

## Original Article

## The Upper Normal Limit of Serum Alanine Aminotransferase in Golestan Province, Northeast Iran

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**Background:** The objective of this study was to determine the upper normal limit of serum alanine aminotransferase level in a population-based study in Golestan Province, northeast Iran.

**Methods:** From the randomly invited individuals (2,292), 698 out of the 916 males and 1,351 out of the 1,376 females participated in the study (participation rate: 76.2% and 98.1%, respectively). One hundred and twenty-one participants were excluded due to positive hepatitis B surface antigen or hepatitis C virus antibody and/or drinking more than 20 grams of alcohol per day. A total of 1,928 participants (1300 females) were included. The upper normal limit of serum alanine aminotransferase level was defined as the 95<sup>th</sup> percentile.

**Results:** The upper normal limit of serum alanine aminotransferase level in normal weight and nondiabetics was significantly lower than the total study group (36 versus 45 U/L). Serum alanine aminotransferase level was independently associated with male gender, body mass index, and diabetes mellitus (OR=2.05; 95%CI: 1.44 – 2.94, OR=2.76; 95%CI: 1.84 – 4.13, and OR=2.96; 95%CI: 1.56 – 5.61, respectively).

**Conclusion:** Considering the lower calculated upper normal limit in normal weight nondiabetic participants in this study, we recommend setting new upper normal limit for serum alanine aminotransferase level. It seems reasonable to set upper normal limit for serum alanine aminotransferase level in males and females separately.

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**Keywords:** Alanine aminotransferase • body mass index • gender • general population • upper normal limit

### Introduction

Serum alanine aminotransferase (ALT) concentration is the most commonly-used variable for assessment of the risk of mortality in liver disease.<sup>1,2</sup> The current mean upper normal limit (UNL) for ALT was 40 (range: 30 – 50) U/L in previous studies.<sup>1,3–8</sup> Such

thresholds were introduced when ALT testing was a surrogate marker for the screening of non-A non-B hepatitis among blood donors and before antihepatitis C virus (HCV) testing, and restrictive behavioral criteria for donor selection were implemented.

The so-called “reference” population was likely to include many persons with nonalcoholic fatty-liver disease (NAFLD), now recognized as the most prevalent cause of chronic liver disease in developed countries.<sup>9–11</sup> The current reference ranges for ALT levels probably underestimate the frequency of chronic liver disease.

The UNL varies in different laboratories according to the commercial kit used and the reference population chosen by each manufacturer to establish the normal range. In Iran, no UNL has yet been established in a large-scale population-

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based study, despite its importance as an aid to the classification and management of patients with liver diseases.

Recent studies have shown that serum ALT levels can be modulated by a number of factors, including gender, body mass index (BMI), fasting blood glucose, and serum triglyceride levels.<sup>12-14</sup> These factors are usually not taken into account when the normal ALT range is determined. Considering the fact that serum ALT level varies in different populations, this study was undertaken in an Iranian general population for the first time.

## Materials and Methods

Golestan Province is located in northeast Iran with 1,700,000 populations. Gonbad and Kalaleh are located in Golestan Province. Gonbad has a population of 437,960 and the population of Kalaleh is 154,349.

In autumn 2006, a total of 2,292 (1,376 females, aged 18 – 75 years) inhabitants of villages and cities of Gonbad and Kalaleh were randomly selected by systematic clustering, according to the house hold number in the health houses, and were invited to take part in this study. The protocol used in the present study was approved by the Ethical Committee of the Digestive Disease Research Center at Tehran University of Medical Sciences based on the Declaration of Helsinki.

The participants were informed about the objectives of the study. A written informed consent was obtained and a comprehensive questionnaire including history of known hepatic disease, medications used currently or during the last six months, hypertension, and diabetes mellitus, was completed by a trained physician interviewer. Morning blood samples of each participant were collected and transferred to Iranian Blood Transfusion Organization Research Center under appropriate condition. Separation of serum was done under aseptic conditions. The sera were checked for ALT, hepatitis B surface antigen (HBs Ag), and hepatitis C virus antibody (HCV Ab). The sera were tested for ALT level using Hitachi autoanalyzer 704 (Roche, Switzerland) with Pars Azmoon reagents kit (Tehran, Iran). HBs Ag was measured by Enzygnost HBs Ag 5.0 kit (Dade Behring, Germany). HCV Ab was detected by enzyme-linked immunosorbent assay (ELISA) method using Anti-HCV-EIA-Avicenna kit (Moscow, Russia). HCV recombinant immunoblot assay III using INNOLIATM HCV-Score kit

(Innogenetics, Gent, Belgium) was carried out on the positive samples of HCV by ELISA method. Those who had positive HCV recombinant immunoblot assay III test were considered as exposed to HCV. BMI was calculated by dividing weight in kilogram to the squared height in meter. We considered a BMI  $\geq 25$  kg/m<sup>2</sup> as overweight.<sup>15</sup> Diabetes mellitus and hypertension were defined if the participant was a known case of those diseases (diagnosed by a physician, received medications, or had nutritional restrictions). UNL introduced by the manufacturer for ALT kit was 40 U/L. UNL of serum ALT was considered as 95<sup>th</sup> percentile for the serum ALT level, and was measured separately considering gender, BMI, and diabetes mellitus status. All data were analyzed by SPSS software, version 13 (Chicago, IL, USA). Proportions were compared by Chi-square test. Multivariate analysis was performed to identify factors independently associated with serum ALT  $> 40$  U/L. *P* values  $< 0.05$  were considered statistically significant.

## Results

Of the invited individuals (2,292), 698 out of the 916 males and 1,351 out of the 1,376 females participated in the study (participation rate: 76.2% and 98.1%, respectively). One hundred and twenty-one participants were excluded due to positive HBs Ag, positive HCV Ab, and/or drinking more than 20 grams of alcohol per day.

A total of 1,928 participants (1300 females) were included. The mean age of the participants was 40.7  $\pm$  14.7 (range: 18 – 75) years. The mean serum ALT level was 20.48  $\pm$  13.1 U/L and the mean BMI was 26.12  $\pm$  5.09 kg/m<sup>2</sup>.

The mean serum ALT level was significantly higher in males (23.03  $\pm$  13.93 U/L) than females (19.24  $\pm$  12.5 U/L) ( $P < 0.001$ ); in subjects with BMI  $\geq 25$  (22.94  $\pm$  14.27 U/L) than those with BMI  $< 25$  (17.51  $\pm$  10.82 U/L) ( $P < 0.001$ ); and also in diabetics (28.70  $\pm$  19.93 U/L) than nondiabetics (20.12  $\pm$  12.62 U/L) ( $P < 0.001$ ). Table 1 indicates UNL for serum ALT level considering BMI and diabetes mellitus in total study sample and also separately according to gender.

Figure 1 shows the correlation between serum ALT level and BMI in men and women.

Logistic regression analysis showed that male gender (OR=2.05; 95%CI: 1.44 – 2.94), BMI  $\geq 25$  (OR=2.76; 95%CI: 1.84 – 4.13), and diabetes mellitus (OR=2.96; 95%CI: 1.56 – 5.61) were independent risk factors for serum ALT level  $> 40$

**Table 1.** The upper normal limit for serum alanine aminotransferase levels considering body mass index and diabetes mellitus status.

Status	UNL for serum ALT levels (U/L)		
	Male	Female	Total
BMI<25 kg/m <sup>2</sup> and nondiabetics (n=859)	37.5	36	36.1
BMI<25 kg/m <sup>2</sup> (n=875)	37.85	36	36.2
BMI ≥ 25 kg/m <sup>2</sup> (n=1053)	59	45.25	51
Diabetics (n=79)	51	80.5	77
Diabetics and BMI ≥25 kg/m <sup>2</sup> (n=63)	60	85	79.4
Nondiabetics (n=1849)	48.5	39.85	45
<b>Total (n=1928)</b>	<b>50.55</b>	<b>41.95</b>	<b>45</b>

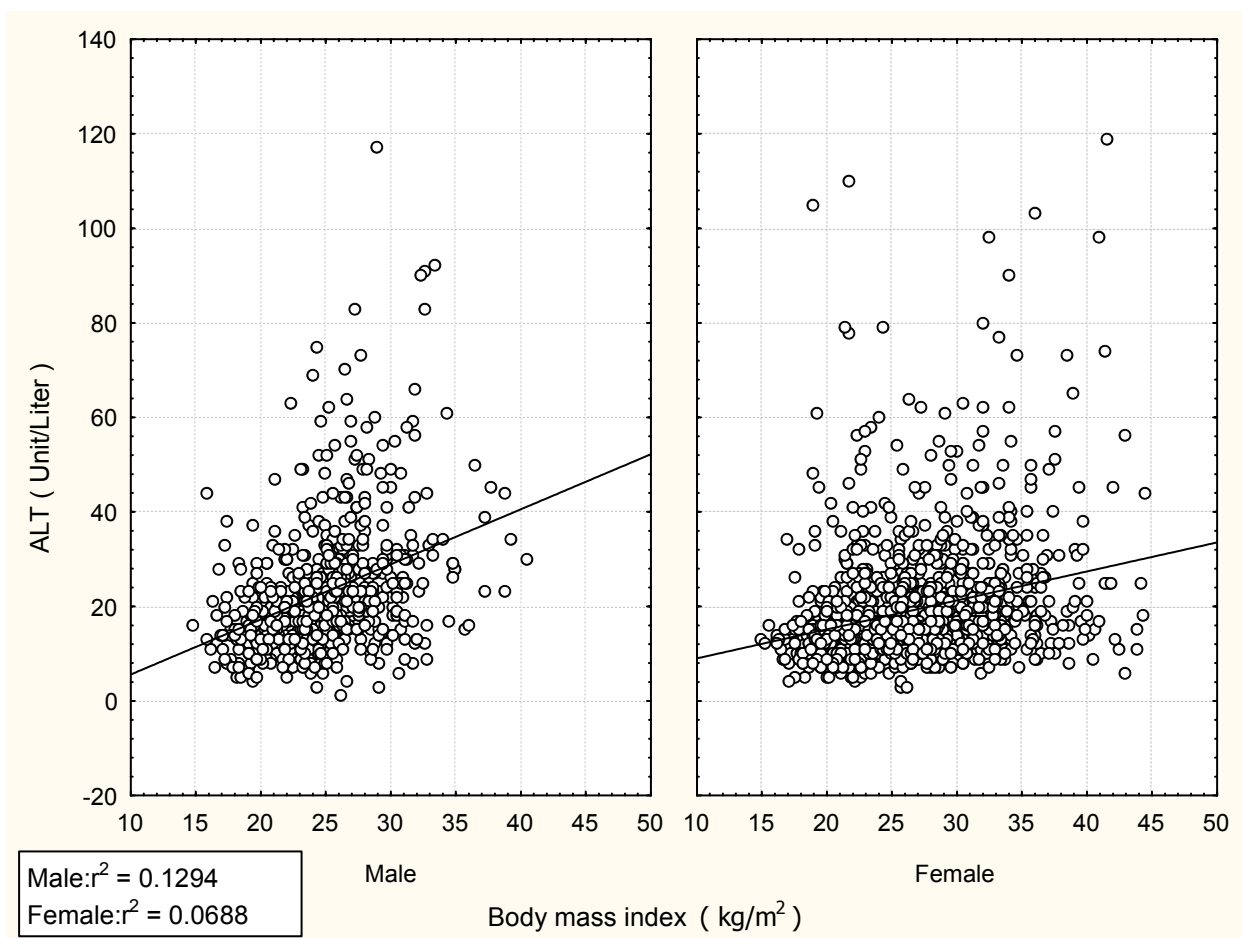
UNL=upper normal limit; ALT=alanine aminotransferase; U/L=unit per liter; BMI=body mass index; kg/m<sup>2</sup>=kilogram per square meter.

U/L. Hypertension did not have any correlation with serum ALT level.

### Discussion

Serum ALT level, a sensitive indicator of liver cell injury, has been used to identify patients with liver disease for almost 50 years.<sup>3</sup> It has been recently suggested as a predictor of overall mortality and medical costs.<sup>16</sup> Several studies have recently questioned whether the previously-

established values for normal ALT range are still accurate. They have suggested that the upper limit of normal range should be revised.<sup>12-14</sup> To the best of our knowledge, this is the first population-based study conducted in Iran that determined the UNL for serum ALT level. A previous study conducted in Iran on 1939 blood donors showed that UNL in Iranian blood donors was 40 U/L in men and 34 U/L in women.<sup>12</sup> However, the sample studied was not representative of the general population. Blood donors in Iran are volunteers



**Figure 1.** Association between serum alanine aminotransferase levels and body mass index in males and females.

with healthy behaviors and usually do not include obese or underweight subjects, alcohol or medication users, or those with severe chronic diseases.

A large-scale population-based study by Kariv et al. showed that the UNL of serum ALT level in a subgroup of the study sample who had normal triglyceride, cholesterol, glucose, negative viral and autoimmune markers, normal BMI, and those who used no hepatotoxic drugs or alcohol (37.5 U/L) was lower than the UNL calculated in total study sample which was selected at random from the general population (50.1 U/L).<sup>13</sup>

Prati et al. calculated the "healthy" range for serum ALT level in blood donors who had a normal BMI and normal serum cholesterol, triglyceride, and glucose levels and were not taking medications. The calculated UNL for ALT level decreased from 40 U/L to 30 U/L in men and from 30 U/L to 19 U/L in women.<sup>14</sup> In this study, after excluding the subjects with overweight or diabetes, the UNL of serum ALT level was significantly lower than the total study group (36 versus 45 U/L). This finding is comparable to the previous studies.<sup>13,14</sup>

Considering the high prevalence of overweight (54.6%) in our study, which is comparable to the previous studies conducted in this area,<sup>17,18</sup> the calculated UNL for the whole study sample (45 U/L) cannot be used as the UNL for healthy individuals. The UNL calculated for the subgroup of the study sample with BMI < 25 kg/m<sup>2</sup> (36.2 U/L) was lower than that for the total study sample and even the current upper thresholds (40 U/L) proposed by the ALT kit manufacturer.

The UNL in those with normal weight and non-diabetic individuals (men: 37.5 U/L; women: 36 U/L) was near to the previously reported UNL for Iranian blood donors (men: 40 U/L; women: 34 U/L).<sup>12</sup> The results also showed that UNL in non-diabetics (45 U/L) was lower than diabetics (77 U/L). This most probably reflects the effect of diabetes mellitus as a component of metabolic syndrome by inducing NAFLD on serum ALT level. There is evidence showing that abnormal carbohydrate metabolism results in elevation of serum ALT level.<sup>14,19-20</sup>

In this study, the UNL for serum ALT level was significantly higher in overweight males than females (59 versus 45.25 U/L). This is comparable with the previous study showing the more strong effect of overweight in males in increasing the serum ALT level than in females.<sup>21</sup> The previous

study for determining UNL in Iranian blood donors also clarified that serum ALT level was independently associated with BMI and male gender. And association of ALT with BMI was more prominent in males than in females.<sup>12</sup>

Our study also determined the correlation between serum ALT levels and male gender, BMI  $\geq$  25 kg/m<sup>2</sup>, and diabetes mellitus. It showed new thresholds for the UNL of serum ALT level according to gender, BMI, and the presence of diabetes mellitus. This study further emphasized the findings of the previous studies regarding the strong association of serum ALT level with male gender and BMI.<sup>12,14,22-25</sup>

Diabetes mellitus and overweight are both components of metabolic syndrome. NAFLD is the hepatic manifestation of metabolic syndrome and the commonest etiology of elevated serum ALT level in Iran.<sup>26,27</sup> This explains the observed elevation of serum ALT level in overweights and diabetics in this study. The study of Iacobellis et al.<sup>19</sup> showed that elevated serum ALT level was associated with increased fasting insulin and not with obesity per se. They suggested that the presence of insulin resistance, rather than BMI alone, plays a role in mediating the increased aminotransferase levels.<sup>19,20</sup>

We should emphasize that UNL calculated in this study is the 95<sup>th</sup> percentile for serum ALT level in the studied population and does not appropriately set the healthy serum ALT level which may predict the over all mortality. Lack of measurement of serum triglyceride, cholesterol, and fasting glucose, which influence on serum ALT level is the limitation of this study.

The previous studies in Iran, Korea, and Jordan have shown that the prevalence of diagnosed diabetes mellitus in adult population was about 4% and if screening with fasting plasma glucose was used, the prevalence would be increased to 7%.<sup>28-30</sup> Although screening with fasting plasma glucose was not performed in our study, the prevalence of diagnosed diabetes mellitus was 4%, which is comparable with the previous studies mentioned above.

In conclusion, considering the lower calculated UNL in normal-weight, nondiabetic participants in this study, we recommend setting new UNL for serum ALT level. The new threshold for serum ALT level increases the sensitivity of the test for early diagnosis and follow-up of patients with liver diseases. It seems reasonable to set UNL for serum ALT level in males and females separately.

However, considering the BMI for redefinition of the UNL is not recommended because elevated levels in overweights may be a clue to the diagnosis of NAFLD. Therefore, adjusting the UNL for overweights will result in underdiagnosis of some patients with NAFLD.

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