Assessment of NAFLD cases and its correlation to BMI and metabolic syndrome in healthy blood donors in Kerman

Sadroddin Lahsaei, Alireza Ghazizade, Mahnaz Yazdanpanah, Ahmad Enhesari, Reza Malekzadeh

1Kerman University of Medical Science, Kerman Iran
2Blood Donation Center, Afzalipoor Hospital, Kerman University of Medical Science, Kerman Iran
3Digestive Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Aim: The aim of this study was to review cases of non-alcoholic fatty liver disease cases and to determine the prevalence of non-alcoholic fatty liver disease as a cause of elevated alanine aminotransferase in healthy blood donors in the Permian area and also assess risk factors of NAFLD such as BMI and correlation with metabolic syndrome in these subjects.

Background: Non-alcoholic fatty liver disease has been increasingly recognized as the most common pathological conditions affecting the liver. Non-alcoholic fatty liver disease is now recognized as the hepatic component of the metabolic syndrome, which includes hyperlipidemia, glucose intolerance, obesity, and systemic hypertension.

Patients and methods: 2002 randomly selected blood donors were recruited for this study. Subjects with elevated serum ALT level (greater than two times the upper limit of normal) were chosen for further follow up. Subjects with a persistently elevated ALT level, evidence of steatosis on computerized tomography and a negative cirrhosis screen (viral hepatitis B and C serology, autoimmune hepatitis, transferrin saturation <45% and a no history of excess alcohol consumption or hepatotoxic medication) were presumed to have non-alcoholic fatty liver disease.

Results: 378 donors (20.5% of all subjects recruited) had elevated ALT levels at first measurement. 35 cases had persistently elevated serum ALT level. In 22 of these 35 cases (62.9%) non-alcoholic fatty liver disease was the diagnosis. The mean body mass index of the 22 cases was 31.18 ±5.7 and non-alcoholic fatty liver disease was associated with the metabolic syndrome in these subjects.

Conclusion: Non-alcoholic fatty liver disease is the most common diagnosis for subjects with elevated serum ALT level in healthy blood donors in Kerman, Iran.

Keywords: Non-alcoholic fatty liver disease, Body mass index, Metabolic syndrome, Blood donors.

Introduction

The spectrum of non-alcoholic fatty liver disease (NAFLD) encompasses simple fatty liver, NASH (non-alcoholic steatohepatitis) and NAFLD associated cirrhosis. Although large epidemiologic studies of NAFLD are lacking and the prevalence of NAFLD in the general population is undefined, NAFLD probably is the most common liver disorder in the world, affecting 2.8% to 24% of the general population, including overweight children and adolescents (1).
Non-alcoholic fatty Liver disease (NAFLD) has been increasingly recognized as the most common pathological conditions affecting the liver in conjunction with the increase in Body Mass Index (BMI) and prevalence of the metabolic syndrome in developed countries that has occurred during the last decades. Increasingly, individuals with abnormal liver tests are found to have NAFLD (2-3).

A minority of patients with NAFLD will develop liver cirrhosis. NAFLD is probably the most common underlying cause of cryptogenic cirrhosis (2-5). In addition to the risk of developing cirrhosis, patients with NAFLD have an increased cardiovascular mortality as well as increase in liver related complication compared to the general population (2). Non-alcoholic steatohepatitis is the most common cause of persistently elevated serum ALT in the asymptomatic Iranian blood donors in Tehran (6).

Most patients with NAFLD have multiple risk factors for cardiovascular disease, including obesity, type 2 diabetes mellitus and hyperlipidemia. NAFLD is now recognized as the hepatic component of the metabolic syndrome (hyperlipidemia, glucose intolerance obesity and systemic hypertension) (1). Current management of patients with NAFLD targets visceral obesity, hyperglycemia, type 2 diabetes mellitus and hyper-triglyceridemia (2).

This study determined the prevalence of NAFLD as a cause of elevated alanin aminotransferase in healthy blood donors in Kerman and also risk factors of NAFLD, such as raised BMI and the metabolic syndrome in these subjects.

**Patients and Methods**

A prospective study was carried out in the Blood Donation Center of Kerman, Iran in one year, 2002 healthy blood donors were recruited in this study.

According to the regulations of the Blood Transfusion Organization of Iran, the following individuals are not allowed to donate blood: those who have had major chronic disease, those with any mild to severe acute disease, intravenous drug users and those with a past history of non-neonatal jaundice.

We also excluded persons with positive viral markers and alcohol drinkers (intake of more than 20g alcohol per day).

**First Phase:**

After explanation about the aim of study and possible need for further blood tests and follow-up by a general physician, information about demographic characteristics, detailed drug history during the past 3 months, alcohol consumption (the number and type of drink per day) and cigarette smoking was obtained. BMI (Body Mass Index) was measured as weight (kg) divided by height squared (m²) and categorized according to the classification of National Heart, Lung and Blood Institute of the USA as follows: under weight (<18.5 kg/m²), normal weight (18.5-24.9 kg/m²), overweight (25-29.9 kg/m²) and obese (30-34.9 kg/m²) (7).

Serum level of ALT was measured and a value greater than the upper limit of the laboratory reference range (41 U/L) was considered elevated.

From 2002 blood donors in the first step; 9 subjects were Hbs Ag+, one was HIV+, 14 were HCV Ab+, and 95 consumed excess alcohol; we excluded these 119 donors. 50 donors did not want to participate in our study and were also excluded.

From these 1839 donors, 378 had high serum ALT levels and entered the second phase of the study.
Second Phase
We invited all subjects with elevated ALT levels at the first phase to enter the study and have a further blood test in six months to recheck serum ALT level. Subjects with ≥2 times elevated ALT level (high ALT level in re-checking) considered as having persistent elevated ALT (6), and followed in this step.

From 378 persons who agreed to be entered into the second phase, we could only follow up 105 persons (because of non-clinical problems such as time of data collection in the first step, incorrect contact details and distance from study center). As a result of the challenges, we followed 105 subjects from the 378 in the first phase. From these 105 subjects, 35 persons had high serum ALT level in re-checking and followed-up in third phase.

Third Phase
All subjects with persistently elevated ALT Levels (n=35) were invited for physical examination, waist circumference measurement, ultrasound of liver and CT-Scan of liver and the complete laboratory tests including: serum protein electrophoresis, aspartate aminotransferase, serum iron, total iron binding capacity, fasting blood glucose, total cholesterol, high density lipoprotein, low density lipoprotein alpha 1 antitrypsin, anti TTG, serum caeruloplasmin, ANA and bilirubin (direct, total), Alkaline phosphatase.

Criteria for the metabolic syndrome was defined according to the NCEP ATP3 2005 definition of the metabolic syndrome ≥3 of these items: Glucose level ≥100 mg/dl or treatment for elevated blood glucose, HDL cholesterol <40 mg/dl men, <50 mg/dl for women or drug treatment for low HDL. Triglycerides≥150mg/dl or drug treatment for elevated triglycerides, obesity waist ≥102cm for men or ≥130/85 cm for women hypertension: blood pressure ≥130/85 mmHg or drug treatment for hypertension (8). The definition of high cholesterol and triglycerides level were based on the Nation Cholesterol Education Program (ATP III) criteria (8).

Diabetes mellitus was defined according to the provisional report to the provisional report of the World Health Organization Consultation (9).

All ultrasonographies were done by an expert sonographer and fatty infiltration of the liver was graded from I to III (mild to severe) (10).

Subjects with persistently elevated ALT levels and negative test results for viral hepatitis B and C, autoimmune hepatitis, transferring saturation <45% and a negative history of alcohol and medication: with or without fatty infiltration in their liver sonography were invited to have liver CT-Scan. At CT-Scan, the hepatic attention was measured by means of a random selection of 25 circular regions of interest (ROIS) on both lobes on five transverse sections at different hepatic levels (liver ROIS per section). We avoided areas of visible hepatic vascular and biliary structure. ROIs ranged from 200 to 400 mm2. The ROI values were averaged as a mean hepatic attenuation. To provide an internal control the mean splenic attenuation was also calculated by averaging three random ROI values of splenic attenuation measurement on three transverse sections at different splenic levels (ROI per section). The largest possible ROI (size range, 200-400 mm2) was also selected to represent splenic parenchymal attenuation. The liver attenuation index (LAI) was derived from the difference between mean hepatic attenuation and mean splenic attenuation, and was used as a parameter liar prediction of the degree of macro vesicular steatosis. LAI that was below 5HU was considered diagnostic of liver steatosis (11).

These subjects, with persistently elevated ALT level and negative laboratory results who had evidence at liver steatosis on CT-Scan were presumed NAFLD and were referred to a dietician and received medication if necessary.
Results

In this study, which performed in one year, from 2002 healthy blood donors in the blood donation center of Kerman, information about demographic characteristics, detailed drug history during the past 3 months, alcohol consumption (the number and type of drinks per day) and cigarette smoking were obtained. BMI (Body Mass Intake) was measured and blood samples were sent to the laboratory of the blood donation center for checking serum ALT level.

80.8% (n=1617) were male and 18.8% (n=376) were female and 0.4% (n =9) were not defined due to a lack of data. The relative frequency of participants according to demographic characteristic BMI and sex is shown in Table 1. There is a statistically significant difference between mean BMI of male and female.

Table 1. The relative frequency of participants (n=2002) according to demographic characteristics BMI and sex

| Characteristic     | Male            | Female           | Total          |
|--------------------|-----------------|------------------|----------------|
| Age (years)        | 31.65±11.27     | 32.29±11.75      | 31.76±11.31    |
| BMI (kg/m²)        | 25.7±4.4        | 27.2±4.9         | 26.2±4.6       |
| Underweight*       | 2.26%           | 0.6%             | 2.2%           |
| Normal weight      | 44.8%           | 37.2%            | 43.4%          |
| Over weight        | 37.3%           | 33.3%            | 36.6%          |
| Class I obesity    | 12.4%           | 20.7%            | 13.9%          |
| Class II obesity   | 2.2%            | 7.4%             | 3.2%           |
| Class III obesity  | 0.7%            | 0.8%             | 0.7%           |

*Mean±SD

†Underweight:<18.5; Normal weight: 18.5-24.9; Over weight: 25-29.9; Class I obesity: 30-34.9; Class II obesity: 35-39.9; Class III obesity: ≥40

Mean age of participants was 31.76±11.31 years in all participants, 31.65±11.27 in men and 32.29±11.75 in women. There was no significant difference between the two groups (P>0.3). Education levels were scored (from early school leaver to graduate) and did not differ significantly between the two groups (p>0.2).

BMI (Body Mass Index) in these 2002 subjects ranged from 15.73 to 57.60 and mean of BMI in all subjects was 26.2±4.6.

Mean of BMI in women was 27.2±4.9 and in men was 25.7±4.4 and was different between men and women (P<0.0005) (Table 1). 19.4 (n=388) of 2002 subjects were cigarette smoker, 5.3% (n=107) were opium addicts and 4.7 (n=95) were alcohol drinkers.

Table 2. Liver ultrasonography on NAFLD

| Ultrasoundography | CT Scan | NAFLD | Total |
|-------------------|---------|-------|-------|
| Normal            | 6(31.6%)| 13(68.4%)| 19(100%)|
| NAFLD             | 16(100%)| 0     | 16(100%)|
| Total             | 22(62.9%)| 13(37.1%)| 35(100%)|

In keeping with the study protocol, subjects who were HIV+ (n=1), HBS Ag+ (n=9), HCV Ab+ (n=14) or alcohol drinkers (n=95) were excluded from study and also 50 subjects who did not want to participate. As a result, 1839 subjects included to the study.

Of 9 subjects who were HBS Ag+, only 6 subjects had elevated serum ALT level (66.66%). In 14 subjects who were HCV Ab+, one subject had elevated serum ALT level (7.1%). The subject who was HIV+ had a serum ALT level that was normal.

Serum ALT level was measured in all subjects and ranged from 5 to 256; the mean of serum ALT level was 34.56±20.22 (standard error 0.48). From 1839 case, 378 persons had elevated serum ALT level. The prevalence of once elevated serum ALT level was 20.55% of healthy blood donors.

From 378 persons who had elevated serum ALT level, 105 persons completed the second phase of the study. Subjects were lost due to non clinical problems, such as data collection of contact details, timing of recruitment and reluctance of individuals to travel to the study centre.

From 105 subjects who completed the second step, 70 persons had normal serum ALT level on re-checking and 35 subjects had elevated serum
ALT level. In these 70 subjects with normal ALT level on re-checking, 15.7% (n=11) were female and 84.3% (n=59) were male. The mean age of these 70 subjects was 35.2±11.7 and it ranged from 19 to 69 years. The mean of age in women was 34±12.95 and in men, it was 35.4±10.9 it did not differ (P>0.7). Education level was under diploma in 31.4% (n=22), diploma to license 67.1% (n=470 and 1.4% (n=1) after license.

Mean of BMI in this group was 25.6±3.9 kg/m² in women, 27.7±3.9 in men and it did not differ between men and women (P>0.2).

At third phase, there were 35 subjects with persistently elevated serum ALT level. One of these 35 subjects was female (2.9%) and 34 subjects were male (97.1%). Education level ranged from under diploma (25.7% (n=9)) and to diploma to license (74.3% (n=26)). 8.6% (n=3) of these 35 subjects were cigarette smoker. The mean of BMI for this group was 28.8±5.7 (SD).

We assessed these 35 subjects with liver ultrasound and liver CT-Scan according to methods we explained before. These subjects with persistently elevated ALT level and negative laboratory results for viral hepatitis B and C, autoimmune hepatitis, transferring saturation <45% and a negative history of alcohol and dedication; who had evidence of liver steatosis on CT-Scan were presumed NAFLD.

As a result 22 of these 35 (62.9%) persons presumed NAFLD and 13 subjects (37.1%) had normal CT-Scan. Only 45.7% (n=16) of these persons had evidence of steatosis on sonography. Demographic characteristics and BMI of subjects with NAFLD diagnosis is shown in Table 2.

Although the gold standard test for diagnosis of NAFLD is liver biopsy, since we could not use biopsy as a diagnostic test in voluntary blood donors; we used liver CT-Scan as the last step of our study for diagnosis of NAFLD in subjects whose laboratory tests were negative for other aetiologies of elevated ALT. By using CT-Scan and diagnostic test for NAFLD, sensitivity and specificity of sonography for diagnosis of NAFLD in our study was 72.7% and 100% respectively. Positive predictive value was 100% and negative predictive value was 68.4% (Figure 1).

In assessment the association between NAFLD and metabolic syndrome in these 35 subjects 37.1% (n=13) of these subject had metabolic syndrome and 54.5% (n=12) of subjects whom diagnosed NAFLD had metabolic syndrome but only 7.7% of patients who did not diagnosed NAFLD had metabolic syndrome. And so NAFLD was correlated with metabolic syndrome (Figure 2).
Discussion

The prevalence of an isolated elevated ALT levels (cut-off value of 41/Ul for both men and women) after excluding individuals with viral hepatitis, alcohol and drug etiologies was 20.55%.

In the study which carried out on Tehran blood donation center prevalence reported 5.71% (6) this prevalence in the Third National Health and Nutrition study of the population of the United States was 2.8% (14).

Mean BMI of our participants in this study was 26.02, comparable to previous studies (27.14 in Tehran study and 25.6kg/m2 in US study). A previous study in Tehran had shown a strong correlation between NAFLD and BMI, we could not show a correlation between BMI and ALT level.

We could not find prevalence of NAFLD in healthy blood donors because, for reasons already outlined, we could not follow-up 273 patients; In 35 subjects with persistently elevated serum ALT level the prevalence of NAFLD was 63%. It seems that NAFLD is the most common cause of a persistently elevated ALT in blood donors from Kerman.

We also assessed subjects with elevated serum ALT level for rare disease such as Wilson’s disease, α1-antitrypsin deficiency (1 in 30000), autoimmune hepatitis, celiac and primary biliary cirrhosis. We could not found any of these diseases as the cause of elevated serum ALT level in subjects of our study. We found a correlation between NAFLD and metabolic syndrome and this is regarding that NAFLD is the hepatic component of metabolic syndrome.

Results of our study show that NAFLD, which the hepatic manifestation of metabolic, is presently the most common liver disease in Kerman and this is in keeping with a previous study in Tehran (although the methodology differed from this study and prevalence of NAFLD was higher in Tehran study). The majority of people with metabolic syndrome have evidence of NAFLD. This is a cause of significant concern as studies suggest that one out of every 10 persons with NAFLD progress to a more serious stage of liver disease called NASH (Non Alcoholic Steatohepatitis) and some may progress to cirrhosis.

References

1. Reid AE. Nonalcoholic fatty liver disease. In: Felderman M, Friedman LS, Brandt LJ, eds. Sleisenger and Fordtran’s gastrointestinal and liver diseases. 8th edition. New York: Saunders Elsevier; 2006. P.1793-94.
2. Biomssen E. The clinical aspects of non-alcoholic fatty liver disease. Minerva Gastroentrol Dietol 2008; 54:7-18.
3. Jov D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary? Eur J Gastroenterol Hepatol 2003;15:539-43.
4. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999;29:664-69.
5. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, et al. Expanding the natural history of non-alcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;23:134-40.
6. Poursamens A, Malekzadeh R, Monavvari A, Akbari MR, Mohamadkhani A, Yarahmadi S, et al. Prevalence and etiology of persistently elevated alanine aminotransferase level in healthy Iranian blood donors. J Gastroenterol Hepatol 2005;20:229-33.
7. US Department of Health and Human Services. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. Washington, DC: US Department of Health and Human Services; 1998.
8. National Cholesterol Education Program (NCEP) Expert on detection, evaluation and treatment of high blood cholesterol in adults. (Adult Treatment Panel III). Third report of the National Cholesterol Education Program (NCEP) Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-44.
9. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complication. Part 1: diagnosis and classification of diabetes mellitus provisional report of WHO consultation. Diabetes Med 1998; 15:539-53.

10. Parulekar SG, Bree RL. Liver. In: MC Gahan JP, Goldberg BB. Diagnostic ultrasound: a logical approach. Philadelphia: Lippincott-Raven 1998;599-91.

11. Limanond P, Raman SS, Lassman C, Sayre J, Ghobrial RM, Busuttil RW. Macro vesicular Hepatic Steatosis in living related liver donors: correlation between CT and histologic findings. Radiology 2004; 230:276-80.

12. Raptopoulos V, Karellas A, Bernstein J, Reale FR, Constantinou C, Zawacki JK. Value of dual-energy CT indifferenating Focal Fatty infiltration of the liver from low-density masses. AJR AMJ Roentgenol 1991;157:721-25.

13. Kamel IR, Kruskal JB, Raptopoulos V. Imaging for right lobe living donor liver transplantation. Semin Liver Dis 2001;21:271-82.

14. Anderson NA, Raafat A, Shwe KH, Barbara J, Contreras M, Fraser ID, et al. UK multicenter study on blood donors for surrogate marker of non-A nin-B hepatitis. Part I: alanine transferase and anti-HBC testing. Transfus Med 1992; 2:301-10.