Clinical Studies

Re-evaluation of serum alanine aminotransferase upper normal limit and its modulating factors in a large-scale population study


Abstract: Background: The upper normal limit (ULN) of serum alanine-aminotrasferase (ALT) normal range was recently challenged, because patients diagnosed with liver diseases may have ‘normal’ or near-‘normal’ ALT levels, and because possible modulators are often ignored in determining normal range. Aim: To estimate the ULN for serum ALT and to identify factors modulating it. Subjects and methods: We reviewed medical records of subjects aged 15–90, who underwent standard panels of laboratory tests, including serum ALT, over 6 months at a central laboratory. Three groups were defined: Group 1, comprised total study population (N = 272 273). Group 2 (N = 87 020) comprised total study population, excluding those receiving potentially hepatotoxic drugs, or diagnosed with liver disease, or had any abnormal laboratory test results other than for triglycerides, cholesterol, glucose, or HbA1c. Group 3 (N = 17 496) the ‘healthy’ population, from whose ALT values we established the new ULN, comprised Group 2 subjects with normal triglycerides, cholesterol, glucose, and HbA1c levels. Results: The 95th percentile ALT values, corresponding to the ULN, in groups 1, 2, and 3 were 50.1, 40, and 37.5 U/l, respectively. 6.2% (16 943/273 273) of subjects whose ALT was below ULN listed by the test manufacturer (52 U/l), had ALT level above our new ULN. Linear and logistic-regression analyses showed that ALT levels were significantly modified by gender, age, glucose, cholesterol, triglycerides, and overweight/obesity diagnosis. Significant interaction was found between gender, glucose and cholesterol levels. Conclusions: In this first large-scale study of ‘healthy’ population, serum ALT ULN was far lower than currently accepted value. Age and gender may be considered when determining the ULN for ALT.

Serum alanine aminotransferase (ALT) is a valid laboratory parameter for evaluation and follow-up of liver diseases and hepatocellular damage. The upper limit of the normal range (ULN) varies in different laboratories according to the commercial kit used and the reference population chosen by each manufacturer to establish the normal range.

The currently accepted range of normal values for serum ALT levels was recently challenged (1, 2) by research groups, who claimed that the true normal values are significantly lower than those listed by kit manufacturers, and that an updated, reliable ULN is needed. No such ULN has yet been established in a large-scale population-based study, despite its importance as an aid to the classification and management of patients with liver diseases, and in particular with hepatitis B (HBV) and C (HCV). In addition, serum ALT is a surrogate marker for the diagnosis of patients with non-alcoholic fatty liver disease (NAFLD), the most common cause of elevated serum ALT levels in otherwise serologically negative patients (3, 4). In the absence of proper screening tools (such as a reliable ‘healthy’ ULN for serum ALT) for NAFLD, its diagnosis remains problematic.
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Recent studies (1, 5) have shown that serum ALT levels can be modulated by a number of factors, including age, gender, body mass index (BMI), fasting blood glucose, and serum triglyceride (TG) levels. These factors are usually not taken into account when the normal ALT range is determined.

This study was undertaken with the aim to show that the ‘healthy’ ULN for serum ALT, based on samples taken from a large community-based population, is lower than that of the manufacturer, and to identify potential modulating factors for ALT levels.

Patients and methods

Maccabi Health Care Services is a Health Maintenance Organization (HMO) that provides health services to more than 1.5 million members in Israel.

Patients’ medical records, including their medical history and diagnoses, laboratory results, and medications, are kept in a data warehouse. Sera are tested at one central laboratory by technicians who always use the same brand of commercial kit for any specific test. Fresh (same-day) sera are used, thus avoiding artifacts because of freezing and thawing.

The results of all consecutive ALT and other relevant laboratory tests (blood count, liver and kidney function tests, lipids, glucose and HbA1c levels, and serological tests for viral, metabolic and autoimmune liver diseases; see Table 1, panel A), performed at the central laboratory between 1 January, and 30 June 2002 on sera from adult subjects (aged 15–90 years), were retrieved from Maccabi’s data warehouse and analyzed. All of the ALT tests had been performed using an Olympus (OSR 6107) kit and reagents (Olympus Diagnostica, GmBH, Lismeehan, Ireland). Data on patients’ clinical diagnoses and prescribed medications were also retrieved. Laboratory tests were performed as either routine tests or because of a medical indication.

A total of 346,530 ALT tests were performed in sera from 273,273 subjects in the selected age group. In the case of subjects with more than one ALT result, we have chosen the lower value for the analysis.

Three groups were defined: Group 1 consisted of the total population of 273,273 subjects. Group 2 (87,020 subjects) comprised the total population after exclusion of subjects with abnormal values of one or more of the laboratory parameters, medical diagnoses that may affect liver function tests (listed in Table 1, panels A and B), or a medication profile consisting of potentially hepatotoxic drugs (Table 1, panel C). Not excluded from this group were subjects with abnormal levels of serum TGs, cholesterol, glucose, or HbA1c. Group 3 (17,496 subjects) comprised only those subjects from Group 2 whose values of glucose, HbA1c, cholesterol, and TG were normal. This last group therefore comprised the ‘healthy’ population from which our best estimate of normal ALT values was determined.

Out of the 273,273 participants (Group 1), about 97,000 had abnormal cholesterol level, 61,000 had abnormal TG level, 51,000 had abnormal glucose level, 37,000 had abnormal Hemoglobin (Hb) level and 32,000 had leucocytosis. We excluded all subjects with at least one abnormal laboratory test, such as those just mentioned, because those abnormal tests may be associated with illnesses that may affect ALT level, and lead to a biased estimation of ALT distribution in the ‘healthy’ population.

Table 1. Criteria for exclusion from the ‘healthy’ population (study group 3)

<table>
<thead>
<tr>
<th>Criteria for Exclusion</th>
<th>Study Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Exclusion by abnormal laboratory results</td>
<td>Group 3</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), Alkaline phosphatase, albumin, bilirubin, creatinine, white blood count (WBC), hemoglobin, platelets, ceruloplasmin, ferritin, Glucose, HbA1c, triglycerides (TG), cholesterol, HbsAg, anti HCV, Anti mitochondrial antibodies (AMA), anti nuclear antibodies (ANA), anti tissue transglutaminase (TTG).</td>
<td></td>
</tr>
<tr>
<td>B. Exclusion by medical diagnosis</td>
<td></td>
</tr>
<tr>
<td>Obesity, overweight, ethanolism*, chronic liver disease, cirrhosis, hepatitis b or c, liver neoplasms.</td>
<td></td>
</tr>
<tr>
<td>C. Exclusion by medications†</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptives, hypolipidemic agents (statins and bezofibrates), any anti-epileptic or Antifungal agents.</td>
<td></td>
</tr>
</tbody>
</table>

*Ethanolism was defined as alcohol consumption in excess of 30 g/day for men and 20 g/day for women. †Most common categories for potentially hepatotoxic drugs were chosen for exclusion.

Statistical analysis

Statistical analysis was performed using the SPSS package version 12.0 and Matlab version 7.0. The 5th, 25th, 50th (median), 75th, and 95th percentiles for ALT levels were calculated on the basis of the empirical distribution of the data. We set the ULN at the 95th percentile, a common practice for distribution of a continuous variable.

To examine the factors that modulate serum ALT level, serum ALT values were fitted with univariate and multivariate general linear models and various neural net models (6), according to age, cholesterol, TG, and glucose (analyzed as continuous variables), and according to gender, use of medications, and medical diagnosis (analyzed as dichotomous variables: males were denoted by 1 and females by 0; the use of at least
one medication listed in Table 1 was denoted by 1, and ‘no use’ was denoted by 0; a patient diagnosed with at least one of the listed diagnosis was denoted by 1, and ‘no such diagnosis’ by 0). Values of $P$ less than 0.05 were considered statistically significant.

For the logistic regression, subjects were defined as having an abnormal serum ALT pattern if at least one recorded ALT level was above the current ULN of the commercial kit used by Maccabi Health Care Services Laboratory (52 U/l). Interaction between each modulating factor and gender was also analyzed. All analyzes were conducted in the total study population (Group 1) excluding only patients with HCV, HBV, and positive serology for autoimmune liver disease.

**Results**

Demographic data for subjects included in the study groups (1–3) are shown in Table 2.

**ALT percentiles**

A total of 346,530 ALT tests were evaluated from 273,273 subjects in the selected age group. The ALT 95th percentile of groups 1–3 were: 50.1, 40, and 37.5 U/l, respectively. 88.9% (242,940/273,273) out of the total population (Group 1) had ALT level below 37.5 U/l (the new ULN of ALT), where 95.5% (260,976/273,273) of the total population has ALT level below the current ULN (52 U/l). 89.4% (144,743/161,905) out of all females had ALT level below 34.9 U/l (the 95th percentile of the empirical distribution of ALT in females) and 97.6% (158,019/161,905) out of all females had ALT level below 52 U/l (the current ULN of ALT). 88.5% (97,676/110,368) out of all males had ALT level below 44.9 U/l (the 95th percentile of the empirical distribution of ALT in males) and 92.4% (101,980/110,368) out of all males had ALT level below 52 U/l (the current ULN of ALT).

The mean, median, and SD values as well as 5, 25, 75 and 95 percentiles for ALT results in all three groups are shown in Table 3.

**Factors modulating serum ALT**

As the distribution of ALT is skewed, we used the log function and noticed that log(ALT) have the normal distribution. We used stepwise linear regression with log(ALT) the dependent variable, to examine the significance of all covariates. The adjusted $R^2$ of the regression was 16%. The two important independent variables were gender and

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean age (SD)</th>
<th>95% CI for age</th>
<th>% Males</th>
<th>% Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>272,273</td>
<td>45.09 (20.07)</td>
<td>45.015-45.165</td>
<td>41</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>87,020</td>
<td>43.1 (18.33)</td>
<td>42.978-43.222</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>17,496</td>
<td>31.91 (17.07)</td>
<td>31.657-32.163</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>

**Table 3. Serum ALT level in groups 1–3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>272,273</td>
<td>161,905</td>
<td>110,368</td>
<td>87,020</td>
<td>52,015</td>
<td>35,005</td>
<td>17,496</td>
<td>11,168</td>
<td>6,328</td>
</tr>
<tr>
<td>Mean</td>
<td>23.5</td>
<td>20.2</td>
<td>28.5</td>
<td>20.5</td>
<td>17.6</td>
<td>24.9</td>
<td>18.8</td>
<td>16.7</td>
<td>22.7</td>
</tr>
<tr>
<td>Median</td>
<td>19</td>
<td>16.7</td>
<td>23</td>
<td>18</td>
<td>16</td>
<td>22</td>
<td>16.3</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>SD</td>
<td>25.6</td>
<td>20.1</td>
<td>31.5</td>
<td>10.3</td>
<td>7.8</td>
<td>11.9</td>
<td>9.8</td>
<td>7.9</td>
<td>11.7</td>
</tr>
<tr>
<td>5th</td>
<td>9.2</td>
<td>8.8</td>
<td>11</td>
<td>9.2</td>
<td>8.8</td>
<td>11.1</td>
<td>8.7</td>
<td>8.1</td>
<td>10</td>
</tr>
<tr>
<td>25th</td>
<td>13.9</td>
<td>12.6</td>
<td>16.7</td>
<td>13.4</td>
<td>12.1</td>
<td>16.5</td>
<td>12.3</td>
<td>11.6</td>
<td>14.7</td>
</tr>
<tr>
<td>75th</td>
<td>27</td>
<td>23</td>
<td>33</td>
<td>25</td>
<td>21</td>
<td>30</td>
<td>23</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>95th</td>
<td>50.1</td>
<td>40.6</td>
<td>60.8</td>
<td>40</td>
<td>32.4</td>
<td>48</td>
<td>37.5[†]</td>
<td>31.8</td>
<td>44.9</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; ULN, upper normal limit. *The 95th percentile denotes the upper limits of normal ALT for each group. †$P<0.0001$ (comparing with Group 1 95th percentile).
whether the patient had a diagnosis of obesity or overweight. Cholesterol, TG, glucose and the use of hepatotoxic medication (listed in Table 1) were also significant. The logistic regression analysis is summarized in Table 4. The results obtained with the linear regression model, and with the neural net model were similar to those shown with the logistic regression model.

After stratification of group 3 ALT results by age and gender, the 95th percentiles for serum ALT are shown in Fig. 1 for gender-specific age groups. Examination of the interaction between gender and other parameters revealed a significant interaction between gender and glucose values, and between gender and cholesterol values. The interaction of cholesterol and ALT levels was significantly stronger in males than in females, whereas the interaction of glucose and ALT levels was significantly weaker in males than in females. No significant interaction was detected between TG values and gender.

Discussion

This is the first study evaluating ALT levels in a large-scale population, based on a central laboratory database. Maccabi is the second largest national health plans in Israel, which provides health services for over 1.5 million people (about 25% of Israel’s population). Study population well represents the general Israeli population in terms of demographic, socioeconomic and medical status.

ALT results were available for 272,273 subjects (group 1). The strict exclusion criteria for blood tests results that exceeded the ULN, resulted in a very high exclusion rate (93.4%), so finally, group 3 consisted of 17,496 subjects. Nevertheless, the large exclusion rate did not significantly reduce the statistical power of our results.

We believe that all subjects with at least one abnormal result of the above-mentioned laboratory tests, should not be included in the sample by which we estimate the distribution of ALT in the healthy population. Abnormal laboratory results may reflect underlying acute or chronic illness, or associated conditions as NAFLD (often accompanied by hyperglycemia or hypertriglyceridemia), that may shift ALT distribution, resulting in a biased estimation of the distribution in the ‘healthy’ population. As our ‘healthy’ population excluded, as far as possible, patients with acute illnesses, liver diseases, and relevant systemic diseases, we can reasonably assume that it is representative of the healthy Israeli population.

The results of this study indicate that the ULN for ALT in the ‘healthy’ population is significantly ($P<0.0001$) lower than that listed by the manufacturer of the biochemical test for ALT.

The new ULN, corresponding to the 95th percentile of our healthy population, was 37.5 U/l, compared with the value of 52 U/l specified by the manufacturers of the laboratory test. According to the criteria of the NCCLS (7), a globally recognized organization that develops and disseminates standards and guidelines for laboratory medical testing, the normal ranges for laboratory parameters (such as serum ALT) are based on questionnaires completed by a very small reference population (100–200 subjects), and a limited laboratory workup. These criteria do not necessarily rule out individuals with NAFLD or other liver diseases, and so, the reference population may not be representative of the general healthy population.

The advantage of our study was the determination of the ‘healthy’ ALT range in a very large population, in which hepatotoxic drugs, and relevant liver and systemic were reasonably excluded.
Our study is unique by its population, which is representative of the general population, while other studies (1, 2) suggested new ULN for ALT in a very selected population such as blood donors.

Potential limitations to our study include the collection of retrospective data, lack of several blood tests results such as anti-HCV or HBsAg for many subjects, and no BMI data, as it was not available in the medical records. Therefore, our ‘healthy’ population may be ‘contaminated’ with patients with viral liver disease or NAFLD. This ‘contamination’ may have led to an overestimation of ALT ULN, however, by excluding those patients, the ‘true healthy’ ALT ULN may be even lower that that we have found.

We believe that ‘healthy’ ALT ULN is 37.5 U/l or less, and that the current ULN is indeed too high. The precise value for ULN for a healthy ALT should be determined in a prospective study.

Several authors have addressed the role of factors that may modify serum ALT levels (1, 5). Some of these are ‘partitioning factors’, i.e., parameters that divide the reference population into statistically significant subclasses, such as age and gender, and should therefore be taken into account when determining the normal range of a laboratory parameter. Our study confirmed the observation (1, 7) that normal females have significantly lower serum ALT levels than normal males. Serum ALT levels in women remain constant throughout life, whereas in men they gradually increase up to the 5th decade and then decline. A reciprocal association between ALT levels and age has been reported (5).

NAFLD is considered to be part of the metabolic syndrome (8). Certain biochemical parameters associated with the metabolic syndrome, including blood glucose, insulin, and TG levels, have been found to affect serum ALT (5). Our study confirmed some of those findings. Surprisingly, we found that cholesterol, which is not considered part of the metabolic syndrome, was a significant predictor for ALT levels in a multivariate logistic regression analysis. This finding may reflect a possible confounder effect of BMI.

Body weight and BMI are reportedly also important determinants of serum ALT levels (1, 5), independently or via biochemical parameters such as glucose and TGs. Data for BMI was not available in this study, nevertheless, the strict exclusion of all abnormal laboratory results of abnormal blood lipids and glucose has managed to exclude many subjects with high BMI. This potential bias would be expected to increase ALT levels in Group 3, thereby overestimating ALT ULN beyond its true level.

We ruled out excess alcohol consumption as a factor in our ‘healthy’ population by excluding subjects with a diagnosis of ethanolism. Moreover, alcohol consumption in Israel is relatively low. According to a national health and nutrition study (Mabat National Health and Nutrition Survey, unpublished data), only about 1.4% of men and 0.06% of women consume alcohol in quantities considered to be risk factors for NAFLD (in excess of 30 g/day for men and 20 g/day for women). Furthermore, a normal $\gamma$ glutamyl transferase (GGT) finding should exclude the majority of excessive drinkers.

3.5% of the study population was tested for HCV and 5.4% for HBV. As the endemicity for HBV in the Israeli population is relatively low with an annual carrier rate of 1–3% (9, 10), and the prevalence of HCV infection is ~0.5% (Israel’s national blood center, personal communication), the contribution of those patients’ ALT levels to the overall ALT results in Group 3 is negligible, and as mentioned above this would only lower the true level for healthy ALT.

The high specificity (95%, by taking the 95th percentile in the healthy population or higher if the UNL is greater than the 95th percentile) of elevated serum ALT levels makes this enzyme an important marker for the presence of liver disease in the absence of clinical symptoms or signs (11, 12). However, its sensitivity as a marker for the presence of liver pathology is only 83% (12), as some patients with chronic HCV infection (13) or NAFLD (14), in whom ALT levels (according to the current standards) are normal, are found to have significant histological abnormalities of the liver. Furthermore, a recent study suggested that higher ALT levels, even within the normal range, may result in increased mortality (15). A new ULN for ALT might increase sensitivity, allowing earlier diagnosis and treatment of liver diseases.

On the other hand, lowering of the ULN might decrease the test specificity, leading to unnecessary investigations involving higher costs and increased patient anxiety (16). In any case, until final conclusions are drawn, physicians should probably change their clinical approach towards a patient whose ALT levels are borderline according to the accepted ULN, as these levels may indicate a significant liver pathology.

Analysis of the distribution of serum ALT levels in our population disclosed that approximately 6% of the population has ALT levels that are currently defined as normal but, in fact, are abnormal, according to our new ‘healthy’ ULN. This group should be targeted for further research.

New normal upper limit for ALT
Future prospective studies should address the question of whether the ‘normal’ ranges for ALT should be modified and to what extent, in terms of cost effectiveness. Partitioning factors such as gender and age should be taken into account.

In conclusion, our study suggests that the currently accepted upper limit of the normal range for serum ALT is too high. Serum ALT values are significantly affected by gender, age, serum lipids, and glucose levels. Our new normal ULN is significantly lower than that given by the manufacturer of the laboratory test currently used by our HMO. It is likely that a prospective population-based study would show that the normal true ULN for serum ALT levels is even lower.

Acknowledgement
We thank S. Smith for her significant contribution in editing this article.

References