# Prevalence of Nonalcoholic Steatohepatitis in Iran: A Population based Study

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## ABSTRACT

**BACKGROUND**

Non-alcoholic fatty liver disease/steatohepatitis (NAFLD/NASH) is the most common form of chronic liver disease worldwide and is no longer considered a benign disease. Its prevalence has not been determined in a large-scale population-based study in Iran.

**METHODS**

A total of 6583 individuals aged 18 to 65 were randomly selected from three geographically distinct provinces in Iran. Blood samples were obtained from each subject and a questionnaire was completed exploring data including self-admitted regular alcohol use. Serums were tested for anti-HCV antibody (anti-HCV), hepatitis B surface antigen and anti-hepatitis B core antibody. Positive samples for anti-HCV antibody were re-tested and those positive in a repeat ELISA were confirmed by a recombinant immunoblot assay (RIBA) test. Serums were also tested for ALT levels. Subjects with elevated ALT defined as serum ALT $\geq$40 IU/L with no history of alcohol consumption and negative HBV and HCV infection were considered as "presumed NASH".

**RESULTS**

In this study 5589 subjects were analyzed. Two hundred and forty two individuals (4.3%) were diagnosed with elevated ALT levels. Among individuals with elevated ALT, 15 (6.2%) were diagnosed with either hepatitis B or hepatitis C. The overall weighted prevalence of presumed NASH was 2.9%. According to multivariate analysis, male sex, urban lifestyle, and being overweight or obese were significantly associated with "presumed NASH".

**CONCLUSION**

Obesity and metabolic syndrome, the most predictive factors of fatty liver disease, are increasing in Iran, therefore the prevalence of NAFLD/ NASH and related complications are expected to increase in the future. This population based study gives a crude estimate of the prevalence of NASH around the country. Studies with more accurate surrogates of NASH need to be done. The disparity among different provinces merits special consideration.

**KEYWORDS**

Fatty liver; alanine transaminase; prevalence, Iran

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## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered to be the most common form of chronic liver disease in many parts of the world. NAFLD is defined as the presence of fat accumulation in the liver parenchyma in the absence of significant alcohol consumption.

It encompasses a spectrum of clinicopathologic entities from simple steatosis with mild or no inflammation, to the more significant condition steatohepatitis (NASH), characterized by active inflammation and hepatocytes damage.

Simple steatosis by itself is generally considered to have a benign prognosis and it is estimated that only a small portion of these patients will develop NASH.[1]

On the other hand, once patients have developed NASH, histologic progression is noted in approximately 32% to 41% of individual over 4.3 to 13.7 years of follow-up.[2] In fact, evidence shows that approximately 9% of NASH patients may develop cirrhosis.[3] There is evidence that hepatocellular carcinoma (HCC) is a rare, yet worrisome complication of NAFLD.[4] Recent evidence indicates that patients with NASH are at higher risk of both overall and liver-related mortality compared to the general population.[5]

The prevalence of NAFLD has been increasing along with the rise in obesity since the term nonalcoholic steatohepatitis (NASH) was coined by Ludwig in 1980.[6] NAFLD is considered to be the hepatic manifestation of the metabolic syndrome. Roughly 90% of NAFLD cases have at least one characteristic feature of the metabolic syndrome, and one third of them fulfill the complete diagnostic criteria. Patients with NASH are more likely to have the metabolic syndrome than are those with mere steatosis.[7]

Ethnic variation in the prevalence of NAFLD/NASH has been described; several studies have indicated less common prevalence in African Americans.[8] Interestingly, the correlation between steatosis and obesity as well as insulin resistance is weaker in this subpopulation.

The estimated prevalence in the general population depends on the type of screening test and ranges from 2.8% to 46% in unselected populations worldwide.[9]

NAFLD is probably the most common cause of liver disease in Iran.[10] However a large scale population based study has not been performed in this country.

The aim of this study is to find the estimated prevalence of NASH in the Iranian population.

## MATERIALS AND METHODS

### Survey design and study sample

This cross-sectional study was performed among the adult general population of three geographically distinct provinces of Iran: Hormozgan (south), Tehran (north/center), and Golestan (northeast).

Subjects between 18 and 65 years of age were included. Being a permanent inhabitant of the household was an inclusion criterion. Non-Iranian nationals were excluded. Both urban and rural populations were included in Tehran and Golestan. In Hormozgan, due to practical considerations, only the urban population was studied. The census information of Iran and the three provinces are presented in Table 1.

### Data collection

Clustered random sampling was used. One hundred clusters were selected from each province with a cluster size of 20 or 25. Postal code or family registry code was used to randomly select the first household for each cluster. Blood samples were obtained from each subject and a questionnaire was completed which included demographic and anthropometric data and risk factors for hepatitis.

A family history of diagnosed liver disease was also documented. Further details have been reported elsewhere.[11]

### Laboratory assessment

Morning serum was obtained and transferred to the Iran Blood Transfusion Organization (IBTO) Research Center where samples were tested for anti-HCV antibody (anti-HCV) with the use of a third generation ELISA test (Ortho HCV 3.0 Enhanced SAVe ELISA, Ortho-Clinical Diagnostics, Amersham, UK and Hepanostika HCV Ultrakit, Beijing United Biomedical Co., Beijing, China).

Positive samples were re-tested and those positive by repeat ELISA were additionally confirmed

**Table 1:** Census information - Iran the three provinces studied*.

| | Area (km²) | Population | Percent urban | Percent male | Literacy |
|---|---|---|---|---|---|
| Iran | 1,648,195 | 70,495,782 | 68.5% | 50.9% | 84.0% |
| Tehran | 18,814 | 13,422,366 | 91.3% | 51.4% | 91.3% |
| Hormozgan | 70,697 | 1,403,674 | 47.1% | 51.7% | 82.4% |
| Golestan | 20,367 | 1,617,087 | 49.2% | 49.7% | 82.1% |

*Source: Iran national population and housing census, 2006,
http://www.sci.org.ir/portal/faces/public/sci_en, reproduced from Merat et al.[11]

by a recombinant immunoblot assay (RIBA) test (INNO-LIA HCV Score, Innogenetics, Ghent, Belgium).

Serum levels of HBsAg and anti-HBc were also evaluated by an Enzygnost HBsAg 5.0 kit (Dade Behring, Germany) and Hepanostica anti-HBc-Uni-Form kit (Biomerieux, France), respectively. Serum was tested for ALT level using a Hitachi autoanalyser 704 (Roche Diagnostics, Switzerland) with Pars Azmoon reagent kit (Tehran, Iran).

**Definitions**

Subjects with repeated reactive HCVAb-ELISA underwent a RIBA test and were considered as HCV patients if both tests were positive. HBsAg patients were defined as HBV infected. ALT levels above 40 IU/L were considered to be elevated.

Elevated ALT in individuals not infected with HCV or HBV was presumed as NASH.

**Statistical analysis**

The overall weighted prevalence of presumed NASH was calculated based on the fractional population of each province. Weighted logistic regression analyses were used to calculate the odds ratio (OR) and 95% confidence interval (95% CI) for each risk factor. A final logistic regression analysis was performed by a backward model selection for age, sex, province, BMI, level of education and urban/rural residence. Data was analyzed by SPSS version 17.0 (SPSS Inc, Chicago, IL).

**Ethical issues**

Written informed consent was obtained from all subjects. Data were stored in the database with no reference to subjects' names. The study protocol and the consent forms were reviewed and approved by the Institutional Review Board of the Digestive Disease Research Center, Tehran University of Medical Sciences.

**RESULTS**

A total of 6583 subjects were interviewed across the three provinces. Of these, 381 subjects from Golestan or Hormozgan did not meet the age criterion of 18 to 65 years of age and their data were excluded. Additionally, 613 subjects refused to give blood samples or had inadequate samples and were also excluded. The remaining 5589 subjects were analyzed. Demographic characteristics of the study population are provided in Table 2.

In all, 49 individuals were infected by HCV and 181 had HBV, respectively. Two patients from Hormozgan province were found to be infected with both HCV and HBV.

With a cut-off value of 40 IU/L, 242 individuals (4.3%) had elevated ALT levels which included 133 men and 109 women. Among individuals with elevated ALT, 15 (6.2%) had either hepatitis B or hepatitis C.

Among virus-free subjects, 227 had elevated ALT levels. These individuals were presumed to have

**Table 2:** Demographic data of the study population.

| | Participants interviewed | Number valid | Samples collected | Percent male | Percent rural | Mean age±SD (yr) |
|---|---|---|---|---|---|---|
| Tehran | 2561 | 2561 | 2290 | 41.5% | 5.1% | 35.6 ± 13.6 |
| Hormozgan | 1987 | 1745 | 1403 | 44.6% | 0% | 33.4 ± 11.9 |
| Golestan | 2035 | 1896 | 1896 | 32.0% | 48.2% | 38.8 ± 12.9 |
| Total | 6583 | 6202 | 5589 | 39.3% | 18.4% | 36.1 ± 13.1 |

NASH. The prevalence of presumed NASH in the three provinces is shown in Table 3.

**Table 3:** Prevalence of presumed NASH in three Iranian provinces [n (%)].

| | Tehran | Hormozgan | Golestan | Total* |
|---|---|---|---|---|
| Total | 56 (2.5 %) | 23 (1.6 %) | 148 (7.8 %) | 277 (2.9%) |
| Male | 35 (3.7 %) | 14 (2.2 %) | 73 (12.0 %) | 122 (4.4%) |
| Female | 21 (1.6 %) | 9 (1.2 %) | 75 (5.8 %) | 105 (2.0%) |

*Weighted prevalence according to the fractional population of each province.

The weighted prevalence of presumed NASH in different age groups is given in Table 4.

**Table 4:** The weighted prevalence of presumed NASH in different age groups.

| Age group (years) | Male | Female |
|---|---|---|
| 18-29 | 4.8% | 1.8% |
| 30-45 | 7.2% | 2.1% |
| 46-65 | 1.1% | 1.9% |

Mean BMI was significantly higher in presumed NASH patients compared to non-NASH (27.7 vs. 24.9; $p<0.001$). Mean age was not significantly different between patients with and without presumed NASH. Overall, presumed NASH was significantly more prevalent in males than females (5.6% vs. 3.1%; OR=1.9; 95% CI: 1.4-2.4).

Before the age of 45, presumed NASH was significantly more prevalent in men than women (6.5% vs. 2.7%; $p<0.01$).

Among individuals older than 45, the prevalence of presumed NASH was not significantly different between sexes. There was no association between the level of education and the prevalence of presumed NASH. Data was not collected in rural areas of Hormozgan due to technical confinements.

The prevalence of presumed NASH among the other two provinces was not significantly different between rural and urban areas (4.7% vs. 4.9%).

A previous study by Mohammadnejad et al. suggested the upper limit of normal ALT in Iranian male and female healthy blood donors to be set at 40 U/L and 34 U/L, respectively.[12] With the set cut-off values taken into account, more women would be categorized as having abnormal ALT levels, which translates into 265 individuals (54% female) with

presumed NASH. The overall weighted prevalence of presumed NASH would be 3.1% with this definition.

In univariate analysis, presumed NASH was significantly more prevalent in Golestan compared to Tehran province (OR=3.4; 95% CI: 2.5-4.6).

In multivariate analysis, compared to Tehran, the prevalence of presumed NASH was significantly higher in Golestan and significantly lower in Hormozgan. Male sex, urban living and being overweight or obese remained significantly associated with presumed NASH. Interestingly, age was not an independent risk factor in multivariate analysis (Table 5).

**Table 5:** Risk factors associated with presumed NASH in multivariate analysis.

| | OR (95% CI) | *p*-value |
|---|---|---|
| **Province*** | | |
| Golestan | 4.7 (2.7-8.1) | <0.001 |
| Hormozgan | 0.5 (0.3-0.9) | <0.05 |
| Male sex | 2.2 (1.5-3.2) | <0.001 |
| Urban residence | 2.2 (1.3-3.6) | <0.01 |
| **BMI**** | | |
| Overweight (25≤BMI<30) | 1.6 (1.1-2.5) | <0.05 |
| Obese (30≤BMI) | 2.9 (1.8-4.7) | <0.001 |

*Compared to Tehran province
**Compared to BMI<25

## DISCUSSION

The true prevalence of NAFLD and its different stages is hard to determine. The definition of NAFLD remains clinicopathological with well-defined criteria of the patterns of liver injury. The condition is asymptomatic until very late in its course and most patients are incidentally diagnosed.

Moreover, the gold standard for diagnosis and staging is liver biopsy, which is not feasible in population based studies.[13] Liver biopsy is an invasive procedure and has the potential for sampling and interpretation error.

Thus the use of liver biopsy for epidemiologic studies is limited. For operational purposes, most studies make use of surrogate indicators of NASH, such as elevated liver enzymes or imaging studies, including ultrasonography or computed tomography.

In our study, the overall prevalence of presumed NASH was determined at 2.9%. Our study was based on a single measurement of serum ALT, and a

diagnosis of presumed NASH was based on raised ALT Levels in patients shown to be HBV and HCV-free. Muslims virtually comprised our entire study population.

Alcohol consumption is prohibited for Muslims and also by law, but it is available in the black market. Thus regular alcohol consumption is rare but not nil.[14] Nevertheless, none of our subjects reported alcohol consumption. Furthermore, other causes of liver disease, including metabolic and autoimmune liver disease, are extremely rare, thus it is safe to assume that this number (2.9%) is a crude estimate of the prevalence of NASH in Iran.

A previous study on healthy blood donors in Tehran reported a slightly less prevalence of 2.35% for presumed NASH.[15] This could partly be explained by different subjects population and the fact that in this study, NASH was diagnosed only if ALT was elevated on two occasions.

In an autopsy study performed on 896 postmortem subjects at the Forensic Medicine Center in Tehran who died of acute incidents not related to hepatic disorders, 2.1% of cases were found to have NASH upon histological evaluation.[16]

Another recent cross-sectional study on over two thousand Golestan residents estimated the prevalence of NAFLD as 2.04% in Golestan.

This study involved liver ultrasonography in subjects with persistent ALT elevation, defined as ALT > 40 U/L on two occasions six months apart. This number is considerably less than our finding.

A different study design (repeated measurement) can probably explain this difference. Furthermore, 66% of their patients were female and not all patients returned for follow-up and ultrasonography.

The rate of presumed NASH varies across different provinces. The prevalence was significantly higher in Golestan (northern of Iran) and lower in Hormozgan (southern Iran). In multivariate analysis, this remained as an independent associated factor after adjustment for age, sex, BMI, as well as other relevant factors.

It remains unclear if there are any genetically defined metabolic characteristics or environmental factors-including nutritional habits and other life style patterns-which can explain this difference.

The prevalence of presumed NASH was highest in subjects between 30-45 years of age. This was observed in both sexes. However age was not an independent associated factor after adjusting for other important risk factors including sex and BMI.

Our data indicate that presumed NASH is almost twice as prevalent among males than females. Although early studies have emphasized that NASH was more common in women,[17] recent studies have shown a more even distribution between men and women.

The possibility that female hormones protect against NASH has been postulated and is supported by the fact that NASH is more common in post-menopausal women than premenopausal women by a factor of two.[18] Those who receive hormone replacement therapy are significantly less likely to have NASH in comparison with women who do not.[19] Gender-specific fat distribution may also be contributory, as men tend to have more visceral fat than women.[20]

The presence of presumed NASH was significantly associated with higher BMI. This association was seen overall, when looked at separately among male and female subjects, and with both definitions of raised ALT levels as mentioned above.

Obesity and metabolic syndrome, the most predictive factors of fatty liver disease, are on the rise in Iran[21-22] so the prevalence of NAFLD/NASH and related complications are expected to increase in the future. Health promoting campaigns advocating a healthy life style, including balanced diet, adequate physical exercise, and weight maintenance programs are of essential importance for the future comprehensive health plans, in order to control a rather prevalent cause of liver morbidity.

There are several limitations to this study. First, ALT was measured only once. A study of the data from a national cross-sectional sample of 1864 U.S. adults who had liver tests performed twice (mean, 17.5 days apart) showed that initial ALT levels were high in 6% of the people and remained elevated in 68%. This population included individuals having consumed ≥ 12 alcoholic drinks in the previous year (52%)as well as subjects with positive HCVAb (3%), and positive HBsAg (0.5%).[23]

Second, elevated ALT is only a gross surrogate for NASH. Demonstrating fatty infiltration of the liver in imaging studies is important for diagnosing NAFLD/NASH.

Our data did not include ultrasonography or CT imaging and some patients might have had reasons other than NASH for elevated ALT. We admit that this is a rough estimate and should certainly be verified by more accurate criteria.

## ACKNOWLEDGMENT

## CONFLICT OF INTEREST

None declared.

## REFERENCES

1. Teli MR, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: A follow-up study. *Hepatology* 1995;**22**:1714-9.

2. Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G,et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006;**44**:865-73.

3. Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology* 2002;**36**:1349-54.

4. Bugianesi E. Non-alcoholic steatohepatitis and cancer. *Clin Liver Dis* 2007;**11**:191-207, x-xi.

5. Soderberg C, Stal P, Askling J, Glaumann H, Lindberg G,Marmur J,et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology* 2010;**51**:595-602.

6. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980;**55**:434-8.

7. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and meta- bolic syndrome. *Hepatology* 2003;**37**:917-23.

8. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;**40**:1387-95..

9. Lazo M, Clark JM. The epidemiology of nonalcoholic fatty liver disease: A global perspective. *Semin Liver Dis* 2008;28:339-50.

10. Jamali R, Khonsari M, Merat S, Khoshnia M, Jafari E, Bahram Kalhori A,et al. Persistent alanine aminotransferase elevation among the general Iranian population: prevalence and causes. *World J Gastroenterol* 2008;**14**:2867-71.

11. Merat S, Rezvan H, Nouraie M, Jamali A, Assari S, Abolghasemi H,et al. The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in Iran: A population-based study. *Arch Iran Med* 2009;**12**:225-31.

12. Mohamadnejad M, Pourshams A, Malekzadeh R, Mohamadkhani A, Rajabiani A, Asgari AA, et al. Healthy ranges of serum alanine aminotransferase levels in Iranian blood donors. *World J Gastroenterol* 2003; **9**:2322-4.

13. Clark JM, Diehl AM. Defining nonalcoholic fatty liver disease: implications for epidemiologic studies. *Gastroenterology* 2003;**124**:248-50.

14. Nasrollahzadeh D, Kamangar F, Aghcheli K, Sotoudeh M, Islami F, Abnet CC,et al. Opium, tobacco, and alcohol use in relation to oesophageal squamous cell carcinoma in a high-risk area of Iran. *Br J Cancer* 2008;**98**:1857-63.

15. Pourshams A, Malekzadeh R, Monavvari A, Akbari. MR, Mohamadkhani A, Yarahmadi S, et al. Prevalence and etiology of persistently elevated alanine aminotransferase levels in healthy Iranian blood donors. *J Gastroenterol Hepatol* 2005;**20**:229-33.

16. Sotoudehmanesh R, Sotoudeh M, Ali-Asgari A, Abedi-Ardakani B, Tavangar SM, Khakinejad A,et al. Silent liver diseases in autopsies from forensic medicine of Tehran. *Arch Iran Med* 2006;**9**:324-8.

17. Sheth SG, Gordon FD, Chopra S. Nonalcoholic steato-hepatitis. *Ann Intern Med* 1997;**126**:137-45.

18. Carulli L, Lonardo A, Lombardini S, Marchesini G, Loria P.Gender, fatty liver and GGT. *Hepatology* 2006;**44**:278-9.

19. Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. *Gastroenterology* 2002;**122**:1649-57.

20. Enzi G, Gasparo M, Biondetti PR, Fiore D, Semisa M, Zurlo F. Subcutaneous and visceral fat distribution according to sex, age, and overweight, evaluated by computed tomography. *Am J Clin Nutr* 1986;**44**:739-46.

21. Esteghamati A, Khalilzadeh O, Mohammad K, Meysamie A, Rashidi A, Kamgar M, et al. Secular trends of obesity in Iran between 1999 and 2007: National surveys of risk factors of non-communicable diseases. *Metab Syndr Relat Disord* 2010;**8**:209-13.

22. Hosseinpanah F, Barzin M, Eskandary PS, Mirmiran P, Azizi F .Trends of obesity and abdominal obesity in Tehranian adults: A cohort study. *BMC Public Health* 2009;**9**:426.

23. Lazo M, Selvin E, Clark JM. Brief communication: clinical implication of short-term variability in liver function test results. *Ann Intern Med* 2008;**148**:348-52.