의학석사 학위논문

대사적 및 조직학적 정상 인구에서 건강한 알라닌 아미노전이효소 수치의 새로운 정의

Updated Definition of Healthy Alanine Aminotransferase Levels in a Metabolically and Histologically Normal Population

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이 논문을 의학석사 학위 논문으로 제출함

2024년 2월

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Abstract

Background and Aims: This study re-evaluates the upper limit of normal (ULN) for alanine aminotransferase (ALT), traditionally set at 40 U/L, using histological and metabolic parameters in Asian living liver donor.

Methods: We conducted a retrospective analysis of 5,455 potential living liver donors from 2005 to 2019. Patients were screened for hepatitis B, C, HIV, and alcohol use. Histologically and metabolically healthy participants was assessed using the modified Prati criteria (body mass index <23 kg/m^2, triglyceride ≤200 mg/dL, fasting glucose ≤105 mg/dL, total cholesterol ≤220 mg/dL). The new healthy ULN of ALT was determined at the 95th percentile among participants without hepatic steatosis or metabolic dysfunction.

Results: The median age of the cohort was 30 years with a predominance of males (66.2%). Among all participants, 3,162 (58.0%) were without hepatic steatosis, and 1,553 (49.1%) met the modified Prati criteria, being metabolically healthy. The new healthy ULN of these 1,553 individuals was 34 U/L for males and 22 U/L for females, significantly lower than the conventional 40 U/L. A ‘borderline’ ALT category (34–40 U/L for males, 22–40 U/L for females) was also introduced to participants at risk of hepatic steatosis or metabolic dysfunction.

Conclusion: The traditional ALT ULN is higher than healthy levels for a metabolically and histologically verified Asian population. The proposed ULN values are 34 U/L for males and 22 U/L for females. The introduction of a ‘borderline’ category aids in better disease risk stratification, highlighting the need for an updated ULN for ALT.

Keywords: Alanine aminotransferase, hepatic steatosis, upper limit of normal.

목 차

표 및 그림 목차

Introduction

Alanine aminotransferase (ALT) has been widely used in clinical practice as a surrogate marker for liver disease. Since its introduction in the 1950s, the upper limit of normal (ULN) for ALT has uniformly been accepted as 40 U/L, irrespective of sex.[1-3] However, this value was determined before the introduction of hepatitis C virus (HCV) testing and the concept of fatty liver disease was developed.[4,5] Several studies have therefore proposed new definitions of normal ALT values based on population or hospital-based data.[6,7] However, the fixed ULN remains in widespread use in real-world clinical practice.

Theoretically, normal ALT values should be calculated based on individuals deemed healthy and without liver disease. Although viral hepatitis can be easily detected through serologic tests, fatty liver disease—the most common liver disease—often shows the current normal ALT values, leading to undiagnosed cases. Furthermore, several studies have reported that the ULN of ALT, specifically 30–40 U/L, is associated with a higher prevalence of metabolic syndrome, fatty liver, and increased mortality.[8-11] However, most studies that define normal ALT values are based on the presence of fatty liver on imaging tests. These tests are sub-optimal for detecting mild degrees of fatty liver, as opposed to histological examinations, which are considered the gold standard.

Normal ALT, currently defined as the value within the 95th percentile of the presumed healthy reference population, could include individuals with subclinical liver disease. Hence, healthy ALT levels may require a stricter definition to truly represent ‘health’. Consequently, healthy ALT values should be established using a population that has been metabolically and histologically verified as healthy. In a previous study, we proposed healthy ALT values of 33 U/L for males and 25 U/L for females, based on histologic confirmation of 665 Korean liver donors.[12]

With this background, we aimed to comprehensively explore and update the definition of healthy ALT levels, focusing on metabolically and histologically healthy Asian patients using a large liver donor database.

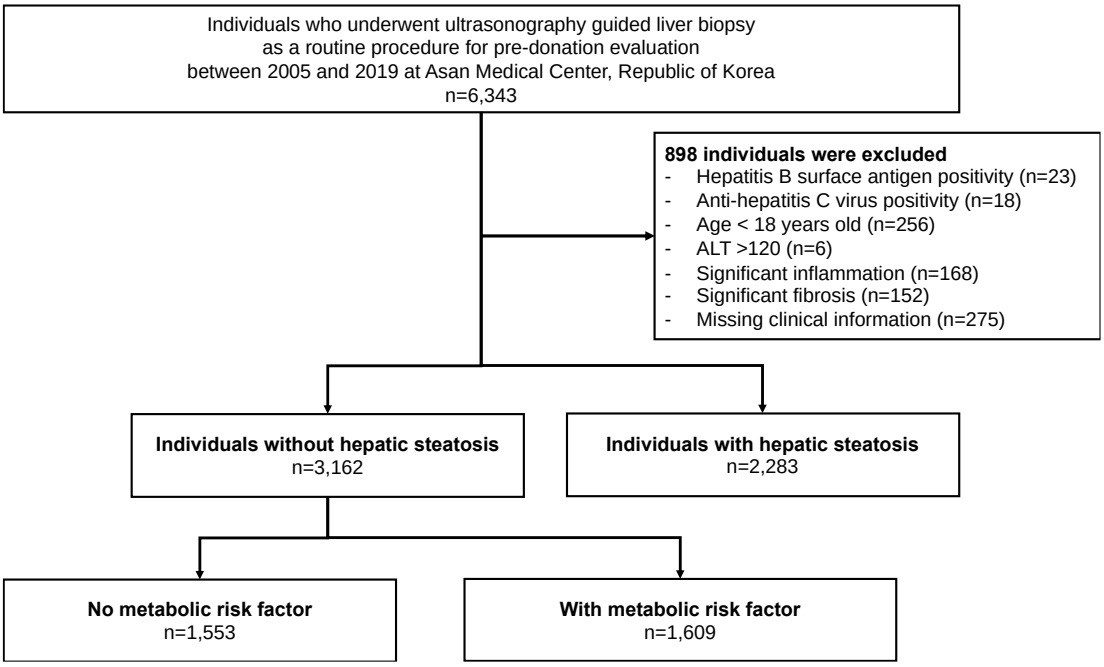
Patients and Methods

1. Study Design and Study Population

This study received approval from the Institutional Review Board (IRB) of Asan Medical Center (IRB No. 2023-0613). Due to the retrospective nature of the study, the need for informed consent was waived by the IRB.

We included a total of 6,343 consecutive voluntary potential living liver donors from the period between 2005 and 2019 as the source population for this study (Figure 1). Screening for alcohol use, hepatitis B surface antigen (HBsAg), anti-HCV, and antibody to HIV were conducted for all patients as a routine pre-operative evaluation for living donor liver transplantation at Asan Medical Center, Seoul, Republic of Korea. We excluded patients who met any of the subsequent criteria: age under 18 years old (n=256); HBsAg positive (n=23); anti-HCV positive (n=18); ALT > 120 U/L (n=6); significant inflammation (n=168), or significant fibrosis (n=152) on histologic examination; and those with missing clinical data (n=275, Figure 1).

Figure 1. Study flow



2. Clinical, biochemical, and histologic variables

Data were sourced from the electronic medical records available from the electronic database at Asan Medical Center. Baseline demographics gathered included age, sex, height, weight, body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP). Comprehensive biochemical tests were conducted on all potential liver donors, including hemoglobin, platelet, prothrombin time, aspartate aminotransferase (AST), ALT, alkaline phosphatase, total bilirubin, protein, albumin, creatinine, total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and fasting glucose. Ultrasound-guided percutaneous liver biopsy was routinely performed as part of the pre-donation evaluation after obtaining written informed consent. A more comprehensive methodology for liver biopsy has been previously described elsewhere.[12] The degree of total hepatic steatosis was also described separately as macrovesicular and microvesicular steatoses expressed as a percentage. Hepatic steatosis was histologically diagnosed when the macrovesicular fatty changes affected ≥5% of the biopsied liver parenchyma.

3. Study outcome and statistical analysis

We defined the healthy ALT thresholds at the 95th percentile, suitable for the distribution of a continuous variable in a presumed metabolically healthy population. We assessed metabolic risk with the modified Prati criteria (BMI <23 kg/m^2, TG ≤200 mg/dL, fasting glucose ≤105 mg/dL, TC ≤220 mg/dL) for comparative purposes.[6] For metabolic risk assessment, we also used the NCEP ATP III criteria, comprising: obesity determined by waist circumference, fasting glucose of ≥100 mg/dL, TG of ≥150 mg/dL, HDL of <40 mg/dL in males or <50 mg/dL in females, and SBP of >130 mmHg or DBP of >85 mmHg. Metabolic syndrome was defined as having at least three of these five criteria.[13] We used the BMI 23 kg/m^2 to define obesity instead of waist circumference because the data were not available for our participants.

The primary outcome assessed the upper reference limit of healthy ALT at the 95th percentile within the metabolically and histologically verified population. We also determined the upper reference limit of ALT based on the presence of metabolic risk factors among histologically normal populations. All results are presented as the mean ± standard

deviation, median (interquartile range [IQR]), or frequency with its corresponding proportion. The baseline characteristics between sexes was assessed using Student's *t*-test or chi-square test, as appropriate. Both univariate and multivariate linear regression analyses were employed to discern independent variables influencing ALT levels. For all statistical analyses, P-values of <0.05 were considered statistically significant, and all statistical analyses were conducted using R version 4.3.0 (https://www.r-project.org).

Results

1. Baseline characteristics of the study participants

The baseline characteristics of the included participants are described in Table 1. There was a predominance of males (3,607; 66.2%), with a median age of 30.0 years and BMI of 23.2 kg/m^2. The mean ALT in males and females was 22.4 and 14.3 U/L, respectively, while the median ALT was 19 in males and 13 U/L in females. Among all participants, 293 (5.4%) had ALT levels over 40 U/L. Hepatic steatosis was present in 2,283 (41.9%) participants.

Within the population, 2,052 (37.7%), 211 (3.9%), and 20 (0.4%) participants had steatosis grades of mild (5–33%), moderate (34–66%), and severe (>66%), respectively, while 3,162 (58.1%) showed no steatosis. Compared with participants with hepatic steatosis, those without were significantly younger and had a significantly lower BMI, SBP, DBP, TC, TG, fasting glucose, and ALT (Table 1). In the no hepatic steatosis group, 59.8% were male, with and median age of 28.0 years and median BMI of 22.3 kg/m^2. The median ALT in males and females was 16 and 12 U/L, respectively.

2. Metabolic risk factors among participants without hepatic steatosis

Of the 3,162 participants without hepatic steatosis, 2,072 (65.5%) met at least one of the five criteria by NCEP ATP-III. Metabolic syndrome, having more than 3 components of the five criteria, was present in 261 (8.3%) participants without hepatic steatosis. According to the modified Prati criteria, 1,553 (49.1%) participants were metabolically healthy, without any component of the modified Prati criteria. Comparison of baseline characteristics between participants without hepatic steatosis according to the Prati criteria are summarized in Table 2.

Table 1. Baseline characteristics of the study population

Characteristics	All participants				No hepatic steatosis			
	Total (n = 5,445)	**Male (n = 3,607)**	**Female (n= 1,838)**	**P-value**	**Total (n = 3,162)**	**Male (n = 1,891)**	**Female (n= 1,271)**	**P-value**
Demographic characteristics								
Age, years	30.0 [24.0;37.0]	28.0 [23.0;35.0]	33.0 [26.0;40.0]	<0.001	28.0 [23.0;36.0]	26.0 [22.0;33.0]	32.0 [25.0;39.0]	<0.001
Height, cm	170.0 [162.6;175.6]	173.8 [169.8;177.8]	160.4 [156.4;164.1]	<0.001	169.5 [161.7;175.2]	174.0 [170.0;178.0]	160.6 [156.7;164.5]	<0.001
Weight, kg	66.8 [58.7;75.0]	71.0 [64.4;78.0]	57.0 [52.0;63.0]	<0.001	63.2 [56.0;71.9]	68.9 [62.5;76.0]	55.1 [51.0;60.6]	<0.001
BMI, kg/m^3	23.2 [21.2;25.3]	23.6 [21.8;25.6]	22.1 [20.3;24.2]	<0.001	22.3 [20.5;24.2]	22.8 [21.0;24.8]	21.6 [19.8;23.5]	<0.001
SBP, mmHg	115.0 [106.0;126.0]	118.0 [109.0;128.5]	110.0 [102.0;120.0]	<0.001	113.0 [105.0;124.0]	117.0 [108.0;127.0]	110.0 [100.0;118.0]	<0.001
DBP, mmHg	74.0 [67.0;81.0]	75.0 [68.0;82.0]	72.0 [65.0;79.0]	<0.001	73.0 [66.0;80.0]	74.0 [67.0;81.0]	71.0 [64.0;78.0]	<0.001
Laboratory findings								
WBC, $\times 1000/mm^3$	6.2 [5.2;7.3]	6.3 [5.3;7.5]	6.0 [5.0;7.1]	<0.001	6.0 [5.1;7.2]	6.1 [5.2;7.3]	5.8 [4.9;7.0]	<0.001
Hemoglobin, g/dL	14.8 [13.5;15.7]	15.4 [14.8;16.0]	13.0 [12.4;13.6]	<0.001	14.6 [13.2;15.5]	15.3 [14.7;15.9]	12.9 [12.4;13.5]	<0.001
Platelet, $\times 1000/mm^3$	245.0 [214.0;279.0]	241.0 [210.0;272.0]	253.5 [219.0;291.0]	<0.001	243.0 [212.0;275.0]	238.0 [208.5;269.0]	248.0 [217.0;283.5]	<0.001
AST, IU/L	19.0 [16.0;23.0]	20.0 [17.0;24.0]	18.0 [15.0;20.0]	<0.001	18.0 [16.0;21.0]	19.0 [16.0;23.0]	17.0 [15.0;20.0]	<0.001
ALT, IU/L	16.0 [12.0;24.0]	19.0 [14.0;27.0]	13.0 [10.0;16.0]	<0.001	14.0 [11.0;19.0]	16.0 [13.0;22.0]	12.0 [9.0;15.0]	<0.001
ALP, IU/L	61.0 [51.0;73.0]	65.0 [55.0;77.0]	52.0 [44.0;62.0]	<0.001	59.0 [49.0;72.0]	65.0 [55.0;77.0]	52.0 [44.0;61.0]	<0.001
Protein, g/dL	7.2 [6.9;7.5]	7.2 [6.9;7.5]	7.2 [6.9;7.5]	0.394	7.2 [6.9;7.5]	7.2 [6.9;7.4]	7.2 [6.9;7.5]	0.976
Albumin, g/dL	4.3 [4.1;4.5]	4.4 [4.2;4.6]	4.2 [4.0;4.4]	<0.001	4.3 [4.1;4.5]	4.4 [4.2;4.6]	4.2 [4.0;4.4]	<0.001
PT, INR	1.0 [1.0;1.0]	1.0 [0.9;1.0]	1.0 [1.0;1.0]	<0.001	1.0 [1.0;1.1]	1.0 [1.0;1.1]	1.0 [1.0;1.1]	0.041
Total bilirubin, mg/dL	0.8 [0.6;1.1]	0.9 [0.7;1.1]	0.7 [0.6;1.0]	<0.001	0.8 [0.6;1.0]	0.9 [0.7;1.1]	0.7 [0.6;0.9]	<0.001
Creatine, mg/dL	0.8 [0.7;0.9]	0.9 [0.8;1.0]	0.7 [0.6;0.7]	<0.001	0.8 [0.7;0.9]	0.9 [0.8;1.0]	0.7 [0.6;0.7]	<0.001
Calcium, mg/dL	9.3 [9.0;9.6]	9.4 [9.1;9.6]	9.2 [8.9;9.4]	<0.001	9.3 [9.0;9.6]	9.4 [9.1;9.6]	9.2 [8.9;9.4]	<0.001
Phosphorus, mg/dL	3.8 [3.4;4.1]	3.7 [3.4;4.1]	3.8 [3.5;4.1]	<0.001	3.8 [3.4;4.1]	3.8 [3.4;4.1]	3.8 [3.5;4.1]	0.010
Cholesterol, mg/dL	171.0 [151.0;194.0]	172.0 [151.0;196.0]	171.0 [152.0;193.0]	0.558	167.0 [148.0;188.0]	164.0 [146.0;187.0]	170.0 [150.5;190.0]	0.004
HDL-C, mg/dL	51.0 [43.0;60.0]	49.0 [41.0;57.0]	57.0 [47.0;66.0]	<0.001	54.0 [46.0;64.0]	51.0 [44.0;60.0]	59.0 [50.0;69.0]	<0.001
Triglyceride, mg/dL	83.0 [58.0;123.0]	92.0 [63.0;138.0]	70.0 [50.0;99.0]	<0.001	73.0 [53.0;103.0]	79.0 [58.0;113.0]	65.0 [48.0;90.0]	<0.001
Fasting glucose, mg/dL	93.0 [87.0;100.0]	94.0 [88.0;101.0]	92.0 [86.0;99.0]	<0.001	92.0 [87.0;99.0]	93.0 [87.0;99.0]	92.0 [86.0;98.0]	<0.001

Table 2. Comparison of baseline characteristics of participants with and without metabolic risk factors

	No steatosis but outside Prati criteria				No steatosis within Prati criteria			
Characteristics	**Total**	**Male**	**Female**	**P-value**	**Total**	**Male**	**Female**	**P-value**
Demographic characteristics								
Age, years	29.0 [24.0;37.0]	27.0 [23.0;34.0]	34.0 [27.0;42.0]	<0.001	27.0 [22.0;35.0]	25.0 [21.0;31.0]	31.0 [25.0;38.0]	<0.001
Height, cm	170.5 [162.5;176.1]	174.1 [170.0;178.0]	160.0 [156.1;163.8]	<0.001	168.0 [161.3;174.4]	174.0 [170.1;177.9]	161.1 [157.1;164.9]	<0.001
Weight, kg	70.5 [62.0;77.5]	74.2 [68.8;80.1]	60.7 [56.0;66.5]	<0.001	58.0 [52.7;63.7]	63.3 [59.2;67.5]	53.0 [49.2;56.4]	<0.001
BMI, kg/m^3	24.2 [23.2;25.7]	24.5 [23.3;25.9]	23.8 [22.4;25.3]	<0.001	20.8 [19.5;21.9]	21.0 [19.9;22.1]	20.5 [19.1;21.7]	<0.001
SBP, mmHg	116.0 [107.0;127.0]	119.0 [110.0;129.5]	110.0 [103.0;120.0]	<0.001	111.0 [102.0;121.0]	114.0 [106.0;123.0]	109.0 [100.0;117.0]	<0.001
DBP, mmHg	74.0 [68.0;81.0]	75.0 [69.0;82.0]	71.0 [65.0;79.0]	<0.001	72.0 [65.0;79.0]	73.0 [66.0;80.0]	71.0 [63.0;78.0]	0.001
Laboratory findings								
WBC, $\times 1000/mm^3$	6.1 [5.2;7.3]	6.2 [5.3;7.5]	6.0 [5.1;7.1]	0.001	5.8 [4.9;6.9]	5.9 [5.1;7.1]	5.8 [4.8;6.8]	0.003
Hemoglobin, g/dL	14.8 [13.5;15.7]	15.3 [14.7;16.0]	12.9 [12.3;13.6]	<0.001	14.2 [13.0;15.4]	15.3 [14.7;15.9]	13.0 [12.4;13.5]	<0.001
Platelet, $\times 1000/mm^3$	244.0 [214.0;280.0]	239.0 [211.0;273.0]	257.0 [224.0;296.0]	<0.001	241.0 [209.0;270.0]	236.0 [205.0;266.0]	245.0 [213.0;276.0]	0.001
AST, IU/L	19.0 [16.0;22.0]	20.0 [17.0;23.0]	18.0 [15.0;21.0]	<0.001	18.0 [15.0;21.0]	19.0 [16.0;22.0]	17.0 [15.0;19.0]	<0.001
ALT, IU/L	16.0 [12.0;21.0]	18.0 [14.0;24.0]	13.0 [10.0;16.0]	<0.001	13.0 [10.0;17.0]	15.0 [11.0;20.0]	11.0 [9.0;14.0]	<0.001
ALP, IU/L	61.0 [51.0;74.0]	65.0 [55.0;77.0]	54.0 [45.0;64.0]	<0.001	58.0 [48.0;70.0]	65.0 [55.0;77.0]	50.0 [43.0;59.0]	<0.001
Protein, g/dL	7.2 [6.9;7.5]	7.2 [6.9;7.4]	7.2 [6.9;7.5]	0.444	7.2 [6.9;7.4]	7.2 [6.9;7.4]	7.1 [6.9;7.5]	0.534
Albumin, g/dL	4.3 [4.1;4.5]	4.4 [4.2;4.6]	4.2 [4.0;4.4]	<0.001	4.3 [4.1;4.5]	4.4 [4.2;4.6]	4.2 [4.1;4.4]	<0.001
PT, INR	1.0 [1.0;1.0]	1.0 [1.0;1.0]	1.0 [1.0;1.0]	0.610	1.0 [1.0;1.1]	1.0 [1.0;1.1]	1.0 [1.0;1.1]	0.202
Total bilirubin, mg/dL	0.8 [0.6;1.0]	0.8 [0.6;1.1]	0.7 [0.5;0.9]	<0.001	0.8 [0.6;1.1]	0.9 [0.7;1.1]	0.8 [0.6;1.0]	<0.001
Creatine, mg/dL	0.8 [0.7;1.0]	0.9 [0.8;1.0]	0.7 [0.6;0.7]	<0.001	0.8 [0.7;0.9]	0.9 [0.8;1.0]	0.7 [0.6;0.7]	<0.001
Calcium, mg/dL	9.3 [9.0;9.6]	9.4 [9.1;9.6]	9.2 [8.9;9.4]	<0.001	9.2 [9.0;9.5]	9.3 [9.0;9.6]	9.2 [8.9;9.4]	<0.001
Phosphorus, mg/dL	3.7 [3.4;4.1]	3.7 [3.4;4.1]	3.8 [3.4;4.1]	0.368	3.8 [3.5;4.1]	3.8 [3.5;4.1]	3.8 [3.6;4.1]	0.086
Cholesterol, mg/dL	173.0 [153.0;198.0]	171.0 [151.5;194.5]	177.0 [158.0;206.0]	<0.001	161.0 [144.0;180.0]	158.0 [141.0;176.0]	165.0 [148.0;183.0]	<0.001
HDL-C, mg/dL	53.0 [44.0;62.0]	50.0 [43.0;59.5]	57.0 [49.0;67.0]	<0.001	56.0 [47.0;65.0]	53.0 [45.5;61.0]	60.0 [51.0;70.0]	<0.001
Triglyceride, mg/dL	81.0 [59.0;118.0]	86.0 [61.0;126.0]	74.0 [53.0;104.0]	<0.001	66.0 [49.0;90.0]	72.0 [54.0;99.0]	61.0 [46.0;82.0]	<0.001
Fasting glucose, mg/dL	95.0 [88.0;105.0]	95.0 [88.0;104.0]	95.0 [88.0;105.0]	0.905	91.0 [85.0;96.0]	91.0 [86.0;96.0]	90.0 [84.0;95.0]	0.008

3. Association between ALT and baseline characteristics among all participants

Age, BMI, TC, HDL, and TG were independently associated with ALT levels in both sexes in the univariate analysis (Figures 2A–2F). The multivariable linear regression analysis of the whole study population revealed that TC, HDL, TG, and BMI were independently associated with ALT values in males, and age, HDL, TG, fasting glucose, and BMI were significantly associated with ALT values in females (Table 3). Among the 3,162 participants without hepatic steatosis, TC, HDL, and BMI were associated with ALT level in males, while age, fasting glucose, and BMI were independently associated with ALT level in females within the multivariable analysis (Table 3).

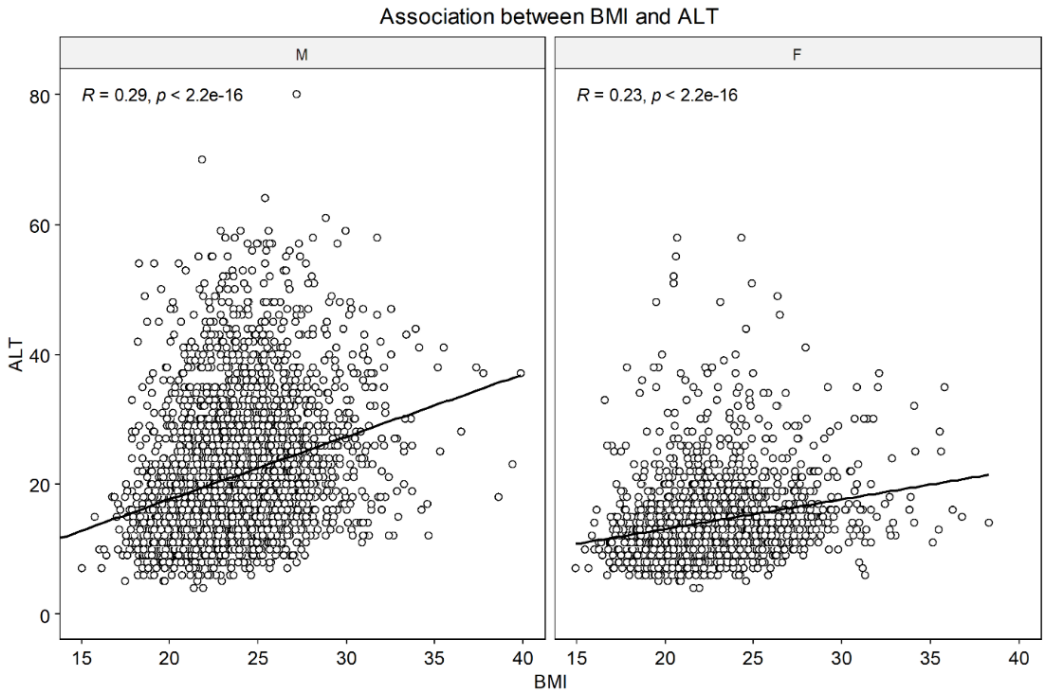
Table 3. Multivariable linear regression analysis by sex among all participants and participants without hepatic steatosis

	Male		**Female**	
	Slope (95% CI)	**P-value**	**Slope (95% CI)**	**P-value**
All participants				
Age			0.068 (0.033–0.103)	<0.001
BMI	0.754 (0.622–0.886)	<0.001	0.235 (0.135–0.336)	<0.001
Total cholesterol	0.069 (0.056-0.081)	<0.001		
HDL	-0.114 (-0.147–-	<0.001	-0.026 (-0.049–-0.002)	0.032
Triglyceride	0.012 (0.007–0.017)	<0.001	0.015 (0.009–0.022)	<0.001
Fasting glucose			0.021 (0.003–0.039)	0.019
Participants without hepatic steatosis				
Age			0.056 (0.021–0.091)	0.002
BMI	0.537 (0.371–0.702)	<0.001	0.178 (0.069–0.286)	0.001
Total cholesterol	0.060 (0.044–0.076)	<0.001		
HDL	-0.044 (-0.082–-0.007)	0.021		
Triglyceride	0.009 (0.000–0.017)	0.055		
Fasting glucose			0.019 (0.000–0.038)	0.049

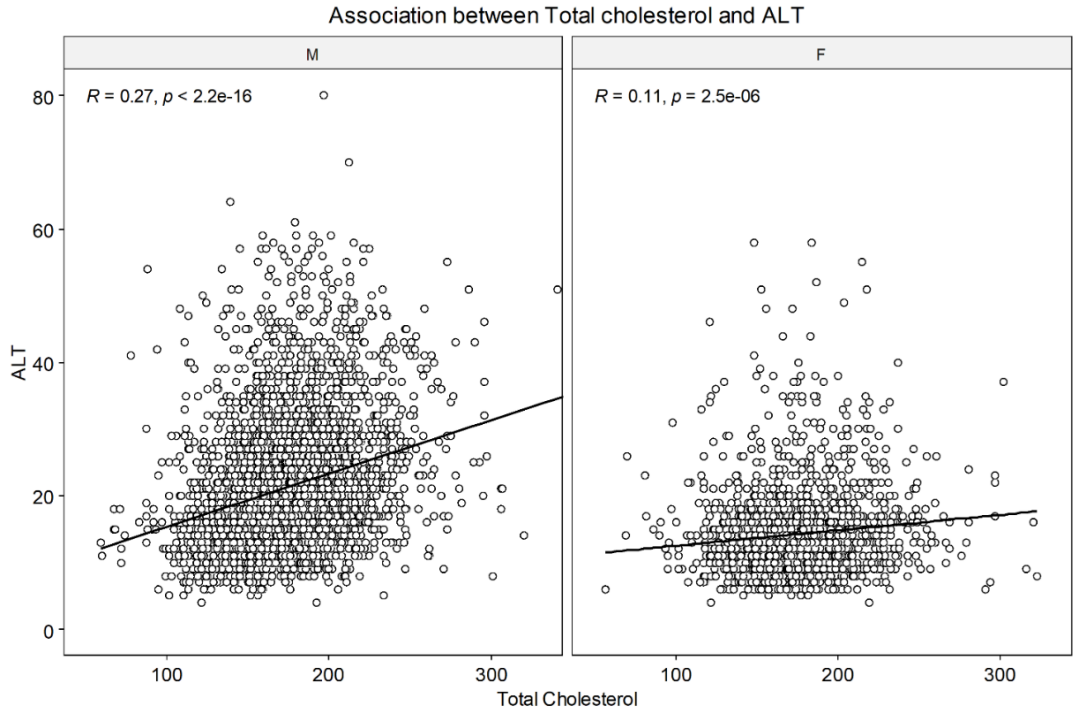
Figure 2. Association between baseline characteristics and ALT level.

A. Age

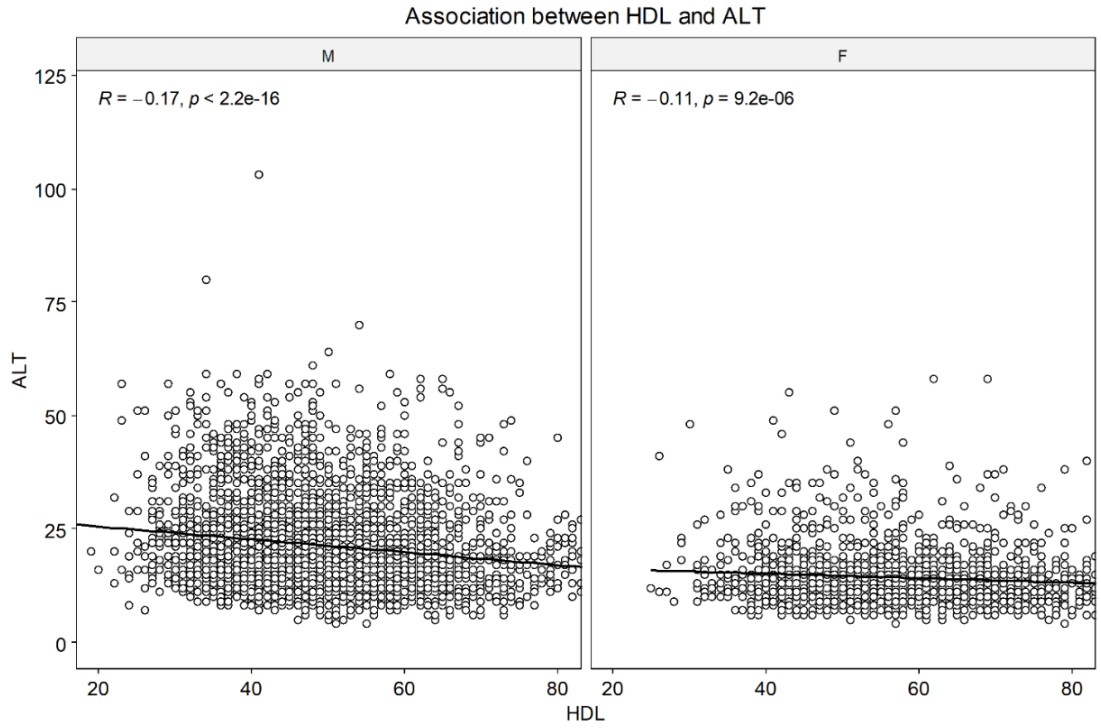
B. Body mass index



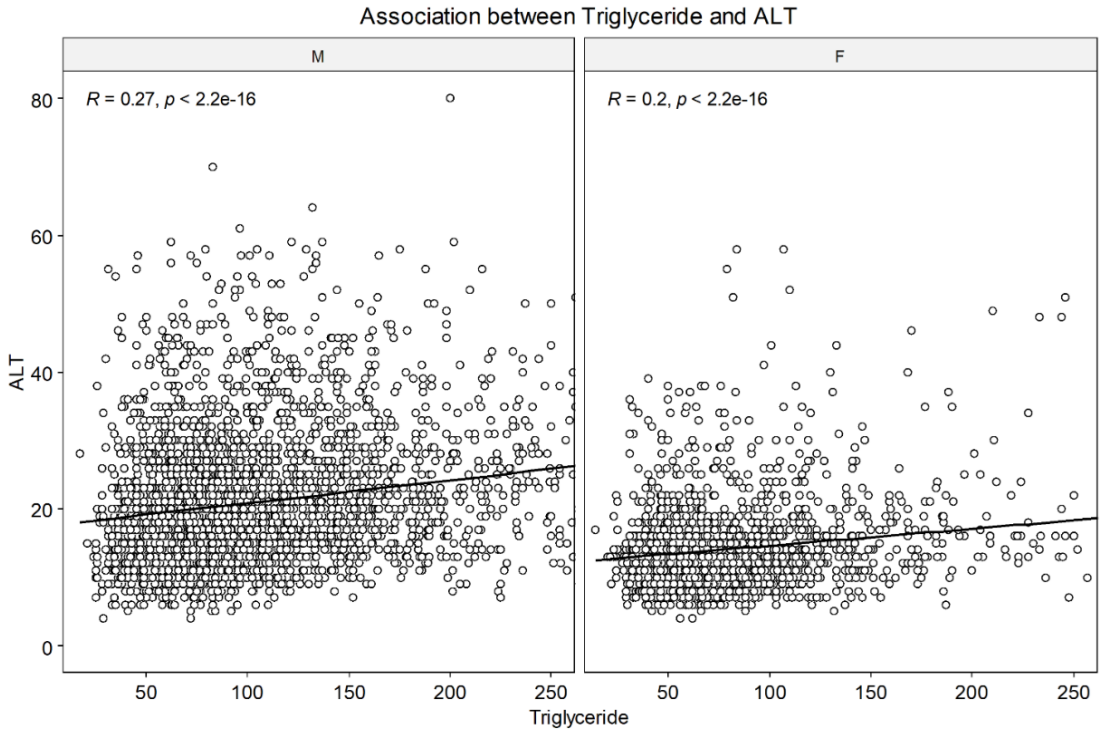
C. Total cholesterol



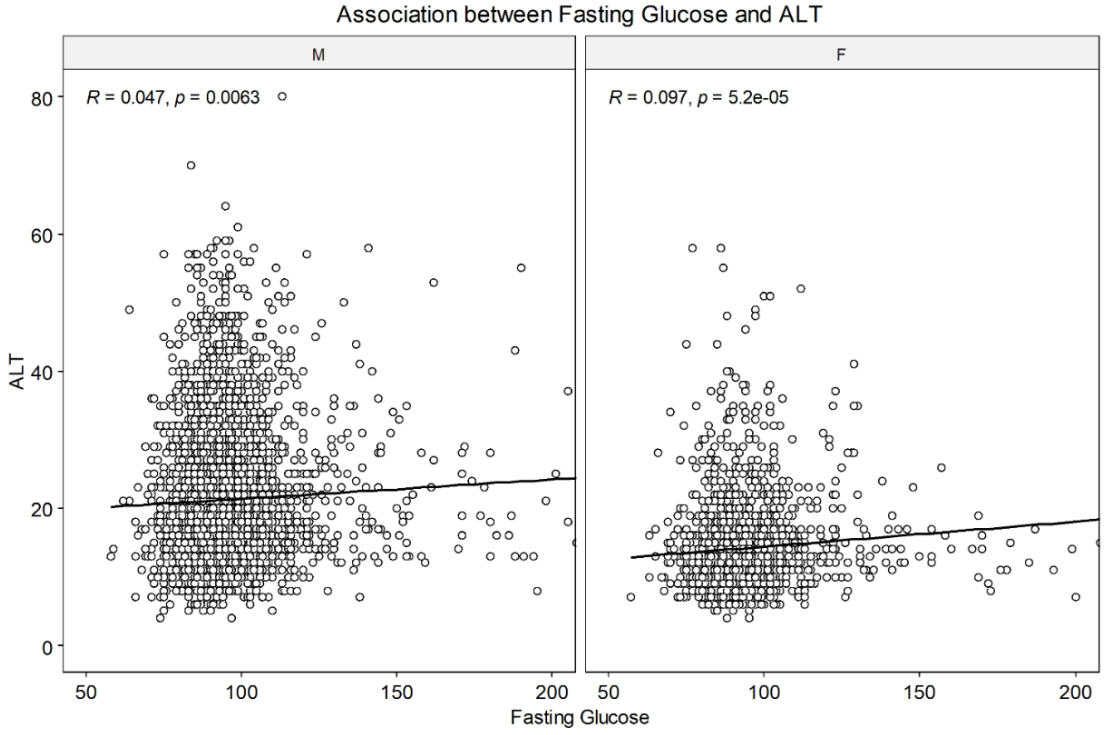
D. High-density lipoprotein



E. Triglyceride



F. Fasting glucose



4. Upper reference limit of ALT in participants without hepatic steatosis

Of the 3,162 participants without hepatic steatosis, the median ALT and the 50th, 75th, 90th, and 95th percentiles were 16, 22, 30, and 36 U/L, respectively, in males and 12, 15, 20, and 24 U/L, respectively, in females (Table 4). Of the 1,553 participants without hepatic steatosis deemed metabolically healthy by the Prati criteria, the median ALT and the 50th, 75th, 90th, and 95th percentiles were 15, 20, 27, and 34 U/L, respectively, in males and 11, 14, 18, and 22 U/L, respectively, in females. Of the 2,892 participants without hepatic steatosis and metabolic syndrome according to the ATP-III criteria, the median ALT and the 50th, 75th, 90th, and 95th percentiles were 16, 22, 30, and 36 U/L, respectively, in males and 12, 15, 19, and 24 U/L, respectively, in females (Table 4).

Table 4. Upper reference limits of alanine aminotransferase level according to the hepatic steatosis and metabolic risk factors

	Mean	**Upper 50%**	**Upper 75%**	**Upper 90%**	**Upper 95%**
No hepatic steatosis + no metabolic risk factors per Prati criteria (n = 1,553)					
Male (n = 815)	16.8	15	20	27	34
Female (n = 738)	12.4	11	14	18	22
No hepatic steatosis + no metabolic syndrome per ATP-III criteria (n = 2,892)					
Male (n = 1,686)	18.7	16	22	30	36
Female (n = 1,206)	13.0	12	15	19	24
No hepatic steatosis only (n = 3,162)					
Male (n = 1,891)	19.0	16	22	30	36
Female (n = 1,271)	13.1	12	15	20	24
No hepatic steatosis + outside the Prati criteria (n = 1,609)					
Male (n = 1,076)	20.4	18	24	32	39
Female (n = 533)	14.1	13	16	21	25

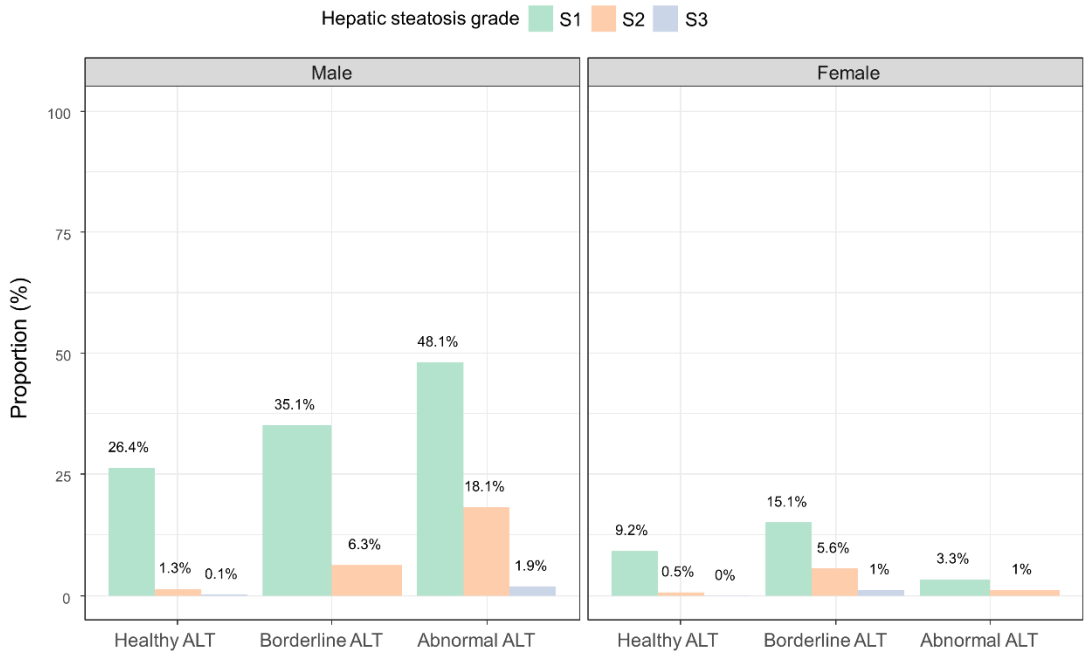
5. Metabolic risk factors and hepatic steatosis per the updated healthy ALT values

We defined 'healthy' ALT as <34 U/L in males and <22 U/L in females based on the 95th percentiles of ALT among participants without hepatic steatosis meeting the Prati criteria. Subsequently, we defined 'borderline' ALT as 34–40 U/L in males and 22–40 U/L in females to assess the distribution of metabolic risk factors and hepatic steatosis. Among the entire population, healthy, borderline, and abnormal ALT was present in 4,743 (87.0%), 409 (7.5%), and 293 (5.4%) of participants, respectively.

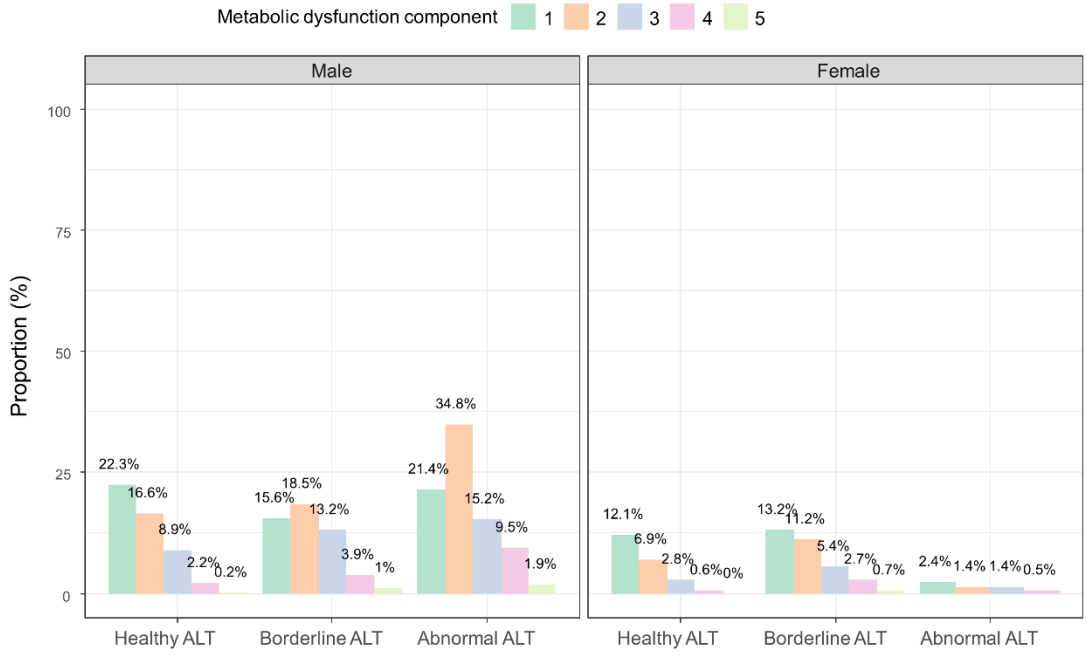
Additionally, 27.8.5%, 41.4%, and 68.1% of male, and 9.7%, 21.7%, and 4.3% of female hepatic steatosis participants had healthy, borderline, and abnormal ALT, respectively (Figure 3A). Moreover, 11.2%, 18.0%, and 26.7% of male and 3.5%, 8.8%, and 1.9% of female participants with metabolic syndrome per the ATP-III criteria had healthy, borderline, and abnormal ALT (Figure 3B).

Figure 3. Prevalence of hepatic steatosis and metabolic syndrome according to the new definition of ALT

A. Hepatic steatosis



B. Metabolic dysfunction components



Discussion

Our study demonstrates that the true healthy ULN levels without identifiable risk factors for liver disease were 34 U/L in males and 22 U/L in females, which are lower than the traditional levels. Across both sexes, BMI, HDL, and TG were significantly associated with ALT levels; however, BMI was the only significant factor among participants without hepatic steatosis. Proportions of hepatic steatosis and metabolic syndrome significantly increased in participants with borderline ALT (34–40 U/L for males and 22–40 U/L for females), and further increased in those with abnormal ALT (>40 U/L for both sexes), compared with participants with healthy ALT (<34 U/L for males and <22 U/L for females).

ALT, which is more specific than AST in assessing hepatocellular injury, is frequently included in comprehensive metabolic profiles and serves as a gatekeeper to identify liver disease. Traditionally, the ULN of ALT is set at 40 U/L, irrespective of sex, with slight variation among laboratories. However, ongoing concerns have led to various efforts to establish an updated normal ULN of ALT. The current ULN was established half a century ago when HCV testing was not routinely conducted and fatty liver disease was not considered a common chronic liver disease. Previous studies have suggested that normal ALT was lower than commonly reported reference ranges and differed by sex.[14] A study by Prati et al. proposed the normal ULN of ALT of 30 U/L for males and 19 U/L for females using a large-scale cohort of blood donors.[6] We also previously proposed an ULN of 33 U/L for males and 25 U/L for females in living liver donors with normal liver histology.[12]Another population-based study using the National Health and Nutrition Examination Survey database proposed an ULN of 29 U/L for males and 22 U/L for females.[15]

Determining the reference population to estimate the ULN of ALT is crucial. If the reference population varies in characteristics potentially associated with ALT values, such as sex, age, and BMI, the reference value of ULN of ALT may differ. Previous studies demonstrated that ALT levels correlated with increasing BMI, as observed in our study.[16,17] To define the healthy reference population, participants with abnormal values of variables significantly associated with ALT levels should be excluded.[14] In this regard, participants with hepatic steatosis, viral hepatitis, or other chronic liver disease, given the prevalence, are

not eligible to be considered a healthy reference population. Therefore, we assessed the ULN of ALT among those who were confirmed to not have hepatic steatosis, and who met the strict criteria of metabolic dysfunction components. In this regard, selected patients in our study may be regarded as metabolically and histologically proven healthy reference populations.

Another important aspect of our findings is that sex differences in the ULN of ALT should be considered, as has been reported in the literature.[6,12,15,18] The traditional ULN of ALT has been identical for both sexes without reasonable cause, despite a tendency to be lower in females than in males. Factors associated with ALT levels differ between males and females. Notably, TC, HDL, and TG were associated with ALT levels in males but not in females among those without hepatic steatosis. Age was also associated with ALT levels in females but not in males according to our findings. This implies that the ALT levels may be differently influenced by these factors according to sex. Therefore, it is imperative to establish the ULN according to distinctions in sex.

Opposition to lowering the ULN of ALT remains given the increased costs associated with potentially superfluous use of testing and medical resources, while reducing the blood donation pool.[14] However, several studies have demonstrated that increasing ALT levels, despite being within the traditional normal range, were associated with increased mortality, especially liver-related mortality.[8,15,19] A study from Korea reported that individuals with ALT between 30–39 U/L had a 9.5 times the relative risk for liver-related death compared with those with ALT <20 U/L.[8] Moreover, a previous study demonstrated that despite ALT levels within the normal change, the risk of metabolic syndrome increased as the ALT levels increased.[10] This suggests that upper normal ALT based on the traditional normal range is not clinically insignificant.

Therefore, we propose a new category of ‘borderline’ ALT, which is beyond the new healthy ULN of ALT, but belongs to the traditional normal ULN. Notably, the proportion of participants with hepatic steatosis incrementally increased among those with healthy ALT, borderline ALT, and abnormal ALT in our study. Additionally, only 1.6% of male participants with healthy ALT had moderate-to-severe steatosis, whereas 7.3% and 23.5% of male participants with borderline and abnormal ALT had moderate and severe steatosis, respectively. For females, a similar trend was observed except for the abnormal ALT group,

likely attributed to the small number of participants (n = 13).

Our study has several strengths. We determined a healthy reference population with strict criteria, excluding participants with common chronic liver disease with serologic testing and metabolic dysfunction. We also analyzed more than 5,000 living liver donors with liver biopsy. Identifying hepatic steatosis by current imaging tests such as ultrasonography or computed tomography is suboptimal if the degree of steatosis is mild. Regardless, liver biopsy is considered the gold standard for the diagnosing hepatic steatosis. We confirmed the presence of hepatic steatosis in our study population based on these biopsy findings, allowing us to confirm and exclude subjects with a mild degree of hepatic steatosis, which may not easily be diagnosed in non-invasive imaging studies.

However, there are also several limitations to our study. The study population only included Korean participants, so we cannot generalize the differences in ULN of ALT to different ethnicity. In addition, we could not evaluate the possible association between the newly proposed ULN of ALT and mortality, especially liver-related mortality. However, this was beyond the scope of our study. Moreover, the age of participants had a skewed distribution, given that living liver donors are usually young.

In conclusion, we estimate the ULN of ALT in metabolically and histologically healthy Asian participants to be 34 in males and 22 in females, which is lower than the traditionally accepted values. Lowering the ULN of ALT should be carefully considered based on our findings in addition to the newly proposed category of borderline ALT to minimize confusion of radical changes.

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국문요약

배경: 이 연구에서는 전통적으로 40 U/L 으로 간주되었던 알라닌 아미노전이효소(ALT) 수치의 정상 상한치를 아시아인 생체 간이식 공여자들의 조직학적 그리고 대사적 척도들을 바탕으로 재평가하였다.

연구방법: 2006 년부터 2019 년까지 서울아산병원의 잠재적 생체 간이식 공여자 5,455 명을 대상으로 후향적 분석을 수행하였다. 모든 환자들의 B 형간염, C 형간염, 인간면역결핍바이러스, 음주력 여부가 평가되었다. 대사적 및 조직학적 정상 참가자들이 modified Prati criteria (체질량지수 <23 kg/m^2, 중성지방 ≤200 mg/dL, 공복혈당 ≤105 mg/dL, 총콜레스테롤 ≤220 mg/dL)를 이용하여 평가되었다. 건강한 ALT 정상 상한치의 새로운 기준은 지방간이나 대사적 기능이상이 없는 참가자들의 95th percentile 값으로 정의하였다.

결과: 코호트 연령의 중간값은 30 세였으며 성별은 남성이 66.2%로 우세하였다. 모든 참가자들 중 3,162 명(58.0%)은 지방간이 없었고 이중 1,553 명(49.1%)은 modified Prati criteria 를 만족하여 대사적으로 건강하였다. 이 1,553 명에서 도출한 건강한 ALT 정상 상한치는 각각 남성에서 34U/L, 여성에서 22U/L 로 모두 통상적으로 사용되어왔던 기준인 40U/L 보다 유의미하게 낮았다. 지방간 또는 대사장애 위험이 있는 참가자들을 평가하기 위해 '경계성' ALT 범주(남성에서 34-40U/L, 여성에서 22-40U/L)가 새롭게 도입되었다.

결론: 전통적인 ALT 정상 상한치는 대사적 및 조직학적으로 검증된 아시아 인구에서 새롭게 도출한 건강한 ALT 정상 상한치에 비해 높았다. 이 연구에서 제시하는 ALT 정상 상한치는 각각 남성에서 34U/L, 여성에서 22U/L 이다. '경계성' ALT 범주의 도입은 간질환 및 대사질환 위험군의 분류에 도움이 되었으며 이는 ALT 정상 상한치의 개정 필요성을 강조하고 있다.

중심단어: 알라닌 아미노전이효소; 지방간; 정상 상한치