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Baseline keratin-18 level and risk of anti-tuberculosis drug-induced liver injury: An individual matched case-control study

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Abstract: Anti-tuberculosis drug-induced liver injury (ATLI) presents a grand challenge to the global control of tuberculosis. Serum keratin-18 may have a certain predictive value for the occurrence of liver injury. The purpose of this study is to examine the correlation between baseline serum keratin-18 levels and ATLI risk among the eastern Chinese Han population. Employing a 1:2 individual matched case-control approach, the study encompassed 88 ATLI cases and 176 controls. Univariate and multivariate conditional logistic regression analyses were performed to evaluate the association between baseline keratin-18 levels and ATLI risk. Furthermore, area under the curve (AUC) was used to assess keratin-18's efficacy in distinguishing ATLI cases from controls. In ATLI cases, baseline keratin-18 levels were significantly lower than controls (188.8 vs. 234.9 ng/L, P = 0.044), with higher levels associated with reduced ATLI risk (OR = 0.995, 95% CI: 0.992-0.999, P = 0.005). Under the optimal keratin-18 cut-off value of 191.6 ng/L, the AUCs were equal to 0.577 (95% CI: 0.513-0.640, P = 0.018) in univariate analysis and 0.597 (95% CI: 0.524-0.670, P = 0.010) in multivariate analysis. The present study indicated that the higher the baseline keratin-18 concentration in tuberculosis patients of eastern Chinese Han population, the lower the risk of ATLI.

Keywords: Anti-tuberculosis drug-induced liver injury; keratin-18; individual matched case-control study

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INTRODUCTION

Tuberculosis (TB), resulting from Mycobacterium tuberculosis infection, is a leading global cause of mortality (Furin *et al.*, 2019). The 2022 Global Tuberculosis Report states that around 10.6 million people infected with TB in 2021, leading to 1.6 million deaths globally (Bagcchi, 2023). China ranked third globally in terms of newly diagnosed cases in 2021, with approximately 784,400 new cases and an incidence rate of 54 per 100,000 people(Bagcchi, 2023). The most common and effective anti-TB medicines are a combination of rifampicin (RIF), isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZA), in the initial two months of treatment, and a combination of INH and RIF in the residual four months(Suárez *et al.*, 2019). Despite its extensive use and a success rate of 86% globally in 2020, this multi-drug combination therapy could cause a variety of adverse drug effects, the most common of which is anti-tuberculosis drug-induced liver injury (ATLI) (Bagcchi, 2023; Lewis *et al.*, 2024). With an estimated incidence rate of 2%-28%, ATLI can bring about prolonged hospitalization and discontinuation of anti-TB drugs, thus leading to the exacerbation or relapse of TB and increasing the risk of drug resistance(Tostmann *et al.*, 2008). Therefore, early detection and prevention of ATLI are pivotal for effective TB control.

Up to now, the liver function test is a helpful tool to detect hepatic dysfunction with aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (TBil) and alkaline phosphatase (ALP), and have long been employed for decades to early ATLI recognition (Clinton *et al.*, 2021). However, abnormal liver function test indicators do not necessarily indicate the onset of ATLI, as these biomarkers lack specificity for ATLI and can be influenced by various clinical conditions unrelated to hepatic injury (Clinton *et al.*, 2021). A recent study identified some new candidate biomarkers such as cytoplasmic aconitate hydratase, argininosuccinate synthase, fumarylacetoacetase by tandem mass spectrometry-tagged quantitative proteomics(Ravindra *et al.*, 2023). In addition, several new biomarkers, such as high mobility group box-1 (HMGB1)(Cheng *et al.*, 2022), microRNA-122 (miR-122) (Howell *et al.*, 2018), glutamate dehydrogenase (GLDH) (Xiang *et al.*, 2023) and keratin-18(Rupprechter *et al.*, 2021), have also gradually entered the public view, with potential clinical application value. It is believed that the application of these new biomarkers can effectively reduce the uncertainty in the diagnosis of drug-induced liver injury

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(DILI), even make accurate predictions of ATLI. However, these new biomarkers still need a lot of verification in different populations to determine their diagnostic value. Among these potential biomarkers, keratin-18, also known as KRT18, is a type-I intermediate filament protein that is highly enriched in parenchymal cells (hepatocytes and bile duct cells), accounting for 5% of total liver protein (Korver *et al.*, 2021). As a structural protein that makes up the cytoskeleton, keratin-18 maintains the integrity of cellular and tissue structures and is able to regulate cell division, migration, differentiation, apoptosis and necrosis (Wang P. *et al.*, 2021). The stability of keratin is largely attributed to its structural characteristics, including alpha-helical structure, disulfide bonds and hydrogen bonds, which together provides essential elasticity, mechanical strength, chemical stability and stability of three-dimensional structure (Wang B. *et al.,* 2016). In the liver, keratin-18 is usually co-expressed with keratin-8 (a type-II intermediate filament protein) to form a keratin-8/ keratin-18 heterodimer, which has the function of protecting hepatocytes from apoptosis and necrosis.

Previous studies have suggested that serum keratin-18 during treatment shows promise as a prognostic biomarker in DILI. Through two human DILI cohorts, one study revealed that the intact full-length form of keratin-18 during treatment is a potential biomarker for DILI superior to ALT regarding sensitivity and specificity (Thulin *et al.*, 2014). Another study suggested that keratin-18 phosphorylation serves as a reliable biomarker for liver-related disease getting better or worse (Tamber *et al.*, 2023).

As a structural protein of liver epithelial cells, the level of keratin-18 is usually increased during liver injury (Thulin *et al.*, 2014), and compared to standard liver function tests and other biomarkers, keratin-18 exhibits greater liver-specificity and early-stage predictive ability for DILI in some patients who overdose on paracetamol (Dear *et al.*, 2018; Tajima *et al.*, 2019). Recently, one British study suggested a correlation between keratin-18 and ALT, which increased in patients experiencing ATLI during anti-TB therapy, while keratin-18 showed a higher level in healthy patients at baseline(Rupprechter *et al.*, 2021). It is worth studying whether this biomarker could be used to predict ATLI. Consequently, the purpose of our case-control study is to examine the correlation between baseline keratin-18 levels and the onset of ATLI, and explore the efficiency of keratin-18 in distinguishing ATLI patients from controls as an early biomarker in Chinese peoples.

MATERIALS AND METHODS

Subjects

The study was approved by the Ethics Committee of Nanjing Medical University (No. [2018]579) and conducted in accordance with the Declaration of Helsinki principles. All the study subjects were TB patients from four designated anti-TB treatment hospitals, spanning from March 2020 to October 2022 in Jiangsu Province. Patients who fulfilled the following criteria were incorporated: (1) newly diagnosed TB patients; (2) received standard short-course anti-TB chemotherapy; (3) willing to join this study; (4) available baseline serum for testing. Patients presenting any of the subsequent conditions were ineligible and therefore excluded: (1) psychiatric patients; (2) patients with one or more abnormalities in serum liver function indexes prior to initiating anti-TB treatment; (3) patients taking other hepatotoxic drugs at the same time; (4) patients with acquired immunodeficiency syndrome (AIDS), syphilis or other infectious diseases; (5) serious diseases, such as tumors.

ATLI case and control definition

ATLI was delineated based on the subsequent criteria: (1) ALT ≥ five times upper limit of normal (ULN); (2) ALP ≥ two times ULN; (3) ALT ≥ three times ULN and TBil ≥ two times ULN. Additionally, causal assessment was performed on each patient with potential liver injury as per the updated Roussel Uclaf Causality Assessment Method (the updated RUCAM). In this study, patients with a score higher than three points were judged as ATLI case (the updated RUCAM total score ranged from -9 to 14)(Kobayashi *et al.*, 2023). The types of liver injury in patients with elevated serum liver function indexes were assessed, and three patterns (hepatocellular, mixed or cholestatic liver injury) were defined based on the R ratio (Yu *et al.*, 2017). R ratios exceeding 5 characterized hepatocellular injury, while below 2 indicated cholestatic injury and R ratios between 2 and 5 suggested a mixed injury.

Patients in accordance with above ATLI criteria during anti-TB treatment were categorized into the case group, and individuals whose liver function test indicators were at the lower ULN throughout anti-TB treatment were identified as potential control subjects. Two controls were randomly selected for each ATLI case, matched based on gender, age (5 years old) and region. After meticulous screening, the study comprised 88 cases and 176 controls.

Quantification of baseline keratin-18

When a person with TB was diagnosed and ready for treatment, liver function tests were generally performed, and blood samples were mainly obtained simultaneously. Subsequently, the baseline serum was separated at room temperature, and stored at -80^{o}C. The detection of serum keratin-18 was conducted strictly in accordance with the instructions of conventional ELISA detection kit (Singampalli *et al.*, 2022). The detection of serum keratin-18 was conducted while blinding the case or control status.

Table 1: Clinical characteristics of anti-TB treatment patients with or without ATLI

Variables	ATLI cases (N=88)	Controls (N=176)	P-value
Age (years), mean±SD	49.4±17.1	49.7±17.0	0.914[a]
Gender (male/female)	60/28	120/56	-
Liver diseases history (yes/no)	6/82	5/171	0.084[b]
Smoke history (yes/no)	10/78	21/155	0.892[b]
Drink history (yes/no)	4/84	7/169	0.831[b]
Hepatoprotectant (use/not use)	50/38	89/87	0.278[b]
Clinical pattern of hepatotoxicity			
Hepatocellular, n (%)	74(84.1)	-	-
Cholestatic, n (%)	11(12.5)	-	-
Mixed, n (%)	3(3.4)	-	-
Baseline value, median (IQR)			
ALT, U/L	14.5(9.8-23.4)	12.0(8.8-16.2)	0.007[c]
AST, U/L	20.1(17.5-25.0)	18.7(15.0-24.0)	0.029[c]
ALP, U/L	82.3(64.6-94.9)	82.3(64.0-93.0)	0.873[c]
GGT, U/L	26.6(22.6-50.0)	26.6(21.0-32.9)	0.033[c]
TBil, μmol/L	10.3(8.1-14.1)	9.0(6.7-12.2)	0.002[c]
DBil, μmol/L	3.2(2.3-4.5)	2.9(2.0-3.8)	0.010[c]
Peak value during treatment, median (IQR)			
ALT, U/L	264.8(202.0-413.8)	11.2(8.1-17.3)	<0.001[c]
AST, U/L	204.5(119.8-344.6)	20.0(15.5-26.1)	<0.001[c]
ALP, U/L	113.4(83.9-162.4)	80.4(65.5-97.0)	<0.001[c]
GGT, U/L	70.7(35.0-140.2)	30.1(20.8-38.0)	<0.001[c]
TBil, μmol/L	16.4(10.8-25.3)	8.0(6.0-10.7)	<0.001[c]
DBil, μmol/L	5.7(3.3-9.2)	2.8(1.8-3.5)	<0.001[c]

[a] Independent-sample t-test.
[b] Conditional logistic regression model analysis.
[c] Wilcoxon rank-sum test.

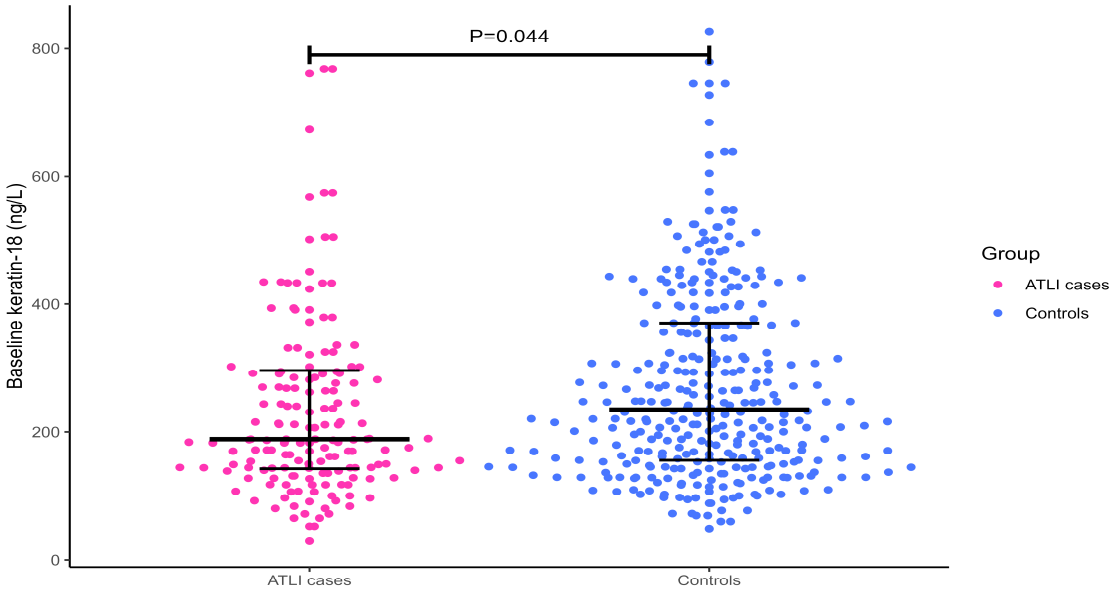

Fig. 1: The distributions of baseline keratin-18 in two groups.
ATLI, anti-tuberculosis drug-induced liver injury.

Table 2: Baseline keratin-18 distribution in patients with or without ATLI and the risks of ATLI

Baseline keratin-18	ATLI cases (N=88)	Controls (N=176)	χ^2	P-value	P-value for trend[e]	Model 1[a]		Model 2[b]	
						OR (95%CI)	P-value	OR (95%CI)	P-value
Overall, median (IQR)	188.8 (142.4-296.9)	234.9 (155.8-370.3)		0.044[c]	-	0.996 (0.992-0.999)	0.006	0.995 (0.992-0.999)	0.005
Quartile, n (%)									
Q1	31 (35.2)	44 (25.0)	3.516	0.319[d]	0.076	1.000		1.000	
Q2	21 (23.9)	44 (25.0)				0.533 (0.247-1.151)	0.186	0.537 (0.245-1.176)	0.120
Q3	20 (22.7)	44 (25.0)				0.267 (0.090-0.791)	0.037	0.248 (0.082-0.749)	0.013
Q4	16 (18.2)	44 (25.0)				0.142 (0.037-0.538)	0.004	0.129 (0.032-0.511)	0.004
Cutoff, n (%)									
≤191.6 ng/L	42 (47.7)	111 (63.1)	5.054	0.025[d]	-	1.000		1.000	
>191.6 ng/L	46 (52.3)	65 (36.9)				0.169 (0.057-0.500)	0.001	0.172 (0.058-0.515)	0.002

[a] Conditional Logistic regression model analysis without covariates.
[b] Conditional Logistic regression model analysis and adjust for drinking history, smoking history, hepatoprotectant use and liver diseases history.
[c] Wilcoxon rank-sum test.
[d] Chi-square test.
[e] Chi-square test for trend.

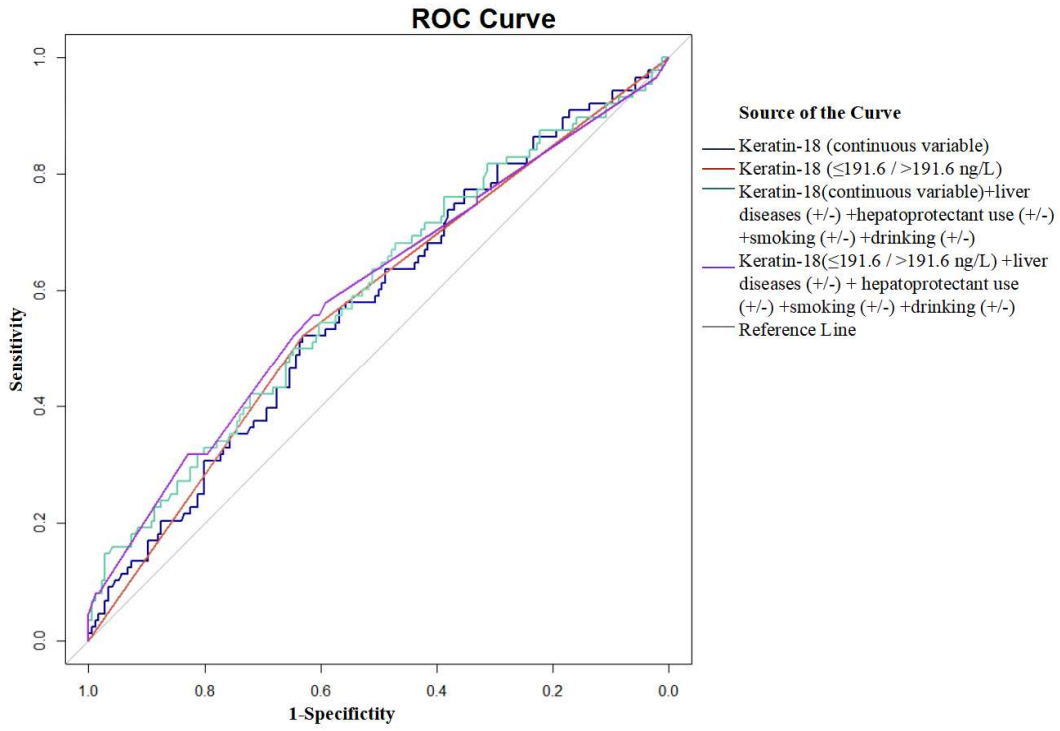

Fig. 2: Logistic ROC curves analysis of baseline keratin-18 for distinguishing ATLI patients. The area under the curve (AUC) for ATLI based on the keratin-18 (measured value), keratin-18 (≤191.6 vs >191.6 ng/L), keratin-18 (measured value) + liver diseases (+/−) + hepatoprotectant use (+/−) + smoking (+/−) + drinking (+/−), and keratin-18 (≤191.6 vs >191.6 ng/L) + liver diseases (+/−) + hepatoprotectant use (+/−) + smoking (+/−) + drinking (+/−) were 0.576, 0.577, 0.595 and 0.597, respectively.
ATLI, anti-tuberculosis drug-induced liver injury; ROC, receiver operating characteristic.

Table 3: Liver function indexes distribution in patients with different baseline keratin-18

Liver function indexes	keratin-18 ≤191.6 ng/L (N=111)	keratin-18 >191.6 ng/L (N=153)	P-value[a]
Baseline value, median (IQR)			
ALT, U/L	13.4 (9.9-20.0)	12.0 (8.8-16.8)	0.091
AST, U/L	20.0 (17.0-25.0)	18.9 (15.0-23.0)	0.032
ALP, U/L	82.0 (64.0-95.0)	83.0 (66.7-93.5)	0.547
GGT, U/L	26.6 (22.2-33.0)	26.6 (20.5-37.0)	0.528
TBil, μmol/L	9.8 (7.2-12.9)	9.8 (6.7-12.7)	0.413
DBil, μmol/L	3.0 (2.2-4.1)	2.9 (2.0-3.9)	0.111
Peak value during treatment, median (IQR)			
ALT, U/L	29.0 (11.5-225.0)	13.6 (8.6-119.3)	<0.001
AST, U/L	30.0 (21.0-142.0)	22.0 (16.0-52.4)	<0.001
ALP, U/L	94.0 (69.0-108.0)	86.5 (67.5-105.8)	0.216
GGT, U/L	35.0 (27.0-71.0)	35.0 (22.0-49.0)	0.084
TBil, μmol/L	9.2 (7.1-15.7)	9.7 (6.9-13.6)	0.885
DBil, μmol/L	3.1 (2.2-6.0)	3.1 (2.0-4.5)	0.332

[a] Wilcoxon rank-sum test.

Table 4: Correlation analysis between baseline keratin-18 and liver function indexes.

Liver function indexes	Baseline keratin-18		
	r_s[a]	95% CI[b]	P-value[a]
Baseline value			
ALT	-0.065	-0.177, 0.045	0.296
AST	-0.115	-0.239, -0.001	0.063
ALP	0.019	-0.111, 0.139	0.764
GGT	-0.022	-0.148, 0.085	0.716
TBil	-0.006	-0.122, 0.119	0.917
DBil	-0.058	-0.175, 0.071	0.345
Peak value during treatment			
ALT	-0.160	-0.278, -0.037	0.009
AST	-0.157	-0.273, -0.031	0.011
ALP	-0.094	-0.215, 0.031	0.127
GGT	-0.064	-0.192, 0.064	0.301
TBil	0.073	-0.045, 0.193	0.239
DBil	-0.036	-0.149, 0.095	0.559

[a]Spearman rank correlation analysis.
[b]95% CI were calculated using the bootstrap method

STATISTICAL ANALYSIS

Mean ± SD (standard deviation) was employed to depict continuous variables that conform to normal distribution, while the median and interquartile range (IQR) was employed to depict continuous variables that do not conform to normal distribution. The statistical tests for differences of those continuous variables between groups were performed using the two-group independent-sample t-test or the two-group Wilcoxon rank-sum test. Numbers (percentages) was employed to depict categorical variables, and conditional logistic regression models was employed to assess the differences of those categorical variables between groups. Two conditional logistic regression models (univariate and multivariate) were employed to analyze the association of baseline keratin-18 levels with ATLI risk. The association strength indices, odds ratios (ORs) with 95% confidence intervals (95%CIs) were calculated, initially using Model 1 without any covariates, and then using Model 2 with covariate adjustments, namely demographics variable as covariates. Discriminative ability of baseline keratin-18 in distinguishing ATLI patients from controls is typically analyzed using the receiver operating characteristic (ROC) curve, and the results were quantified by the area under the curve (AUC) (Bowers *et al.*, 2019). The Spearman rank correlation analyses was utilized to examine the correlation of baseline keratin-18 levels with clinical liver

function indexes. A two-sided P < 0.05 indicated statistically significant. Statistical analyses of data in the present study were processed with R statistical software (v4.3.0, Microsoft, USA).

RESULTS

Patient characteristics

Out of the 88 cases of ATLI, there were 74 cases (84.1%) of hepatocellular type, 11 cases (12.5%) of cholestasis type and 3 cases (3.4%) of mixed type. The median time from initial treatment to the occurrence of ATLI of all cases was 34 days (IQR: 13.0-67.5 days). The basic clinical information of patients in ATLI cases and controls were summarized in table 1. There was no statistically difference in age, sex, smoking history, drinking history, hepatoprotectant usage, and history of liver disease between the ATLI cases and the controls (all P > 0.05). Nevertheless, ATLI cases' TBil, ALT, AST, direct bilirubin (DBil) and gamma-glutamyl transpeptidase (GGT) before anti-TB treatment were notably higher than the controls' (P < 0.05), although all baseline values were within the normal range. Throughout the course of anti-TB treatment, individuals experiencing ATLI exhibited notably elevated peak levels of TBil, DBil, ALT, AST, ALP, and GGT than those in controls (P<0.05) (table 1).

Baseline keratin-18 and ATLI risk

The average baseline concentration of keratin-18 in ATLI cases was lower than that in the controls (median (IQR), 188.8 (142.4-296.9) ng/L vs. 234.9 (155.8-370.3) ng/L, P = 0.044) (Fig. 1). Under two conditional logistic models, patients with relatively high concentrations of keratin-18 were linked with a decreased risk of ATLI (Model 1 without any covariates, OR = 0.996, 95%CI: 0.992-0.999, P = 0.006; Model 2 with covariate adjustments, adjusted OR = 0.995, 95%CI: 0.992-0.999, P = 0.005, respectively). Likewise, patients with a keratin-18 ≥370.34 ng/L (Q4, the highest quartile) were at a lower risk of ATLI than those with a keratin-18 <155.70 ng/L (Q1, the lowest quartile) (Model 1 without any covariates, OR = 0.142, 95%CI: 0.037-0.538, P = 0.004; Model 2 with covariate adjustments, adjusted OR = 0.129, 95%CI: 0.032-0.511, P = 0.004, respectively). According to the maximum Youden's index, 191.6 ng/L was applied as the appropriate cut-off value for keratin-18. Compared to those with a keratin-18 ≤191.6 ng/L, patients with a keratin-18 >191.6 ng/L had a lower risk of ATLI (Model 1 without any covariates, OR = 0.169, 95%CI: 0.057-0.500, P < 0.001; Model 2 with covariate adjustments, adjusted OR = 0.172, 95%CI: 0.058-0.515, P = 0.002, respectively) (table 2).

ROC analysis of baseline keratin-18

Keratin-18 was incorporated in the logistic regression model as a continuous variable, the relevant AUC was equal to 0.576 (95%CI: 0.503-0.649, P = 0.042), while keratin-18 was incorporated as a binary variable (≤ 191.6/ > 191.6 ng/L), the corresponding AUC was equal to 0.577 (95%CI: 0.513-0.640, P = 0.018). After adjusting with demographics and clinical variable as covariates, the relevant AUCs were equal to 0.595 (95%CI: 0.521-0.670, P = 0.012) and 0.597 (95%CI: 0.524-0.670, P = 0.010) (Fig. 2).

Baseline keratin-18 and liver function indexes

Based on the optimal cut-off value, all patients were stratified into two groups (keratin-18 >191.6 ng/L and keratin-18 ≤191.6 ng/L). Although the baseline AST were within the normal range, non-parametric test result indicated that the baseline AST in the keratin-18 ≤191.6 ng/L cases was higher than that observed in the keratin-18 >191.6 ng/L cases (P = 0.032). The peak ALT and AST levels during anti-TB treatment in the keratin-18 ≤191.6 ng/L cases were notably higher than those in the keratin-18 >191.6 ng/L cases (all P < 0.001) (table 3). Spearman rank correlation analysis indicated that baseline keratin-18 has no associations with baseline liver function indexes (all P > 0.05). However, noteworthy correlation was seen between baseline keratin-18 levels and the peak values of ALT and AST during the anti-TB treatment (ALT, r_s = -0.160, P = 0.009; AST, r_s = -0.157, P = 0.011) (table 4).

DISCUSSION

Based on the current 1:2 matched case-control study, significant relationships were found between baseline keratin-18 and ATLI in Chinese patients. Whether used as continuous variable or binary variable (Q4 vs. Q1), keratin-18 showed a significant association with ATLI risk. Likewise, significant findings were corroborated upon the inclusion of additional covariates. As far as our current knowledge extends, this study represents the first investigation into the potential connection between baseline keratin-18 and the susceptibility to ATLI among Chinese individuals undergoing anti-TB treatment. Together with the previous British study(Rupprechter *et al.*, 2021), both studies found that the baseline keratin-18 was at a higher level in the control group, which suggested that patients with a higher baseline keratin-18 were less likely to develop the ATLI.

Past investigations have showed that the lower risk of liver injury in patients with higher baseline keratin-18 may be linked to the reduced deposition of Mallory-Denk bodies (MDB) in the liver (Harada *et al.*, 2007). As a kind of horny nodule that is mainly composed of keratin-8 and keratin-18, MDB's formation is considered to be linked with the onset and progression of hepatic-related diseases, such as alcoholic steatohepatitis (ASH) (Qian *et al.*, 2023; Wu *et al.*, 2024). Under healthy conditions, normal adult hepatocytes express keratin-8 and keratin-18 at a stoichiometric ratio of 1:1, which plays a key role in the formation of MDB (Omary *et al.*, 2002). For example, transgenic mice with a deletion or mutation of the keratin-

8 gene, resulting in a higher keratin-18 ratio compared to keratin-8, are unable to form MDBs (Guldiken, Usachov*, et al.*, 2015). One animal experiment showed that the formation of MDB in transgenic mice overexpressing keratin-18 was inhibited to some extent when treated with hepatotoxic drugs. Furthermore, keratin-18-overexpressing mice displayed reduced levels of ALT and ALP, as well as lower scores for inflammation and cell ballooning, as compared to non-transgenic mice (Harada *et al.*, 2007). Studies have suggested that both keratin-8 and keratin-18, which are encoded by the KRT8/KRT18 gene respectively, together form the cytoskeletal intermediate filament of adult hepatocytes (Lim *et al.*, 2021). There might be an interaction between these two genes, which together protect hepatocytes from apoptosis (Wang P. *et al.*, 2021). Any mutation or deletion of one keratin may indirectly affect the normal expression of the other keratin, thus influencing their protective effects against liver injury (Guldiken, Usachov*, et al.*, 2015). All these studies provided a good theoretical basis for explaining the role of keratin-18 in the progression of DILI.

Although this study and the British study suggest that keratin-18 is linked to the onset and progression of ATLI, there are still some differences between two studies. In the British study, serum samples were collected at five time points: before treatment, and at weeks 2, 4, 6, and 8 post-treatment, and there was a significant correlation between ALT and keratin-18 (r_s = 0.42, P < 0.0001) (Rupprechter *et al.*, 2021). However, our study showed a negative correlation between baseline keratin-18 and peak value of ALT and AST during treatment (r_s = -0.160, P = 0.009; r_s = -0.157, P = 0.011, respectively). After logistic ROC curve analysis and including other covariates, the present result indicated that baseline keratin-18 has a limited predictive ability for ATLI (AUC is equal to 0.597, 95%CI: 0.524-0.670, P = 0.010). However, the British study obtained a higher AUC (AUC is equal to 0.80, 95%CI: 0.72-0.87, P < 0.0001) (Rupprechter *et al.*, 2021). Those differences could have several reasons. Firstly, there were differences between the case groups of the two studies. The present study set stricter criteria for inclusion and exclusion as all patients with unclear diagnosis of TB or combined with HIV infection were excluded and all patients received anti-TB treatment. Contrastively, the British study included not only patients with non-TB mycobacteria infection, but also patients with HIV infection, and some patients received both anti-TB treatment and antiviral therapy simultaneously. Therefore, the role of liver injury cannot be solely attributed to the use of anti-TB drugs(Rupprechter *et al.*, 2021). Secondly, there were also differences between the controls of the two studies. And the controls of the present study were consisted of TB patients with confirmed diagnoses and received standard anti-TB drugs, while the British study used healthy and unmedicated people as their control group (Rupprechter *et al.*, 2021). Differences in cases and controls between the two studies may account for the difference in AUC. The most significant feature of this study is that it adopted a 1:2 individual matched case-control study design. This approach enabled the individual matching of cases and controls based on age, gender and region, which partially controlled for the influence of other covariates and increased the statistical power. Nevertheless, there are still certain limitations that must be acknowledged. Firstly, this study only detected the baseline keratin-18, but did not monitor the change of serum keratin-18 in the process of liver injury. Therefore, the predictive value of baseline keratin-18 in ATLI is still very limited. Secondly, keratin-8 and keratin-18 together constitute the main structural proteins of the liver, but the present study only focused on baseline keratin-18, which may also be a reason for the low discriminative performance of keratin-18 in distinguishing ATLI cases from controls. A study in rodents showed that the variation of keratin-8 could increase the risk of hepatotoxicity induced by acetaminophen to a certain extent(Guldiken, Zhou*, et al.*, 2015). Therefore, the diagnostic and predictive value of keratin-8 in DILI may be worthy of further study. Lastly, due to the strict diagnostic criteria for ATLI, the sample size was limited, and only Han population in eastern China were recruited in present study. A validation cohort with larger samples and more diverse populations is needed to verify our results.

CONCLUSION

In summary, we conducted a preliminary exploratory study to describe the relationship between baseline keratin-18 and ATLI risk among Chinese patients undergoing anti-TB treatment. Through a 1:2 matched case-control analysis, the concentration of keratin-18 in controls was higher than that in ATLI cases, and that the higher the keratin-18 concentration, the lower the risk of ATLI. Further studies involving larger and more cohorts is are warranted to elucidate the potential clinical utility of these findings.

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Conflict of interest

There is no conflict of interest.

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