increasing prevalence of obesity (14-15). The Third national surveillance of risk factors of non-communicable diseases (SuRFNCD-2007) in Iran reported a strikingly high prevalence of some metabolic abnormalities such as diabetes (8.7%), obesity (22.3%), hypertension (26.6%), hypertriglyceridemia (36.4%), hypercholesterolemia (42.9%) and central obesity (53.6%) in our country, as a developing country which was comparable, if not higher, to most developed countries (16). Since all these metabolic abnormalities are risk factors for NAFLD, these results predict a high prevalence of NAFLD in Iran. To the best of our knowledge a few studies are available on the prevalence of NAFLD in Iran (17-19) and most of them have reported NASH prevalence in particular (not NAFLD) or has been conducted on a specific group such as diabetic patients (20). On the other hand there has been no study on the prevalence of NAFLD in Northern provinces of Iran. In fact the distribution of type 2 diabetes, central obesity, hypertension and dyslipidemia is not the same in different regions and it affects on the prevalence of NAFLD. The aims of this study were to determine the prevalence of NAFLD, its potential predictors and their sex distribution in an adult population based study in Amol, northern Iran.

Material and Methods

Study design

This study was conducted within the framework of Amol health cohort study. From 2008, the Gastro Intestinal and Liver Disease Research Center (GILDRC) has conducted a multidisciplinary study on general population of Amol and surrounding areas. Total 6420 subjects were involved in this study by cluster random sampling. Details of the Amol cohort protocol have been published elsewhere (21).

This population based cross-sectional study was conducted on phase 1 of Amol cohort study and included 5023 adult individuals who had full relevant data.

Inclusion criteria
An adult population of 18-90 year old, who gave written informed consent, participated in the study.

Exclusion criteria
The exclusion criteria were patients with chronic liver diseases, the presence of hepatitis B virus surface antigen or hepatitis C virus antibodies, known cases of autoimmune hepatitis or Wilson disease, an alcohol consumption more than 30 g/day in men and more than 20 g/day in women, patients with cognitive diseases and individuals who were incapable of communicating.

Procedure
Details of the Amol cohort health study have been published elsewhere (21) but briefly, after signing a detailed informed consent, a standardized questionnaire was administered to determine the clinical histories, past medical histories, alcohol consumption and the use of any drugs including hepatotoxic drugs in all the participants. Then a physical examination was performed to measure weight (kg), height (m) and waist circumference [WC (cm)] according to the standard protocol (22). Body mass index (BMI) was calculated as body weight in kilogram divided by square of height in meter. Blood pressure was measured by mercury sphygmometer three times at 1-min intervals, with the patient in a sitting position; the average of the second and third measurements was reported. Then a 12-h fasting venous blood sample was taken from each participant to measure biochemical parameters [Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), triglyceride (TG), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), total cholesterol (T-Chol), fasting serum glucose (FSG), fasting serum insulin, hepatitis B virus surface antigens and hepatitis C virus antibodies.

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AST, ALT, triglyceride, LDL, HDL and T-Chol and FSG were measured by auto-analyzer (Bio system kits). Fasting serum Insulin was measured by ELIZA (Monobind kit), hepatitis B virus surface antigens and hepatitis C virus antibodies were measured by ELIZA (Acon kit). Insulin resistance was assessed by HOMA-IR index as follows: 
\[
\text{HOMA-IR} = \frac{\text{fasting serum glucose (mg/dl)}}{\text{fasting serum insulin (mU/l)}}/405.
\]
Finally, all subjects underwent abdominal sonography (by Esaote May lab number 15) for evaluation of fatty liver or other abnormal findings. All sonographies were done by one expert sonographer.

**Definition of Fatty liver**
In sonography, fatty liver was diagnosed with an increase in hepatic echogenicity using renal echogenicity as a reference, the presence of enhancement and a lack of differentiation of periportal and bile duct walls reinforcement because of great hyperechogenicity of the parenchyma (23).

**Definition of Metabolic Syndrome**
Metabolic syndrome was defined according to National Cholesterol Education Program Adult Treatment Panel III criteria (ATPIII) (24). ATPIII defines Metabolic Syndrome, as presence of any three out of five risk factors:
- Fasting Glucose ≥100 mg/dl
- Waist Circumference: (i) Men: >102 cm (40 in); (ii) Women: >88 cm (35 in)
- TG ≥150 mg/dl
- HDL-C: (i) Men: < 40 mg/dl; (ii) Women: < 50 mg/dl
- Blood Pressure ≥130/ or ≥85 mm Hg

**Statistical analysis**
Data were analyzed by SPSS ver. 16 J for Windows. Variables were categorized according to the definition of metabolic syndrome and standard protocols (Table 1) and were described by frequency tables in the total population and in each sex separately. Chi-square test was used to compare the frequency of these variables between two sexes.

### Table 1: The prevalence of NAFLD and its potential predictors in the study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Men n (%)</th>
<th>Women n (%)</th>
<th>Total n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist Circumference (cm) &gt;102 in males and &gt;88 in females</td>
<td>505 (17.7)</td>
<td>1361 (62.6)</td>
<td>1866 (37.1)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fasting Serum Glucose (mg/dl) ≥100</td>
<td>782 (27.5)</td>
<td>709 (32.6)</td>
<td>1491 (29.7)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Hypertension (mm Hg) (SBP≥130 or DBP≥85)</td>
<td>1128 (39.6)</td>
<td>814 (37.4)</td>
<td>1942 (38.7)</td>
<td>0.116</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) ≥150</td>
<td>984 (34.6)</td>
<td>710 (32.6)</td>
<td>1694 (33.7)</td>
<td>0.157</td>
</tr>
<tr>
<td>HDL (mg/dl)&lt;40 in males and &lt;50 in females</td>
<td>1534 (53.9)</td>
<td>764 (35.1)</td>
<td>2298 (45.7)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl) ≥200</td>
<td>853 (30.0)</td>
<td>885 (40.7)</td>
<td>1738 (34.7)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ALT(U/L) ≥40</td>
<td>403 (14.2)</td>
<td>119 (5.5)</td>
<td>522 (10.4)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>AST (U/L) ≥40</td>
<td>140 (4.9)</td>
<td>57 (2.6)</td>
<td>197 (3.9)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HOMA Index ≥3.8</td>
<td>354 (12.4)</td>
<td>453 (20.8)</td>
<td>807 (16.1)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>1039 (36.6)</td>
<td>411 (18.9)</td>
<td>1450 (28.9)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>≥25 -&lt;30</td>
<td>1155 (40.7)</td>
<td>685 (31.6)</td>
<td>1840 (36.7)</td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>647 (22.8)</td>
<td>1073 (49.5)</td>
<td>1720 (34.3)</td>
<td></td>
</tr>
<tr>
<td>Metabolic syndrome (ATPIII)</td>
<td>570 (20.0)</td>
<td>917 (42.2)</td>
<td>1487 (29.6)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Age groups (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>1072 (37.6)</td>
<td>826 (38.0)</td>
<td>1898 (37.8)</td>
<td>0.113</td>
</tr>
<tr>
<td>40-60</td>
<td>1198 (42.1)</td>
<td>972 (44.7)</td>
<td>2170 (43.2)</td>
<td></td>
</tr>
<tr>
<td>Above 60</td>
<td>578 (20.3)</td>
<td>377 (17.3)</td>
<td>955 (19.0)</td>
<td></td>
</tr>
<tr>
<td>Fatty liver</td>
<td>1203 (42.2)</td>
<td>996 (45.8)</td>
<td>2199 (43.8)</td>
<td>0.012*</td>
</tr>
</tbody>
</table>

Abbreviations: NAFLD: Nonalcoholic fatty liver disease, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, BMI: Body Mass Index/ *Statistically significant: P<0.05

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Univariate regression analysis was performed to find the association of NAFLD with its potential predictors for each sex separately. Then all the factors with \( P \text{-value} \geq 0.2 \) were used in multivariate logistic regression (forward LR) analysis. \( P \text{-value} < 0.05 \) was used to identify significant level in statistical tests.

**Ethics approval**
This study received ethics approval from the Human Research Ethics Committee of Iran University of Medical Science.

**Results**

**Descriptive analysis**
This study included 5023 participants (56.7% men and 43.3% women) with a mean age of 45.35 ± 15.87 years (age range: 18 to 90 years). The prevalence of NAFLD and metabolic syndrome (as one of the most important predictors of NAFLD) in the study group was 43.8% and 29.6% respectively. NAFLD was significantly more prevalent in women than men (45.8% vs. 42.2%, \( P = 0.01 \)). The prevalence of metabolic syndrome was also significantly more prevalent in women than men (42.2% vs. 20%, \( P<0.001 \)) (Table 1). The distribution of age groups between the two sexes was not significantly different (\( P=0.113 \)) (Table 1). NAFLD was significantly more prevalent in the age group of 40-60 years in comparison to the other two groups of <40 and > 60 years in total population (55.3% vs. 26.4% and 52% respectively, \( P < 0.001 \)). This result was the same in men (51.8% vs. 30.2% and 44.6% respectively, \( P < 0.001 \)) but in women NAFLD was most prevalent in the age group of >60 years (63.4% vs. 21.5% for<40 year old and 59.6% for 40-60 year old, \( P < 0.001 \)).

As the prevalences of metabolic syndrome and NAFLD were significantly different between the two sexes, the prevalences of potential predictors of NAFLD (metabolic syndrome components and some other related biochemical and anthropometric parameters) were assessed for each sex separately.

The frequency of high WC, FSG, T-Chol, HOMA index, BMI and low HDL were significantly more in women than men (all \( P<0.001 \)). The only two components of metabolic syndrome which were not significantly different between the two sexes were TG and Blood Pressure. At the same time women had significantly lower serum AST and ALT level (all \( P<0.001 \)) (Table 1).

**Univariate analysis**
The risk of having NAFLD in participants with metabolic syndrome was significantly more than the participants without metabolic syndrome (OR: 4.328, CI (95%):3.08-4.92) and the risk was higher in women than men [OR: 5.9, CI (95%): 4.9-7.12 vs. OR:3.43, CI (95%): 2.83-4.16] (Table 2). All components of metabolic syndrome (High Waist Circumference (cm), Fasting Serum Glucose, Triglycerides, Hypertension and low HDL), high total cholesterol, high HOMA Index, serum ALT and AST \( \geq 40 \) U/L, age \( \geq 40 \) years and BMI \( \geq 25 \) significantly increased the risk of NAFLD in both sexes (Table 3) so all these variables were used in Multivariate logistic regression except for BMI. Because of the significant correlation between BMI and WC, BMI was not included in the model.

<table>
<thead>
<tr>
<th>Table 2: Association of metabolic syndrome with NAFLD</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th>NAFLD n(%)</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>Yes 377 (66.1)</td>
<td>193 (33.9)</td>
<td>Yes 641 (69.9)</td>
<td>276 (30.1)</td>
<td>1018 (68.5)</td>
</tr>
<tr>
<td></td>
<td>No 826 (36.3)</td>
<td>1452 (63.7)</td>
<td>Yes 355 (28.2)</td>
<td>903 (71.8)</td>
<td>1181 (33.4)</td>
</tr>
<tr>
<td>OR (CI)</td>
<td>3.43 (2.83-4.16)</td>
<td>5.9 (4.9-7.12)</td>
<td>4.328 (3.08-4.92)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NAFLD: Nonalcoholic fatty liver disease.
Multivariate analysis
In both sexes WC, FSG, TG, HDL, Blood Pressure, age, ALT and HOMA Index were significantly associated with NAFLD (all, \( P<0.001 \)). The association of T-Chol with NAFLD was significant only in men (\( P<0.001 \)) (Table 3).

The strongest predictors of NAFLD in men were WC (OR: 7.99, CI (95%): 6.09-10.48), serum ALT (OR: 3.59, CI (95%): 2.76-4.69) and age 40-60 years old (OR: 2.56, CI (95%): 2.07-3.15).
The strongest predictors of NAFLD in women were WC (OR: 5.93, CI (95%): 4.66-7.55), age 40-60 years old (OR: 3.09, CI (95%): 2.41-3.97) and age>60 years (OR: 2.8, CI (95%): 2.01-3.88).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men</th>
<th>Adjusted OR*</th>
<th>Women</th>
<th>Adjusted OR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Serum Glucose (mg/dl) ≥ 100</td>
<td>2.181 (1.845-2.578)</td>
<td>1.536 (1.254-1.883)</td>
<td>3.933 (3.250-4.759)</td>
<td>1.449 (1.138-1.846)</td>
</tr>
<tr>
<td>Hypertension (mm Hg) (SBP≥130 or DBP≥85)</td>
<td>2.252 (1.931-2.626)</td>
<td>1.545 (1.287-1.855)</td>
<td>2.945 (2.460-3.525)</td>
<td>1.528 (1.224-1.907)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) ≥ 150</td>
<td>3.036 (2.587-3.562)</td>
<td>1.955 (1.588-2.406)</td>
<td>3.767 (3.115-4.554)</td>
<td>1.896 (1.506-2.388)</td>
</tr>
<tr>
<td>HDL (mg/dl)&lt;40 in males and &lt;50 in females</td>
<td>2.149 (1.847-2.500)</td>
<td>1.255 (1.034-1.524)</td>
<td>2.151 (1.791-2.582)</td>
<td>1.264 (1.005-1.590)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl) &gt;200</td>
<td>1.976 (1.679-2.324)</td>
<td>1.263 (1.036-1.539)</td>
<td>2.157 (1.812-2.568)</td>
<td>1.084 (0.840-1.307)</td>
</tr>
<tr>
<td>ALT(U/L) ≥40</td>
<td>3.599 (2.871-4.511)</td>
<td>3.598 (2.761-4.690)</td>
<td>1.945 (1.331-2.841)</td>
<td>1.674 (1.047-2.676)</td>
</tr>
<tr>
<td>AST (U/L) ≥40</td>
<td>2.065 (1.461-2.917)</td>
<td>.793 (0.505-1.245)</td>
<td>2.629 (1.494-4.625)</td>
<td>1.634 (0.733-3.639)</td>
</tr>
<tr>
<td>HOMA Index ≥3.8</td>
<td>3.828 (3.005-4.876)</td>
<td>1.821 (1.353-2.451)</td>
<td>2.890 (2.326-3.592)</td>
<td>1.850 (1.401-2.444)</td>
</tr>
<tr>
<td>age 40-60 years</td>
<td>2.485 (2.091-2.953)</td>
<td>2.563 (2.079-3.159)</td>
<td>5.363 (4.349-6.614)</td>
<td>3.095 (2.412-3.971)</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>1.861 (1.510-2.295)</td>
<td>1.835 (1.417-2.378)</td>
<td>6.305 (4.826-8.236)</td>
<td>2.800 (2.017-3.887)</td>
</tr>
</tbody>
</table>

**Table 3:** Association between NAFLD and potential risk factors according to sex

**Discussion**
In this study, the prevalence of NAFLD in Amol, a city in north of Iran, diagnosed by sonography, was 43.8% which is more than the reported prevalence of 20-30% in western countries. This result can be due to the high prevalence of metabolic syndrome in Iran which has been reported to have one of the highest prevalences of metabolic syndrome worldwide. In this study the prevalence of metabolic syndrome was 29.6% which was in close agreement with the results of Tehran Lipid and Glucose Study (TLGS) which found total age-standardized prevalence of 33.7% in the adult population (24).

In the current study NAFLD was significantly more prevalent in women than a man which was in accordance with the significantly higher preval-
alence of metabolic syndrome in women (42.2%) than men (20%). This is in close agreement with the results of Tehran Lipid and Glucose Study (TLGS) which found metabolic syndrome in 42% of women and 24% of men (25). The initial epidemiologic studies reported the same results (26-27) but some recent studies have reported a higher prevalence of NAFLD in men (28-30).

The prevalence of high WC, FSG, T-Chol, HOMA index, BMI and low HDL was significantly more in women than men which were in accordance with higher prevalence of NAFLD in women. As the distribution of age groups between the two sexes was not significantly different, the higher prevalence of NAFLD and its risk factors in women can not be related to age. This result confirms the existence of gender difference in the prevalence of NAFLD, which has also been shown in previous studies (31-32).

Despite the high prevalence of NAFLD in the study population (43.8%), high serum AST (≥40 U/L) and ALT (≥40 U/L) were only present in 3.9% and 10.4% of them respectively. This result was the same in another study where almost half of the patients with NAFLD had normal transaminases (33) or the studies based on liver biopsy that reported normal transaminases in patients with severe lesions (34-35). This is clinically important and several authors have suggested redefining the normal value of transaminases (12, 36-38). On the other hand, despite the higher prevalence of NAFLD and its risk factors in women, high serum AST and ALT were significantly more prevalent in men. This may be the result of more severe injuries of NAFLD in men despite the lower prevalence of NAFLD in them.

In both sexes WC, FSG, TG, HDL, Blood Pressure, age, ALT and HOMA Index were significantly associated with NAFLD. The association of T-Chol with NAFLD was significant only in men. The close association between different components of metabolic syndrome and NAFLD supports the theory that NAFLD is the hepatic manifestation of metabolic syndrome (39). These results are in agreement with the results of other studies which have reported that metabolic syndrome is an important risk factor for NAFLD (27). But the interesting point is that the association of metabolic syndrome and NAFLD is much stronger in women than men (Table 2) that means metabolic syndrome has a much more potency to result in NAFLD in women than men. More well designed studies are needed to clear the association between sex and the degree of injuries in NAFLD over time.

In this study, the strongest predictors of NAFLD in men were high WC, serum ALT and the age group of 40-60 years old and the strongest predictors of NAFLD in women were high WC, the age groups of 40-60 years old and older than 60 years respectively.

In both sexes the risk of having NAFLD in the age group of 40-60 years old was more than the group who were older than 60 years. This could be related to the difference of dietary habits in these two groups. Usually in Iran older people avoid consuming fast food and prefer traditional food.

This result is in accordance with Italian and Taiwanese studies which showed that an age of above 66 years and 65 years were inversely associated with the prevalence of NAFLD(12, 14). In fact the association of age with NAFLD has been controversial in different studies. In one study, age, especially of above 60 years, was an independent risk factor for NAFLD (33) and in another study it did not present any association between age and NAFLD (13).

This study also has its limitations. The first limitation is that although abdominal sonography is a good diagnostic tool for NAFLD it is not useful when fat accumulation is less than 30% of liver volume(9-10, 12-13, 40-41) or in morbid obesity (42) so this could underestimate the prevalence of NAFLD. The second limitation is that except for insulin resistance and metabolic syndrome components there are many factors such as genetic background, environmental factors and gut microbiota that may lead to the development of NAFLD (43) thus further studies are needed to evaluate the association of these potential risk factors and NAFLD in different communities.
Conclusion

This study showed a high prevalence of NAFLD and metabolic syndrome in Amol and its suburbs with a strong association between these two disorders. The prevalence of both disorders was significantly higher and their association significantly stronger in women but at the same time lower serum AST and ALT level in women may be an indicator of lower severity of NAFLD in this group. The strongest predictors of NAFLD in men were high WC, serum ALT and the age group of 40-60 years old and in women were high WC, the age groups of 40-60 years old and older than 60 years.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

Acknowledgments

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