The Association of Abnormal Liver Enzymes with All-Cause and Cause-Specific Mortality: A Large Prospective Population-Based Study

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Abstract

Background: While the association of elevated levels of liver enzymes (ALT, AST, ALP and GGT) with mortality has been suggested in many studies, evidence of their association appears to be contradictory. The current study aims to investigate the associations of entire ranges of serum liver enzymes with mortality in a large cohort of adults.

Methods: In the current study we used the data of repeated measurement phase in Golestan Cohort Study, which was conducted from 2010 to 2012. All liver enzyme levels were categorized into their quintile values, and the association of liver enzymes with mortality rates were evaluated using Cox proportional hazard regression models. Sensitivity analyses were performed by excluding data of first follow-up year, and patients with history of cardiovascular disease (CVD), cancer or viral hepatitis.

Results: We included 11,106 individuals with mean age of 56.22 (±7.97) years, 46.2% male. Median follow-up period was 7 years. In the total population, ALT demonstrated a U-shaped association with all-cause mortality; whereas AST showed no significant association. ALP and GGT had linear and positive associations with overall mortality in the total population. CVD-mortality showed associations with low ALT (aHR=1.53, 95% CI: 1.1-2.2), high ALP (aHR=1.79, 1.3-2.6) and high GGT (aHR=2.0, 1.4-2.9). Cancer-mortality was associated with high ALP (aHR=1.9, 1.2-2.9, while GGT had a U-shaped association with cancer mortality, with the middle category (Q3) associated with the lowest cancer-mortality risk (aHR=0.58, 0.4-0.9). High ALT and high AST were associated with cancer-mortality in total sample as well; although, these associations disappeared after the sensitivity analysis.

Conclusion: Both low and high levels of ALT are associated with increased all-cause mortality, while only high levels of ALP and GGT have positive associations with all-cause mortality. High levels of all four liver enzymes are associated with cancer mortality. However, only the high levels of ALP and GGT are associated with CVD mortality. Evidence implies that abnormal liver enzymes may be manifestations of metabolic syndrome, CVD, and cancers rather than being their precursors. Yet, they may be useful markers for predicting the survival of patients.

Introduction

The role of liver function tests (LFTs) in predicting long-term survival of individuals has been suggested during the past two decades. These markers include serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP), usually measured as markers of liver function [1]. Amongst LFTs, ALT is mostly secreted by hepatic cells, and its serum levels are elevated in the case of hepatocellular injury. A minor proportion of ALT is secreted by skeletal muscle cells. AST on the other hand, is detected in liver, skeletal muscles, heart, kidney and brain; together with ALT, it is responsible for transfer of amino-groups during amino acid metabolism and gluconeogenesis [2, 3].

GGT can be found on the extracellular plasma membrane of various cells. It is freely detected in serum as well. Since GGT plays a significant role in extracellular hydrolysis of glutathione (GSH), it has great anti-oxidant effects. Paradoxically, in certain situations, GGT could mislead this pathway into producing free radicals, and exert pro-oxidant effects [4]. ALP on the other hand, is expressed mostly in bone, kidneys and liver, and plays a major role in phosphate metabolism [5, 6].

Abnormal levels of liver enzymes have been linked to many liver and non-liver disorders, including cardiovascular diseases (CVDs) and cancers. Many attribute these associations to the increasing trend of Metabolic Syndrome (MetS), and its most prominent liver manifestation, Non-Alcoholic Fatty Liver Disease (NAFLD) [7, 8]. While association of elevated LFTs and mortality has been the focus of attention for many years [3, 9, 10], some recent studies have found low enzyme levels to be associated with increased mortality as well. In 2006, Elinav et al reported ALT values lower than

median to be associated with long-term mortality in elderly men [11]. Furthermore, bimodal U or J-shaped associations between liver enzymes and mortality were reported in some studies as well [12, 13].

The findings of most research to date on the association of serum liver enzymes and long-term mortality have been contradictory, specifically regarding the transaminases. In addition, no prospective study has ever investigated the associations between four liver enzymes and overall/cause-specific mortalities in a general population of Iran, or any country in North Africa and Middle East. It is also important to note that as a middle-income country located in the Middle-East, Iran is experiencing high burden of premature deaths, mostly due to the CVDs and cancers [14, 15]. The current study aimed to evaluate the association of liver enzymes (i.e. ALT, AST, ALP and GGT) with overall and cause-specific mortality. In order to accomplish this purpose, we used the data provided by Golestan Cohort Study (GCS), a large population-based cohort study conducted in Golestan province, North-Eastern Iran [16].

Materials/patients And Methods

Golestan Cohort Study (GCS) design

The present study used the information available in Repeated Measurement (RM) phase of Golestan Cohort Study (GCS). GCS is a large-scale population-based prospective cohort conducted in Golestan province, Northern Iran [16]. At GCS baseline 50,045 healthy adults aged 40–75 were recruited from 2004 to 2008. Participants resided in Golestan province. Demographic data, past medical history, medication history, nutritional data, habitual risk factors, in addition to data of blood pressure and anthropometric measurements were obtained during baseline phase of this study. Most laboratory biomarkers were not evaluated at this point, except for CBC, creatinine, HBs Antigen (HBs Ag) and HCV antibody (HCV Ab). Afterwards, participants were annually followed for death and/or major outcomes (such as occurrence of cancers or cardiovascular events). Details of GCS are described in previous publications [16–18].

During 2010–2012, a random selection of 11,419 GCS participants underwent an additional data collection process named Repeated Measurement (RM), which included repeating the entire GCS baseline processes, plus extra laboratory tests. Serum biomarkers including CBC, creatinine, FBS, AST, ALT, ALP, GGT, lipid profile etc. were measured in this step [19]. For the purpose of current study, we included individuals that had participated in RM phase, and retrieved the data of HBs Ag and HCV Ab from their baseline measurement. Since the percentage of individuals with regular alcohol consumption was low in our primary analyses (n = 33, or 2.7% of the population), we excluded them from our analyses. Therefore, our study targets all of the associations without the influence of alcohol.

Data collection

GCS-RM phase questionnaires retrieved data on age, sex, disease history, medication history and habitual risk factors (opium, tobacco and alcohol consumption), and were filled by trained interviewers. All physical examinations (including blood pressure and anthropometry) and blood samplings were performed by trained health-care workers in standard environments.

Height and waist circumference were estimated using non-elastic tapes with 0.5 cm width and 200 cm length, according to WHO recommendations. Weight was evaluated using a scale with the accuracy of 500 grams. BMI was calculated by dividing weight (kg) to height^2 (m^2). Blood pressure was measured twice during the interviews, with a 10-minutes interval between measurements. The mean of systolic and diastolic values of second measurements of right and left arms were defined as individuals' systolic (SBP) and diastolic blood pressure (DBP) [16].

All laboratory samplings were performed after at least 12 hours of fasting, and 10 ml of blood was obtained from each participant, in both baseline and RM. As mentioned above, baseline laboratory sampling only included measurement of HBs Ag, HCV Ab and CBC, while other biomarkers were only measured in RM phase. Serum ALT (IU/L), AST (IU/L), GGT (IU/L), ALP (IU/L), Fasting blood glucose (FBS) (mg/dl), total cholesterol (mg/dl), high-density lipoprotein (HDL) (mg/dl), and triglyceride levels (mg/dl) were measured [16]. Enzyme activities were measured using a method that estimates change rate of absorbance at 340 nm over a fixed-time interval. The absorbance rate of change is directly proportional to the serum enzyme activity. The concentration of serum triglyceride was determined using a timed-endpoint method in which change in absorbance at 520 nm was measured for a fixed time [20–23].

Assessment of mortality

Since the launch of follow-up phase in 2008, all GCS participants are being contacted annually to determine their health status. In case of a death report, a verbal autopsy is completed, and all the clinical and hospital records are transferred to the follow-up center for review. Afterwards, two independent internists ascertain the cause of death according to the International Classification of Diseases–10th version (ICD–10) codes. Cases of disparity are referred to a senior internist for resolution. GCS follow-up process is described in detail elsewhere [17].

The follow-up data was updated at January 31st 2019, and time to event was defined as the interval from the first serum LFT measurement to death, or the last follow-up session. Current study considered all-cause mortality as the primary end point. We also investigated the cause-specific deaths from cardiovascular diseases (ICD–10 code: I10–I95), deaths from cancers (ICD–10 code: C01–C97), deaths from liver-related diseases (ICD–10 code: K70–77), and deaths from other causes among confirmed causes of death.

Assessment of covariates

Participants were categorized as “smoker” if they had smoked at least 100 cigarettes during their lifetime, and “never-smoker” if not. Regarding opium and alcohol consumption, individuals were categorized into “ever user” (individuals who had used the substance at least once per week for six months in their lifetime) and “never user” (otherwise). As mentioned above, all alcohol “ever users” were excluded prior to any analysis.

Metabolic Syndrome (MetS) was defined as presence of three out of five following criteria, according to ATPIII modified version: (1) waist circumference > 95 cm in men and women (ethnic cut-off recommended by Azizi et al) (2) serum triglycerides ≥ 150 milligrams mg/dl (3) HDL< 40 mg/dl in men or 50 mg/dl in women (4) FBS ≥ l00 mg/dl (5) SBP≥ 130 mmHg and/or DBP ≥85 mmHg [24].

Individuals with positive HBs Ag or HCV Ab at baseline were defined as having exposure to viral hepatitis. Diabetes was defined as an FBS ≥ 126 mg/dl, or using anti-diabetic medications. Participants were classified as hypertensive if they had an average SBP ≥ 140 mmHg or average DBP ≥ 90 mmHg, or if they used antihypertensive agents. Area of residence was dichotomized as urban and rural. Physical activity was categorized into tertiles according to the Metabolic Equivalent of Task (MET, in minute/week) [14].

Statistical analysis

We used descriptive analyses for demographics of the study population. Distribution of quantitative variables was compared with normal distribution using Shapiro-Wilk test. Values are represented as mean ± standard deviation (SD) for normal continuous variables, as median with Q1 and Q3 for skewed variables, and as frequency and percentage for

categorical variables. Baseline characteristics of continuous variables were compared between liver enzyme groups using one-way ANOVA or Kruskal-Wallis, as appropriate. Categorical variables were assessed using chi-square test

For the purpose of analyses, all serum LFTs were classified into quintiles separately for men and women. The association between LFTs and overall/cause-specific mortality were evaluated using Cox proportional hazard regression models. In our models, we considered quintile 3 (Q3) of ALT, and quintile 1 (Q1) of the other three enzymes as reference. The reason for selecting Q3 of ALT was that recent previous studies had suggested ALT to have a bimodal relationship with mortality, which would be better demonstrated when comparing its middle values with the two extremes (low and high) [25]. Since this type of association was mostly suggested for ALT and not the other enzymes, we considered the lowest value (Q1) of the other three enzymes as the reference value. Forest-plot figures were used for demonstrating the hazard ratios (HR) of all associations. In order to demonstrate the shape of association of quantitative values of LFTs with all-cause mortality, a restricted cubic spline regression model was conducted with 4 knots which were set at the quartiles. All associations were adjusted for relevant confounders including age, sex, residence area, BMI, Physical activity, Metabolic Syndrome, total cholesterol, and tobacco and opium abuse. Models were also tested for interaction with age and viral hepatitis according to likelihood ratio test. Furthermore, sensitivity analyses were performed by excluding data of first follow-up year, patients with viral hepatitis, history of CVD or cancer.

All data analyses and figure plot depictions were performed with Stata Statistical Software, Release 12 (College Station, TX: Stata Corp LLC), except for the forest-plot figures, which were illustrated using R 3.6.0 for Windows. Statistical significance was set at p-value < 0.05.

Ethical considerations

The present study was ethically approved by the Institutional Review Board (IRB) of Digestive Diseases Research Institute (DDRI) of Tehran University of Medical Sciences (TUMS). Written informed consent was obtained from all GCS participants, both at baseline and RM.

Results

Characteristics and mortality

Quintile values of LFTs in our population are reported in Table 1, while basic characteristics of the participants are presented in Table 2. The current study included 11,106 of GCS individuals who had participated in RM. The population consisted of 46.2% men, and mean age of participants was 56.22 (±7.97) years (range: 45–82 years). In general, 29.43% of the sample had Metabolic Syndrome, 14.88% regularly smoked cigarette, and 16.63% consumed opium. The majority of the population were of Turkmen ethnicity, who resided in rural areas (81.95%). All covariates were significantly different among men and women (Table 2). In total, 595 (5.3%) of the participants were positive for HBs Ag, HCV Ab, or both (data not shown). Baseline characteristics of participants according to quintile levels of liver enzymes can be found in supplementary tables S1 to S4.

Median follow-up period was 7 years, with a maximum of 8.7 years. Overall, 941 individuals (8.5%) died at the end of follow-up period, and the causes of deaths were confirmed in 889 individuals. CVD events were the cause of death in 346 cases (36.7%), while cancer deaths comprised 243 (25.8%). Liver-related diseases were the cause of death in 19 (0.17%) cases; given their small share, they were grouped with all the other death causes as Other-related deaths (300 deaths). Sensitivity analysis after excluding the data of first follow-up year, viral hepatitis patients and participants with history of CVD or cancer is presented in supplementary Table 3. Overall, 1755 individuals were excluded for the sensitivity analysis.

The association of AST and ALT with all-cause and cause-specific mortalities

The multivariate-adjusted analysis showed that compared with middle quintile (Q3), the lowest (<13 IU/L for men, and <12 IU/L for women) and highest (>31 IU/L for men, >27.6 IU/L for women) quintiles of ALT were significantly associated with higher risk of mortality from all causes (the lowest ALT aHR = 1.34, 1.1–1.7, and the highest ALT aHR = 1.26, 1–1.6) (Figure 1a). Analyzing the association of quantitative ALT and all-cause mortality using Cubic spline regression model showed a non-linear association, with ALT values from 20 to 40 having the minimum mortality risk (Figure 3). When investigating different causes of death, ALT Q1had CVD-related aHR = 1.53 (1.1–2.2) compared to Q3, while ALT Q5 had cancer-related aHR = 1.56 (1.1–2.4) compared to Q3 (Figure 1a). However, performing sensitivity analysis diminished the association of high ALT with all-cause and cancer-mortality (Table 3). Interaction analyses of age and viral hepatitis were non-significant.

AST, on the other hand, was not associated with overall mortality when Q1was set as reference (Figure 1b). However, Cubic spline model showed a non-significant U-shape association with mortality with the minimum risk at 20 IU/L value (Figure 3). There was no significant association between AST and CVD-related deaths in the adjusted model. Cancer-related mortality was initially associated with the highest quintile of AST (aHR for Q5 vs. Q1 = 1.7, 1.1–2.6) (Figure 1b); however, performing sensitivity analysis diminished this association (Table 3). Others-related mortality risk was lowest in the third quintile of AST (aHR for Q3 vs. Q1 = 0.62, 0.4–0.9) (Figure 1b). Interaction analyses were associated with no significant change.

The association of ALP and GGT with all-cause and cause-specific mortalities

Increase in ALP activity showed a positive association with all-cause mortality, as ALP Q5 vs. Q1 aHR of all-cause mortality was 1.88 (1.5–2.3) (Figure 2a). Furthermore, Cubic Spline model showed a logarithm-shaped association with all-cause mortality, with ALP greater than 300 IU/L having the highest risk for mortality (Figure 3). Moreover, Q5 of ALP was directly associated with specific causes of mortality, including CVD (aHR = 1.79, 1.3–2.6), cancer (aHR = 1.9, 1.2–2.9) and even Others-related causes of death (aHR = 1.85, 1.3–2.7) (Figure 2a). Sensitivity and interaction analysis were associated with no significant changes.

Finally, GGT was also positively associated with all-cause mortality (Q5 vs. Q1 aHR = 1.62, 1.3–2) (Figure 2b). Continuous GGT (Cubic Spline model) also proved a non-linear logarithm-shaped association with increasing mortality (Figure 3). Higher values of GGT were associated with two folds of CVD-related mortality risk (Q5 aHR = 2.0, 1.4–2.9), while the Q3 category of GGT (21–27 IU/L for men, and 18–24 IU/L for women) was inversely associated with cancer-related mortality (Q3 vs. Q1 aHR = 0.58, 0.4–0.9). Other-related deaths were associated with increment in GGT values, as Q4 and Q5 had aHR = 1.5 vs Q1 (1–2.2) and aHR = 1.7 vs Q1 (1.2–2.5) of other-related death, respectively (Figure–2b). Sensitivity and interaction analyses were associated with no significant changes (Table 3).

Discussion

The present study is the first to investigate the associations between four serum liver enzymes and prospective mortality in a population of middle-aged Iranian adults. While ALT demonstrated a bimodal association with all-cause mortality in the total population, ALP and GGT were associated with overall mortality in their highest levels. AST was not associated with all-cause mortality in any of the analyses. After excluding the first year of follow-up and individuals with previous hepatitis or CVD or cancer, all-cause mortality and CVD mortality were associated with low ALT, high ALP and high GGT, while cancer-mortality was associated with high ALP, and both low and high GGT. Interestingly, Others-related mortality was associated with low and high AST, high ALP and high GGT. Due to the wide variety of death causes in this group (Others-related), the reason behind observed associations remains unknown.

Literature review on the association of baseline liver enzymes and mortality revealed a high variety of investigations among different populations, with conflicting results.

ALT and mortality

The association between ALT and long-term mortality has been the focus of several studies. Kim et al (2004) and Yun et al (2009) reported a positive association between elevated ALT and overall mortality, attributing this relationship to higher liver-related and CVD mortality associated with ALT elevation [7, 26]. However, later studies reported contradictory results. Elinav et al for the first time reported an association between lower levels of ALT and higher mortality among elderly males [11]. Le Couteur et al proposed a similar association, attributing it to the concept of hepatic frailty (liver aging), relating to poor hepatic capacity and increased mortality risks suggested in previous studies [27–29]. Afterwards, Hernaez (2011) and Ford (2011) specifically suggested an association between low ALT and higher risk of CVD mortality. However, since their study populations consisted of middle-aged and old adults, this association was not completely justified regarding aging and frailty [30, 31].

In order to overcome limitations of the previous studies, Ruhl et al (2013) investigated the aforementioned association in a large sample of US adults with a wide age-range (> 20 years), and evaluated ALT levels using deciles; so that the impact of an entire range of ALT (low-normal, high-normal, and elevated) would be assessed. Through investigating mortality, they found mortality rates across deciles 4–9 of ALT relatively invariable; therefore, they considered deciles 4–9 as the reference group for comparison. Using this method, it was revealed that both low and high values of ALT were strongly associated with overall mortality. Moreover, low ALT (and not high ALT) was associated with increased CVD mortality. By investigating body composition analysis, it was shown that skeletal muscle loss/dysfunction, an impairment known as Sarcopenia, was probably the main factor responsible for the association observed between low ALT and higher CVD mortality [25]. Other studies have also supported an association between Sarcopenia - a component of frailty - and higher cardiometabolic disorders. The exact mechanisms through which Sarcopenia could result in CVD are not well-elucidated; however, decreased energy expenditure, increased visceral fat deposition, induced systemic inflammatory state, insulin resistance and development of atherosclerosis are suggested as possible underlying pathways [32, 33].

Our study also confirms the association of low ALT and high risk of CVD mortality. Furthermore, the association between low ALT and CVD mortality remained significant after sensitivity analysis, indicating that the association of low ALT with CVD mortality observed in the total population is not a result of presence of patients with positive history of CVD in the sample. However, we were not able to evaluate the role of Sarcopenia.

Ruhl et al and Koehler et al both suggested U/J-shaped associations between ALT and all-cause mortality among their sample. We also found a U-shaped association between ALT and overall mortality in our total population. However, contradictory to our research, Ruhl et al reported that high ALT remained significantly associated with all-cause mortality after excluding participants with a history of viral hepatitis [13, 25]. In our study, the associations between high ALT and all-cause mortality faded after excluding patients with viral hepatitis and cancer. In other words, this association was not significant in participants without a positive history of cancer, or viral hepatitis. Therefore, it is not feasible for us to suggest a causal association between high ALT and all-cause mortality.

The association between ALT and cancer mortality is relatively unexplored in the literature. In their meta-analysis, Liu et al suggested an association between extremely low ALT and cancer mortality in elderly (>70 years), which is contradictory to our findings. However, this study also stated that the findings regarding the ALT/cancer mortality association in the literature are greatly inconsistent [34]. A more recent meta-analysis by Kunutsor et al investigated the relationship of ALT with incidence of overall and cause-specific cancer, revealing that this association varies according

to the geography of studies conducted, as corresponding RRs for overall cancer in European and Asian populations were 0.96 (0.94–0.99) and 1.65 (1.52–1.79), respectively. The reason behind this variation remained unclear [35]. Further studies are required to illuminate the nature of association between ALT and forthcoming cancer outcomes.

AST and mortality

Most studies in the literature have reported no significant association between AST and CVD mortality [25, 31, 36], which are in agreement with our results. Two meta-analyses confirmed this finding as well [37, 38].

In our study, the most significant association of AST was observed with cancer mortality in total population, and the highest category of AST showed 70% higher risk of mortality from cancer compared to the lowest category. This association, however, diminished after excluding patients with a history of cancer or viral hepatitis, indicating that the significant primary finding was secondary to either one of these diseases. In this regard, Ruhl et al and Unalp-Arida et al used percentiles of AST for analysis, and considered the middle categories as reference group. Both of these studies showed that lower AST values (deciles 1 and 2) were associated with higher cancer mortality [25, 36], contradictory to our results. Although, it should be noted that compared to our study, the sample of both studies included younger participants. Findings of Koehler et al and Hernaez et al on the other hand, were in agreement with our results. Both of these studies consisted of middle-aged and old individuals, similar to our design [13, 31]. Hernaez et al partly attributed the association of high AST and cancer to the possible role of Non-Alcoholic Fatty Liver Disease (NAFLD) and its association with insulin resistance, which has been suggested as one of greatest risk factors for cancer development, particularly hepatocellular carcinoma (HCC) [31, 39, 40]. Due to the small number of individuals with HCC, as well as lack of evaluation of fatty liver in our participants, we were not able to examine this association. However, in our analysis, we had adjusted the associations for Metabolic Syndrome, which could omit the confounding effect of fatty liver in the associations. Therefore, from our perspective, the association observed between high AST and cancer mortality is mostly attributed to inclusion of patients with previous cancer or viral hepatitis.

ALP and mortality

Most of previous studies agree on a positive association between ALP and mortality in general population. Fleming et al investigated ALP association with cause-specific mortality and reported higher mortality from CVD, cancer, respiratory diseases and liver diseases among those with high ALP [10]. Furthermore, ALP was suggested as an independent predictor of all-cause and CVD mortality in three meta-analyses [37, 41, 42]. The findings of these studies are in agreement with our results. A recent meta-analysis by Rahmani et al also confirmed this association [38].

The mechanism underlying association of ALP and CVD mortality is not clear. However, it has been suggested that elevated ALP may be accompanied with higher levels of phosphates, impaired bone metabolism and vascular homeostasis, which could increase progression and development of vascular calcification and result in higher risks for CVD outcomes [6]. A recent meta-analysis proposed the role of NAFLD as the hepatic manifestation of metabolic syndrome, and a possible mediator in the association between ALP and CVD mortality [41]. As mentioned above in case of AST, in our study we did not have access to the data of NAFLD among the target population; however, we adjusted all associations for Metabolic Syndrome. Therefore, it could be concluded that ALP has a positive association with CVD mortality, which is independent of Metabolic Syndrome, and possibly NAFLD.

Although previous population-based studies have reported a positive association between baseline ALP and long term cancer mortality [10, 13], the mechanisms underlying this association has not been investigated. Cellular and genetic studies have suggested an association between altered expression of ALP and various human malignancies, including

cancers of breast, ovarian, hepatocellular carcinoma, lung etc. [43, 44]. While these are consistent with our findings, further prospective studies would benefit from evaluating the association of ALP with different types of non-fatal and fatal cancer.

GGT and mortality

A large body of evidence exists regarding the association of GGT with different causes of mortality, and our findings are mostly in agreement with the previous data. Several prospective studies have reported positive association of GGT with CVD-related [9, 13, 45, 46], cancer-related [31, 47, 48] and all-cause mortality [25, 36, 49]. A study in Turkey in 2014 even suggested GGT as a marker of predicting in-hospital mortality among patients with acute heart failure [50]. Although a few studies found no association between CVD mortality and GGT, the bulk of findings from different societies are strongly in favor of an existing positive association between GGT and CVD, an indication revealed by several meta-analyses [38, 42, 51].

The associations of GGT with mortality was not influenced by hepatitis in our study, as excluding patients with hepatitis did not affect our results. In a study by Yuwaki et al, the association of GGT with mortality remained present after excluding participants with hepatitis B [52], which is similar to our findings. Age interaction was also not observed in our research; whereas in a 2009 study, the association was only significant among participants under 70 years [53]. This association was also observed in older subgroups (e.g. $\geq$ 55 or 60 years) in other studies [13, 54].

Evidence from prior research suggests that GGT has a role in catabolizing reduced glutathione, and delivery of cysteine for intracellular production of glutathione, the main anti-oxidant thiol. High GGT level has been suggested as a strong marker of oxidative stress, which could be induced by various potential factors, including heavy metals, alcohol consumption or hyperglycemia [55]. However, the association between GGT and CVD mortality appears to be independent of these factors, as represented by more recent studies [45]. Our findings also support the alcohol-independent role of GGT, since participants with regular alcohol consumption were excluded from our sample. Previous studies suggest GGT to have pro-oxidant features in certain situations, including in atherosclerotic plaques, where GGT could be exposed to free and compound iron as well as oxidized lipoproteins, resulting in production of free-radicals and atheroma development [56]. Therefore, apart from correlating with CVD risk factors, elevated GGT is considered as an independent predictor of developing fatal or non-fatal CVD events [57], a finding which is consistent with the present study.

Compared to CVD mortality, the association of GGT with cancer mortality is less studied. Monami et al first investigated this association among diabetic patients, and reported a dose-response relationship between increasing GGT and risk of cancer mortality [9]. Later, other studies on non-diabetic individuals suggested this association as well [31, 48, 54]. The role of GGT in cancer biology is still unclear, and it is unknown whether GGT has direct carcinogenesis role, or it is simply a marker of an underlying pathway. However, most speculations again point to the role of GGT in glutathione metabolism and oxidative stress, resulting from the evidence of increased GGT activity in many neoplastic lesions [35]. Our results are congruent with these findings; however, in our analyses we encountered a U-shaped association between GGT and cancer-mortality, which remained significant after sensitivity analysis. In other words, both high and low values of GGT were associated with cancer-mortality; a finding which was never mentioned in previous studies, and therefore, should be interpreted with caution. Although as research conducted by far has mostly investigated the association of GGT and cancer using either GGT elevation or its continuous values, it is possible that categorization with percentiles (similar to the current study) and considering middle values as the reference for hazard assessment changes the findings. In other words, our results suggest that when investigating the association between GGT and cancer mortality, considering the middle quintile of GGT as reference could be a better choice compared to the lowest quintile.

Nevertheless, future studies on the association of GGT and cancer-related deaths are required to further elucidate the nature of this relationship.

Strengths and limitations

Current study has several strengths, including a longitudinal design and extremely low loss-to-followup. Furthermore, in this study we had access to data of viral hepatitis (HBV and HCV), and therefore we could evaluate the interaction of hepatitis in all associations with mortality: a sensitivity analysis which many of the prior studies had not been able to perform. Substantial follow-up accuracy and approved cause-of-death are other significant strengths of the present study. Evaluating the whole range of all four liver enzymes, and investigating their associations with the main causes of mortality (overall, CVD-related and cancer-related) could be considered another important strength of this study. Also, all of our analyses are performed among individuals with no regular alcohol consumption; therefore, it could be safe to presume our findings are not biased by alcohol confounding effects.

Our main limitation is that we did not have access to the data of NAFLD, insulin resistance, and lean body mass in our participants. Furthermore, GCS mainly consists of a rural Iranian population; therefore, it may not be representative of urban residents who currently comprise 70% of the total Iranian population.

Conclusion

In short, the current study investigated the association between serum ALT, AST, ALP and GGT with different causes of mortality in a large-sample prospective study of middle-aged Iranians. It is rational to conclude that abnormal liver enzymes may be associated with all-cause, CVD- and cancer-related mortality. Abnormal liver enzymes may be a manifestation of metabolic syndrome, CVD, or cancer, rather than precursors. Therefore, in practice, patients who refer to receive outpatient or inpatient services with abnormal liver enzymes most probably already suffer from liver disease, CVD, or cancer and abnormal liver enzymes are the outcome of disease. In any case, liver enzymes can be good predictors of the prognosis in patients suffering from CVD or cancers.

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Tables

Table 1: Percentile values of serum ALT*, AST#, ALP¶ and GGT[] levels for men, women and total sample in the total population

Centiles	ALT (IU/L)			AST (IU/L)			ALP (IU/L)			GGT (IU/L)		
	Men	Women	Total	Men	Women	Total	Men	Women	Total	Men	Women	Total
20th percentile	13	12	12.3	16	15	15.2	194	199	197	16.4	14.014	14.9
40th percentile	17	16	16.2	19	18	18.3	227	239	233	20.7	18.398	19.4
60th percentile	22	20	21	22	21	21.3	259	276	268	26.8	23.822	25.1
80th percentile	31	27.6	29.1	27	26	26	304.4	330	319	37.5	34.846	36.1

ALT*: Alanine aminotransferase; AST#: Aspartate aminotransferase; ALP¶: Alkaline phosphatase; GGT[]: Gamma-glutamyl transpeptidase

Table 2: Baseline characteristics of total population according to gender

Variables		Total (n=11,106)	Gender difference		
			Female (n=5,969)	Male (n=5,137)	P-value
Mean (SD)					
Age (year)		56.22 (7.97)	55.5 (7.54)	57.06 (8.37)	**<0.001**
BMI[] (kg/m^2)		27.15 (5.33)	28.44 (5.57)	25.65 (4.61)	**<0.001**
Median (Q25-Q75)					
Cholesterol (mg/dl)		200 (174-228)	208 (182-236)	191(166-217)	**<0.001**
Triglyceride (mg/dl)		115 (86-161)	118 (88-165)	111 (82-156)	**<0.001**
ALT[*] (IU/L)		18.35 (13.4-26.3)	18 (13-25)	19.5 (14-28)	**<0.001**
AST[#] (IU/ L)		20 (16-24.9)	19 (16-24)	20.4 (17-25)	**<0.001**
ALP[¶] (IU/ L)		250 (207-302)	258 (210-313)	242 (204-289)	**<0.001**
GGT[] (IU/ L)		21.99 (16.06-32.27)	20.7 (15.06-30.77)	23.41 (17.25-33.4)	**<0.001**
Number (%)					
Age ≥ 55 years		5489 (49.55)	2790 (46.86)	2699 (52.68)	**<0.001**
Opium		1847 (16.63)	536 (8.98)	1311 (25.52)	**<0.001**
Tobacco		1653 (14.88)	68 (1.14)	1585 (30.85)	**<0.001**
Metabolic Syndrome		3269 (29.43)	1947 (32.62)	1322 (25.73)	**<0.001**
Hypertension		4,512 (40.63)	2,614 (43.79)	1,898 (36.95)	**<0.001**
Diabetes		1,709 (15.39)	1,011 (16.94)	698 (13.59)	**<0.001**
Rural residence		9105 (81.98)	4936 (82.69)	4169 (81.16)	**0.036**
Physical Activity	**Low**	3911 (35.29)	873 (14.65)	3038 (59.31)	**<0.001**
	Moderate	3431 (30.96)	2311 (38.78)	1120 (21.87)	
	High	3739 (33.74)	2775 (46.57)	964 (18.82)	

BMI[]: Body mass index; ALT[*]: Alanine aminotransferase; AST[#]: Aspartate aminotransferase; ALP[¶]: Alkaline phosphatase; GGT[]: Gamma-glutamyl transpeptidase;

Table 3: The association between baseline liver-enzymes and mortality according to the cause, after excluding the data of first year of follow-up, viral hepatitis patients and participants with history of CVD[&] or cancer (n =9351)

Liver enzymes	All-cause deaths (n=632)		CVD-related deaths (n=202)		Cancer-related deaths (n=167)		Others-cause deaths (n=225)	
	aHR[a] (95% CI)	P-Value	aHR (95% CI)	P-Value	aHR (95% CI)	P-Value	aHR (95% CI)	P-Value
ALT*								
Q1	**1.32 (1.0-1.7)**	**0.028**	**1.48** (1.0-2.1)	**0.047**	1.02 (0.6-1.7)	0.542	1.40 (0.9-2.1)	0.108
Q2	0.99 (0.8-11.3)	0.944	1.10 (0.7-1.7)	0.574	1.18 (0.8-2.1)	0.693	0.97 (0.6-1.5)	0.913
Q3	-------	-------	-------	-------	-------	-------	-------	-------
Q4	0.98 (0.7-1.3)	0.841	1.22 (0.8-2.0)	0.414	0.95 (0.6-1.5)	0.473	0.73 (0.4-1.2)	0.241
Q5	1.12 (0.8-1.4)	0.405	1.16 (0.7-1.9)	0.558	1.23 (0.8-2.0)	0.143	1.08 (0.7-1.7)	0.779
***AST**[#]*								
Q1	-------	-------	-------	-------	-------	-------	-------	-------
Q2	0.81 (0.6-1.1)	0.103	0.82 (0.5-1.2)	0.264	0.91 (0.5-1.6)	0.723	0.86 (0.6-1.3)	0.437
Q3	0.84 (0.6-1.1)	0.157	0.73 (0.4-1.0)	0.071	1.48 (0.9-2.4)	0.111	**0.65** (0.4-0.9)	**0.040**
Q4	0.90 (0.7-1.1)	0.389	0.87 (0.6-1.3)	0.505	1.33 (0.8-2.2)	0.255	0.72 (0.5-1.1)	0.102
Q5	0.92 (0.7-1.2)	0.532	0.94 (0.6-1.5)	0.915	1.36 (0.8-2.3)	0.227	0.76 (0.5-1.2)	0.200
***ALP**[¶]*								
Q1	------	-------	-------	-------	-------	-------	-------	-------
Q2	1.05 (0.8-	0.739	1.13 (0.6-	0.669	1.32 (0.7-	0.347	0.78 (0.5-	0.324

	1.4)		1.9)		2.3)		1.3)	
Q3	**1.39** (1.1-1.9)	**0.021**	1.40 (0.8-2.4)	0.208	1.63 (0.9-2.8)	0.088	1.08 (0.7-1.7)	0.761
Q4	**1.34** (1.1-1.8)	**0.039**	1.59 (0.9-2.6)	0.072	1.50 (0.8-2.6)	0.153	1.05 (0.7-1.6)	0.814
Q5	**1.99** (1.5-2.6)	**<0.001**	**2.28** (1.4-3.7)	**0.001**	**1.90** (1.1-3.2)	**0.022**	**1.74** (0.2-2.6)	**0.008**
GGT[¶]								
Q1	-------	-------	-------	-------	-------	-------	-------	-------
Q2	1.05 (0.8-1.3)	0.711	1.36 (0.8-2.2)	0.206	0.81 (0.5-1.3)	0.386	0.97 (0.6-1.5)	0.872
Q3	0.98 (0.7-1.3)	0.891	**1.62** (1.0-2.6)	**0.045**	**0.59** (0.4-1.0)	**0.050**	0.96 (0.6-1.5)	0.849
Q4	1.22 (0.9-1.6)	0.120	1.53 (0.9-2.5)	0.083	0.80 (0.5-1.3)	0.381	1.37 (0.9-2.1)	0.137
Q5	**1.48** (1.2-1.9)	**<0.001**	**1.90** (1.2-3.0)	**0.008**	1.11 (0.7-1.7)	0.653	**1.57** (1.0-2.4)	**0.032**

aHR[a]=Adjusted Hazard Ratio; CVD[&]: Cardiovascular disease; ALT[*]: Alanine aminotransferase; AST[#]: Aspartate aminotransferase; ALP[¶]: Alkaline phosphatase; GGT[▯]: Gamma-glutamyl transpeptidase

Table S1: Baseline characteristics of participants according to ALT* quintiles in total population							
Variables		ALT*					
		Q1	Q2	Q3	Q4	Q5	P-value
Mean (SD)							
Age		57.76 (9.12)	56.76 (8.2)	56.26 (7.89)	55.64 (7.42)	54.81 (6.87)	**<0.001**
BMI		24.8 (5.31)	26.37 (5.24)	27.27 (5.07)	28.3 (5.06)	28.82 (5.03)	**<0.001**
Number (%)							
Age ≥ 55 years		1115 (55.36)	1272 (52.74)	1057 (49.95)	1069 (47.2)	965 (42.91)	**<0.001**
Male gender		986 (48.88)	1112 (46.01)	994 (46.82)	968 (42.66)	1059 (47.02)	**<0.001**
Metabolic Syndrome		322 (15.96)	581 (24.04)	635 (29.91)	785 (34.6)	937 (41.61)	**<0.001**
Tobacco		467 (23.15)	409 (16.92)	284 (13.38)	231 (10.18)	260 (11.55)	**<0.001**
Opium		642 (31.83)	463 (19.16)	275 (12.95)	251 (11.06)	210 (9.33)	**<0.001**
Diabetes		180 (8.92)	298 (12.33)	313 (14.74)	399 (17.58)	517 (22.96)	**<0.001**
Hypertension		736 (36.49)	979 (40.5)	886 (41.73)	936 (41.25)	1285 (42.94)	**<0.001**
Rural residence		1686 (83.59)	2017 (83.45)	1748 (82.34)	1818 (80.12)	1809 (80.33)	**0.002**
Physical activity	**Low**	801 (39.77)	833 (34.55)	754 (35.62)	710 (31.42)	801 (35.58)	**<0.001**
	Moderate	568 (28.2)	757 (31.4)	665 (31.41)	736 (32.57)	698 (31.01)	
	High	645 (32.03)	821 (34.05)	698 (32.97)	814 (36.02)	752 (33.41)	
Median (Q25-Q75)							
Cholesterol *(mg/dl)*		190 (165-216)	196 (173-222)	202 (176-229)	205 (179-233)	207 (178-236)	**<0.001**

Triglyceride *(mg/dl)*	99 (78-134)	108 (82-148)	115 (86-160)	123 (91-175)	132 (95-185)	**<0.001**

Q= Quintile

ALT*: Alanine aminotransferase; BMI: Body mass index

Table S2: Baseline characteristics of participants according to AST[#] quintiles in total population							
Variables		**AST**					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P-value**
Mean (SD)							
Age		56.71 (8.35)	56.14 (8.01)	56.33 (8.02)	56 (7.86)	55.95 (7.64)	0.075
BMI		27.54 (5.42)	27.34 (5.27)	26.92 (5.21)	26.84 (5.23)	27.16 (5.48)	**<0.001**
Number (%)							
Age ≥ 55 years		1047 (52.12)	1068 (49.24)	1090 (49.28)	1198 (49.2)	1068 (48.0)	0.104
Male gender		909 (45.18)	988 (45.51)	1048 (47.31)	1145 (46.83)	1026 (46.03)	0.595
Metabolic Syndrome		654 (32.5)	681 (31.37)	610 (27.54)	622 (25.44)	686 (30.78)	**<0.001**
Tobacco		366 (18.19)	341 (15.71)	330 (14.90)	318 (13.01)	292 (13.10)	**<0.001**
Opium		415 (20.63)	398 (18.33)	343 (15.49)	368 (15.05)	318 (14.27)	**<0.001**
Diabetes		454 (22.56)	349 (16.08)	1920 (13.32)	285 (11.66)	1905 (14.54)	**<0.001**
Hypertension		850 (42.25)	923 (42.51)	864 (39.01)	955 (39.06)	909 (40.78)	**0.035**
Rural residence		1571 (78.08)	1765 (81.3)	1809 (81.67)	2061 (84.29)	1870 (83.89)	**<0.001**
Physical activity	**Low**	764 (38.07)	776 (35.83)	762 (34.46)	857 (35.17)	737 (33.11)	0.052
	Moderate	604 (30.09)	640 (29.55)	711 (32.16)	751 (30.82)	715 (32.12)	
	High	639 (31.84)	750 (34.63)	738 (33.38)	829 (34.2)	774 (34.77)	
Median (Q25-Q75)							

Cholesterol *(mg/dl)*	198 (173-227)	198 (175-227)	199(174-226)	200 (175-228)	203 (174-232)	**<0.001**
Triglyceride *(mg/dl)*	120 (90-166)	117 (88-163)	112 (85-159)	112 (83-155)	114 (85-163)	**<0.001**
Q=Quintile AST[#]: Aspartate aminotransferase; BMI: Body mass index						

Table S3: Baseline characteristics of participants according to ALP¶ quintiles in total population							
Variables		**ALP**					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P-value**
Mean (SD)							
Age		54.62 (8.19)	55.88 (7.98)	56.37 (7.79)	56.8 (7.9)	57.37 (7.73)	**<0.001**
BMI[]		26.99 (5.23)	27.16 (5.2)	27.39 (5.48)	27.13 (5.32)	27.05 (5.38)	0.290
Number (%)							
Age ≥ 55 years		856 (39.48)	1035 (46.32)	1131 (50.95)	1168 (52.95)	1279 (57.61)	**<0.001**
Male gender		1007 (46.3)	1046 (46.78)	1029 (46.29)	1012 (45.77)	1023 (45.98)	0.971
Metabolic Syndrome		489 (22.48)	570 (25.49)	673 (30.27)	713 (32.25)	806 (36.22)	**<0.001**
Tobacco		257 (11.82)	294 (13.15)	312 (14.04)	366 (16.55)	418 (18.79)	**<0.001**
Opium		243 (11.17)	313 (14.00)	372 (16.73)	419 (18.95)	495 (22.25)	**<0.001**
Diabetes		240 (11.03)	315 (14.09)	339 (15.25)	364 (16.46)	447 (20.09)	**<0.001**
Hypertension		755 (34.71)	849 (37.97)	934 (42.02)	921 (41.66)	1040 (46.74)	**<0.001**
Rural residence		1696 (77.98)	1813 (81.08)	1800 (80.97)	1840 (83.22)	1925 (86.52)	**<0.001**
Physical activity	**Low**	706 (32.49)	784 (35.19)	796 (35.84)	774 (35.1)	837 (37.74)	**<0.001**
	Moderate	637 (29.31)	668 (29.98)	706 (31.79)	720 (32.65)	690 (31.11)	
	High	830 (38.2)	776 (34.83)	719 (32.37)	711 (32.24)	691 (31.15)	
Median (Q25-Q75)							

Cholesterol *(mg/dl)*	195 (171-221)	198 (174-228)	201 (177-229)	201 (175-231)	204 (175-233)	**<0.001**
Triglyceride *(mg/dl)*	106 (80-146)	110 (84-155)	117 (88-160)	119 (87-168)	123 (91-175)	**<0.001**
Q=Quintile ALP¶: Alkaline phosphatase; BMI: Body mass index						

Table S4: Baseline characteristics of participants according to GGT quintiles in total population							
Variables		**GGT[]**					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P-value**
Mean (SD)							
Age		56.19 (8.57)	56.41 (8.25)	56.37 (7.92)	56.08 (7.54)	56.01 (7.52)	0.441
BMI[]		25.15 (5.14)	26.44 (5.13)	27.47 (5.21)	28.29 (5.2)	28.43 (5.24)	**<0.001**
Number (%)							
Age ≥ 55 years		1042 (47.23)	1124 (50.47)	1087 (49.73)	1108 (50.39)	1091 (49.64)	0.19
Male gender		1028 (46.54)	1029 (46.18)	1015 (46.35)	1017 (46.21)	1007 (45.79)	0.992
Metabolic Syndrome		283 (12.81)	467 (20.96)	654 (29.86)	844 (38.35)	994 (45.2)	**<0.001**
Tobacco		324 (14.67)	325 (14.59)	319 (14.57)	350 (15.90)	325 (14.78)	0.694
Opium		362 (16.39)	349 (15.66)	376 (17.17)	351 (15.95)	400 (18.19)	0.157
Diabetes		138 (6.25)	224 (10.05)	295 (13.47)	455 (20.67)	591 (26.88)	**<0.001**
Hypertension		710 (32.14)	858 (38.51)	921 (42.05)	1007 (45.75)	996 (45.29)	**<0.001**
Rural residence		1934 (87.55)	1836 (82.41)	1754 (80.09)	1749 (79.46)	1759 (79.99)	**<0.001**
Physical activity	**Low**	744 (33.76)	751 (33.78)	752 (34.43)	773 (35.2)	856 (39.0)	**<0.001**
	Moderate	663 (30.08)	668 (30.05)	681 (31.18)	711 (32.38)	682 (31.07)	
	High	797 (36.16)	804 (36.17)	751 (34.39)	712 (32.42)	657 (29.93)	
Median (Q25-Q75)							
Cholesterol *(mg/dl)*		187 (164-	197 (172-	210 (175-	205 (180-	212 (183-	**<0.001**

	212)	223)	230)	235)	240)	
Triglyceride *(mg/dl)*	93 (75-123)	107 (80-142)	116 (88-160)	130 (96-180)	139 (101-200)	**<0.001**
Q= Quintile GGT: Gamma glutamyl transpeptidase; BMI: Body mass index						

Figures

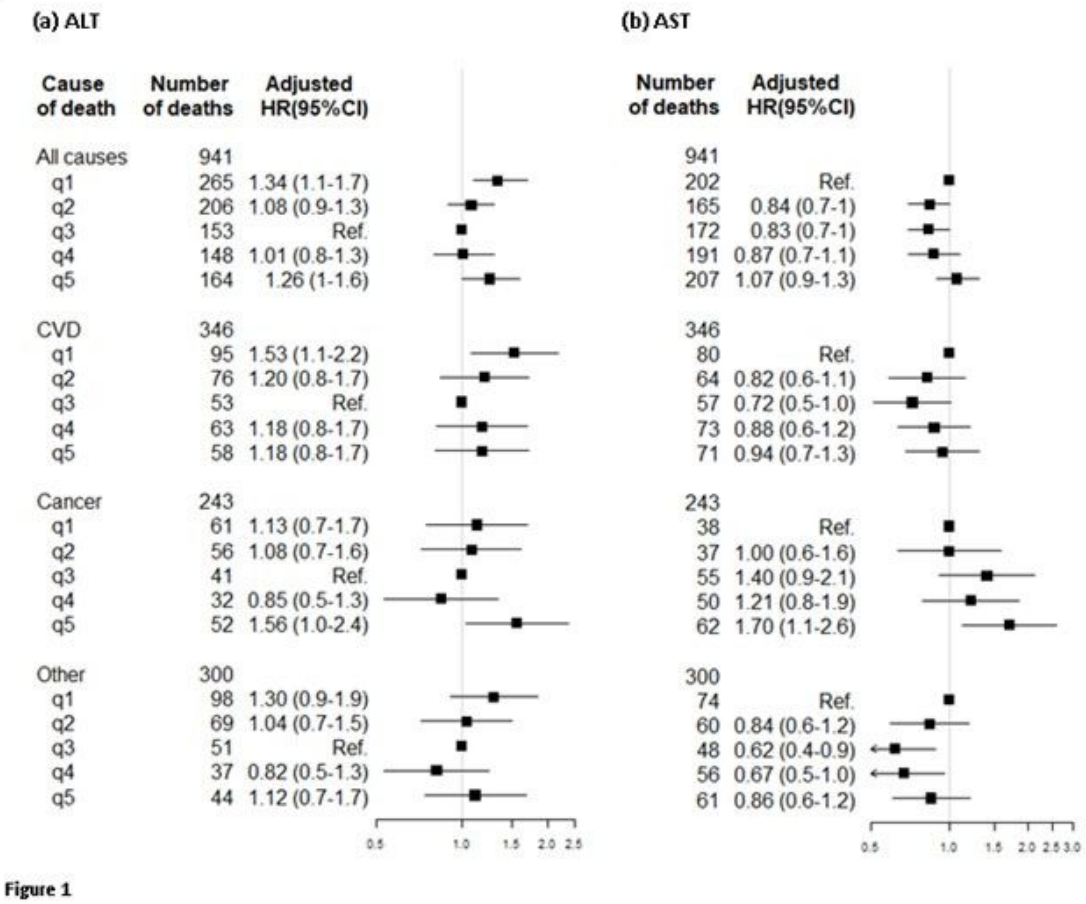

Figure 1

The association of ALT and AST with all-cause and cause-specific mortalities in the total population. (a) Adjusted Hazard Ratios (aHR) for quintiles of ALT, categorized by type of mortality. 3rd quintile is set as a reference. (b) Adjusted Hazard Ratios (aHR) for quintiles of AST, categorized by type of mortality. 1st quintile is set as a reference. All associations are adjusted for age, sex, tobacco consumption, opium consumption, metabolic syndrome, total cholesterol, residence, physical activity and BMI.

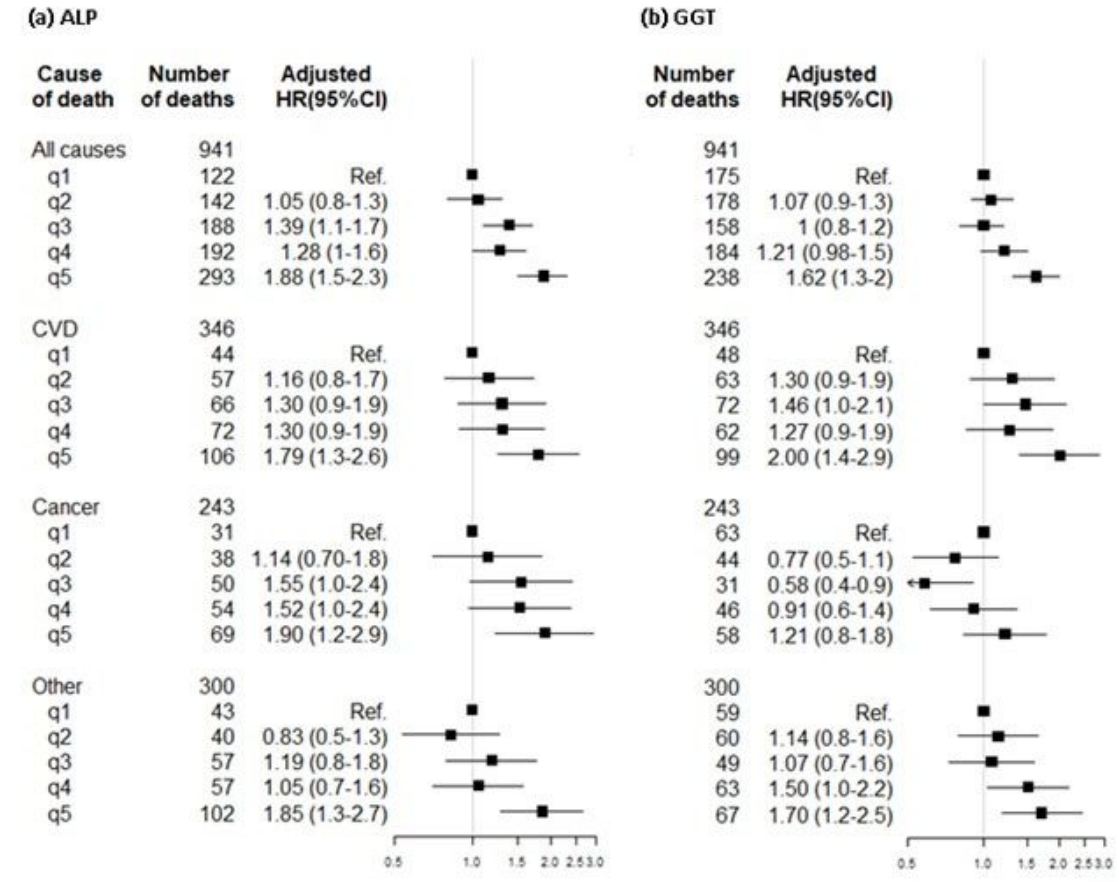

Figure 2

Figure 2

The association of ALP and GGT with all-cause and cause-specific mortalities in the total population. (a) Adjusted Hazard Ratios (aHR) for quintiles of ALP, categorized by type of mortality. 1st quintile is set as a reference. (b) Adjusted Hazard Ratios (aHR) for quintiles of GGT, categorized by type of mortality. 1st quintile is set as a reference. All associations are adjusted for age, sex, tobacco consumption, opium consumption, metabolic syndrome, total cholesterol, residence, physical activity and BMI.

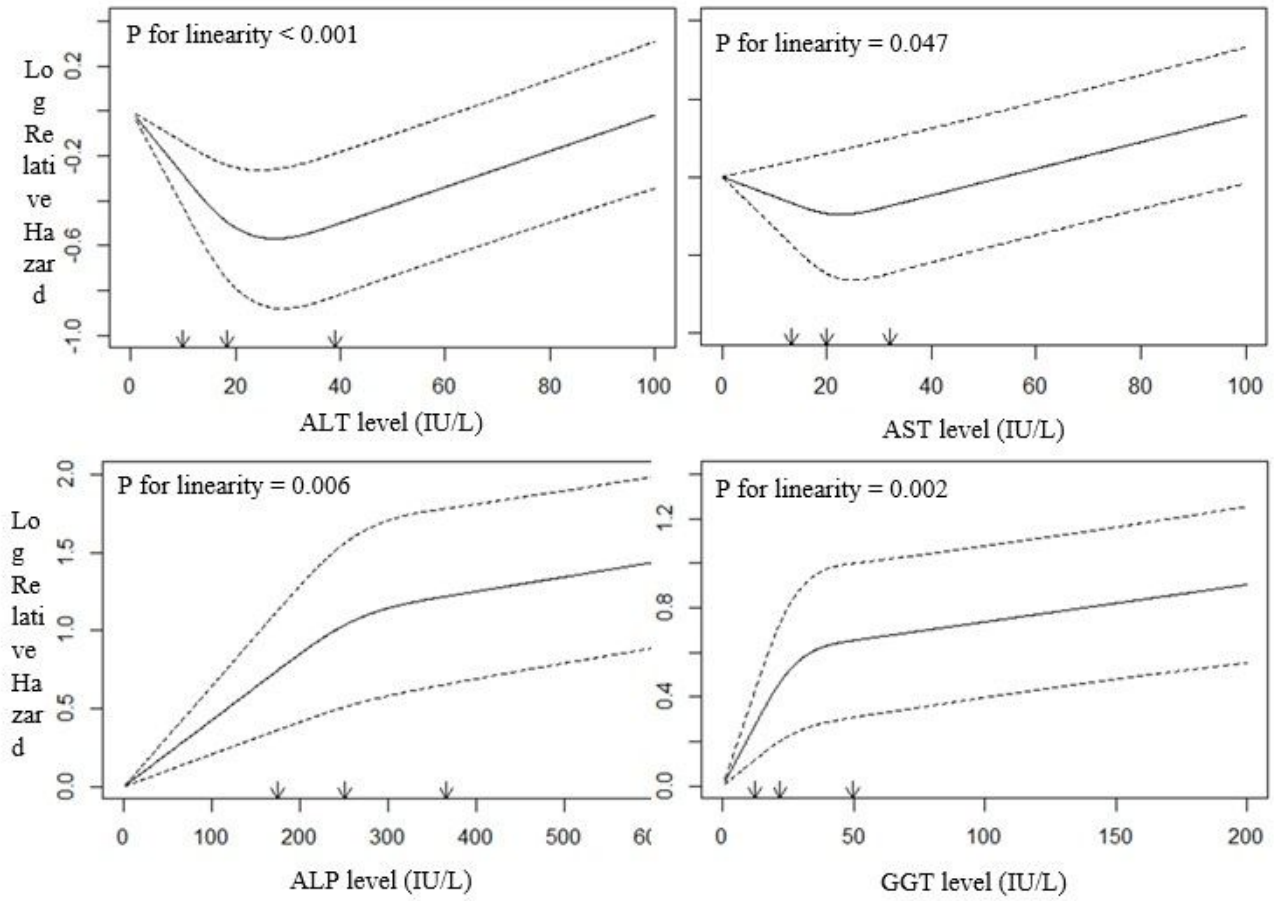

Figure 3

Adjusted association of all-cause mortality with serum level enzymes in total population, illustrated as a restricted cubic spline with the minimum levels as a reference.