PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale national health screening cohort study
AUTHORS	Kim, Kyoung-Nam; Joo, Jungmin; Sung, Ho Kyung; Kim, Chee Hae; Kim, Haebin; Kwon, Yong Jin

VERSION 1 - REVIEW

REVIEWER	Prashant Sakharkar Roosevelt University College of Pharmacy Schaumburg, IL 60173 USA
REVIEW RETURNED	31-Oct-2018

GENERAL COMMENTS	Dear Authors, The research study seems interesting, however, there are some issues that deserves your attention. Please clarify if you are suggesting the association between liver enzymes levels and mortality remained unclear in general or more specifically in population with conditions such CVD, DM or cancer. (Page 6, line 17-22). Since, association of liver enzymes in such condition is well documented in published literature. The exclusion criteria in this study seem to be a limiting step, since exclusion only limited to population with hepatitis and chronic liver disease. Certainly there are lots of other disease conditions (could be a part of data used) that impacts liver enzyme levels that weren't excluded, which may have confounded the study result. In my opinion, readers will benefit more by understanding the impact of high/low levels of liver enzymes on mortality rather than their association based on enzyme level distribution in terms of decile and its association with mortality. It would be great if the cut-off points used for liver enzymes for gender-based analyses, as well the medians and quartiles are reported for the benefit of the readers (page 11, line 34-37). I would be interested in knowing what levels of liver enzymes regardless of decile considered to be normal based on lab data used. This will help readers to understand study results in clinical context and assist them to draw their own conclusions.

	As indicated, use of lowest betas for each outcome of interest in the association analysis as the referent categories suggests these values correspond to decile 4 (ALT 17-18U/L) and for AST (21U/L) based on Table 2. Are these values falls under the normal cut off range for ALT and AST. If not, then what are the clinical implications and how are they significant in terms of mortality? Internationally, values of ALT (>40 U/L) and AST (> 37 U/L) considered being normal, and these values seem to be quite low. Non- monotonic dose response and their relationship to risk assessment is still of questionable relevance. Finding based on inconsistent reporting of liver enzymes levels by different laboratories and sensitivity and specificity of such diagnostics tests is more often questionable and should be included as one of the limitations of study.

REVIEWER	Amedeo Lonardo AOU Modena, Italy
REVIEW RETURNED	14-Nov-2018

GENERAL COMMENTS	GENERAL COMMENT This is an interesting report of a large survey. The manuscript may be improved based on my specific suggestions appended below. SPECIFIC COMMENT MAJOR The rationale of the study is not clear: further to stating that previous studies relating liver enzymes with clinical outcomes have been inconsistent what did these Authors expect to find and why ? non-monotonic dose-response associations□ please define this concept when used for the first time (now this is explained in the discussion only). Both in the box and in the text address another limitation of the present submission, which fails to identify the mechanisms leading from deranged liver enzymes to events/mortality. A short section should discuss - What the chief causes of raised liver enzymes probabilistically are in relation to the overall prevalence of various liver diseases in the general population (Dig Liver Dis. 2010;42:272-82). - What relationship, if any, such causes of raised liver enzymes have on the risk of mortality in the most common liver disease, NAFLD (Dig Liver Dis. 2017;49:471-483; J Hepatol. 2016;65:589-600; Indian J Med Res. 2016 ;143:4-7). MINOR Reference 1 appears to be inappropriate. Please, consider Gastroenterology. 2002;123:1367-84.

REVIEWER	Canbay University of Magdeburg Germany
REVIEW RETURNED	07-Feb-2019

GENERAL COMMENTS	This paper by Kim et al. is describing the associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale

	national health screening cohort study. This paper is well written and there findings are of clinical relevance. Indeed, the liver as central organ of lipid and glucose metabolism is important for the whole organism (Bechmann L J Hep. 2012). 1. The authors should discuss that also liver enzymes even in normal ranges have some metabolic alterations. And we may discuss about normal ranges ! This should be at least discussed. Indeed, the liver enzymes are elevated in higher BMI groups, even in normal ranges! This is important! (Kälsch J et al. Scientific reports 2015). 2. It has also been published that liver enzymes are associated with stenose diameter in acute myocardial infraction (Baars T et al. Medicine 2015). This might also have some impact on mortality!

VERSION 1 – AUTHOR RESPONSE

Reviewers' Comments to Authors:

Reviewer #1

Comments to the Authors

Dear authors, the research study seems interesting, however, there are some issues that deserves your attention.

1. Please clarify if you are suggesting the association between liver enzyme levels and mortality remained unclear in general or more specifically in population with conditions such as CVD, DM or cancer (Page 6, line 17-22). Since, association of liver enzymes in such condition is well documented in published literature.

Thank you for your valuable comment. We aimed to explain that the association between liver enzyme levels and mortality remained unclear in the general population. We agree that the description in the original manuscript was not clear. Therefore, we have revised the manuscript as follows:

In the INTRODUCTION:

"Although liver enzyme levels have been associated with various health outcomes such as cardiovascular diseases,(1) type 2 diabetes mellitus,(2) and cancer,(3) the associations between liver enzyme levels and mortality remained unclear. Previous studies reported positive, null, and inverse associations between liver enzyme levels and mortality (reviewed by Kunstor et al.(4))."

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"Although liver enzyme levels have been associated with various health outcomes such as cardiovascular diseases,(1) type 2 diabetes mellitus,(2) and cancer,(3) the associations between liver enzyme levels and mortality in the general population remained unclear. Previous studies reported positive, null, and inverse associations between liver enzyme levels and mortality in the general population (reviewed by Kunstor et al.(4))."

2. The exclusion criteria in this study seem to be a limiting step, since exclusion only limited to population with hepatitis and chronic liver disease. Certainly, there are lots of other disease condition

(could be a part of data used) that impacts liver enzyme levels that weren't excluded, which may have confounded the study result.

Thank you for your insightful comment, which has helped us to improve the quality of the study. We agree that there are other conditions that impact liver enzyme levels but were not excluded. However, when we further exclude individuals with a history of cancer (due to hepatobiliary cancer, pancreatic cancer, and metastatic cancer, n = 2,732), those with a history of type 2 diabetes (n = 20,691), and those who drank alcohol ≥3–4 times/week (n = 53,350), the results did not change appreciably: compared with the 4th decile of baseline ALT levels (17–18 U/L) and the 4th decile of baseline AST levels (21 U/L), the risk of all-cause mortality was higher in both the higher and lower baseline ALT and AST deciles (10th decile: HR = 1.43, 95% CI: 1.34, 1.54; 1st decile: HR = 1.14, 95% CI: 1.07, 1.21 for ALT; 10th decile: HR = 1.65, 95% CI: 1.53, 1.78; 1st decile: HR = 1.14, 95% CI: 1.05, 1.24 for AST). When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile: HR = 2.04, 95% CI: 1.89, 2.19). Compared with the 9th decile of ALT changes (8–13 U/L), the 6th decile of AST changes (1 U/L), and the 4th decile of GGT changes (-3 to -2 U/L), respectively, the risk of all-cause mortality was higher in both the higher and lower deciles of changes in the corresponding liver enzyme levels (10th decile: HR = 1.36, 95% CI: 1.22, 1.51, 1st decile: HR = 1.47, 95% CI: 1.33, 1.63 for ALT; 10th decile: HR = 1.42, 95% CI: 1.27, 1.60, 1st decile: HR = 1.46, 95% CI: 1.30, 1.64 for AST; 10th decile: HR = 1.77, 95% CI: 1.59, 1.97, 1st decile: HR = 1.72, 95% CI: 1.54, 1.92 for GGT). We have revised the manuscript as follows:

In the METHODS:

"In a sensitivity analysis, we repeated all analyses using sex-specific cut-points for deciles of the baseline liver enzyme levels and changes in liver enzyme levels, instead of the above mentioned sex-non-specific cut-points, to confirm the robustness of the results.[7]"

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"In a sensitivity analysis, because distributions of liver enzyme levels were different by sex (U/L, ALT: median, 25, interquartile range [IQR], 16 among men; median, 18, IQR, 10 among women; AST: median, 25, IQR, 11 among men; median, 22, IQR, 9 among women; GGT: median, 33, IQR, 34 among men; median, 16, IQR, 12 among women), we repeated all analyses using sex-specific cut-off points for deciles of the baseline liver enzyme levels and changes in liver enzyme levels, instead of the above mentioned sex-non-specific cut-off points, to confirm the robustness of the results.[7] Detailed information on the sex-specific cut-off points is presented in the Supplementary Material. In addition, we also conducted analyses further excluding individuals with a history of cancer, history of type 2 diabetes mellitus, and those who drank alcohol ≥3–4 times/week, because these conditions can also impact liver enzyme levels."

In the RESULTS:

"In sensitivity analyses based on sex-specific deciles of the baseline and changes in liver enzyme levels, the results remained robust and did not change appreciably (data not shown)."

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"In sensitivity analyses based on sex-specific deciles of the baseline and changes in liver enzyme levels, the results remained robust and did not change appreciably (data not shown). When we excluded individuals with a history of cancer (n = 2,732), those with a history of type 2 diabetes mellitus (n = 20,691), and those who drank alcohol ≥3–4 times/week (n = 53,350), the results did not change appreciably (data not shown)."

3. In my opinion, readers will benefit more by understanding the impact of high/low levels of liver enzymes on mortality rather than their association based on enzyme level distribution in terms of decile and its association with mortality.

Thank you for your valuable comment, which has helped us to improve the quality and readability of the manuscript. We agree with your comment that readers will benefit more by understanding the impact of high/low levels of liver enzymes on mortality. Although the normal range of ALT, AST, and GGT may vary by age, sex, laboratory, and other conditions, generally, the normal range of ALT is considered as 7–56 U/L, AST as 0–35 U/L, and GGT as 9¬–85 U/L.(1) In the present study, the 1st and 10th decile of ALT, AST, and GGT can be considered to include the abnormal range, and the 2nd to 9th deciles of ALT, AST, and GGT can be considered to conform to the normal range (Table 2). Therefore, even in the normal range, lower or higher levels of ALT and AST and higher levels of GGT were found to be associated with higher mortality risk. We have revised the manuscript as follows:

In the RESULTS:

“Compared with the 4th decile of baseline ALT levels (17–18 U/L), the risk of all-cause mortality was higher in both the higher and lower baseline ALT deciles (10th decile: HR = 1.53, 95% confidence interval [CI]: 1.44, 1.62; 1st decile: HR = 1.16, 95% CI: 1.10, 1.23). Similarly, compared with the 4th decile of baseline AST levels (21 U/L), the risk of all-cause mortality was also higher in both the higher and lower baseline AST deciles (10th decile: HR = 1.70, 95% CI: 1.59, 1.81; 1st decile: HR = 1.15, 95% CI: 1.07, 1.24). When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile: HR = 2.05, 95% CI: 1.93, 2.18) (table 2).”

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“Compared with the 4th decile of baseline ALT levels (17–18 U/L), the risk of all-cause mortality was higher in both the higher and lower baseline ALT deciles (10th decile, ≥43 U/L: HR = 1.53, 95% confidence interval [CI]: 1.44, 1.62; 1st decile, ≤12 U/L: HR = 1.16, 95% CI: 1.10, 1.23). Similarly, compared with the 4th decile of baseline AST levels (21 U/L), the risk of all-cause mortality was also higher in both the higher and lower baseline AST deciles (10th decile, ≥38 U/L: HR = 1.70, 95% CI: 1.59, 1.81; 1st decile, ≤16 U/L: HR = 1.15, 95% CI: 1.07, 1.24). When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile, ≥72 U/L: HR = 2.05, 95% CI: 1.93, 2.18) (table 2). Because the normal range of ALT is generally considered as 7–56 U/L, AST as 0–35 U/L, and GGT as 9¬–85 U/L,[2] even within the normal range, lower or higher levels of ALT and AST and higher levels of GGT were associated with higher mortality risk (Table 2).”

In the RESULTS:

“Compared with the 9th decile of ALT changes (8–13 U/L), the 6th decile of AST changes (1 U/L), and the 4th decile of GGT changes (-3 to -2 U/L), respectively, the risk of all-cause mortality was higher in both the higher and lower deciles of changes in the corresponding liver enzyme levels (10th decile: HR = 1.36, 95% CI: 1.24, 1.48, 1st decile: HR = 1.46, 95% CI: 1.34, 1.59 for ALT; 10th decile: HR = 1.55, 95% CI: 1.40, 1.71, 1st decile: HR = 1.53, 95% CI: 1.38, 1.69 for AST; 10th decile: HR = 1.71, 95% CI: 1.56, 1.88, 1st decile: HR = 1.67, 95% CI: 1.52, 1.84 for GGT) (table 3).”

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“Compared with the 9th decile of ALT changes (8–13 U/L), the 6th decile of AST changes (1 U/L), and the 4th decile of GGT changes (-3 to -2 U/L), respectively, the risk of all-cause mortality was higher in both the higher and lower deciles of changes in the corresponding liver enzyme levels (10th decile, ≥14 U/L: HR = 1.36, 95% CI: 1.24, 1.48, 1st decile, ≤-16 U/L: HR = 1.46, 95% CI: 1.34, 1.59 for ALT; 10th decile, ≥11 U/L: HR = 1.55, 95% CI: 1.40, 1.71, 1st decile, ≤-12 U/L: HR = 1.53, 95% CI: 1.38,

1.69 for AST; 10th decile, ≥21 U/L: HR = 1.71, 95% CI: 1.56, 1.88, 1st decile, ≤-19 U/L: HR = 1.67, 95% CI: 1.52, 1.84 for GGT) (table 3)."

4. It would be great if the cut-off points used for liver enzymes for gender-based analyses, as well the medians and quartiles are reported for the benefit of the readers (page 11, line 34-37).

Thank you for your valuable comment, which has helped us to improve the clarity and readability of the manuscript. We agree that the manuscript would be improved if the cut-off points used for liver enzymes for gender-based analyses as well as the medians and quartiles were reported. According to your suggestion, we have revised the manuscript as follows:

In the METHODS:

"In a sensitivity analysis, we repeated all analyses using sex-specific cut-points for deciles of the baseline liver enzyme levels and changes in liver enzyme levels, instead of the above mentioned sex-non-specific cut-points, to confirm the robustness of the results.[7]"

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"In a sensitivity analysis, because distributions of liver enzyme levels were different by sex (U/L, ALT: median, 25, interquartile range [IQR], 16 among men; median, 18, IQR, 10 among women; AST: median, 25, IQR, 11 among men; median, 22, IQR, 9 among women; GGT: median, 33, IQR, 34 among men; median, 16, IQR, 12 among women), we repeated all analyses using sex-specific cut-off points for deciles of the baseline liver enzyme levels and changes in liver enzyme levels, instead of the above mentioned sex-non-specific cut-off points, to confirm the robustness of the results.[7] Detailed information on the sex-specific cut-off points is presented in the Supplementary Material."

In the SUPPLEMENTARY METHODS:

"In a sensitivity analysis, because distributions of liver enzyme levels were different by sex (U/L, ALT: median, 25, interquartile range [IQR], 16 among men; median, 18, IQR, 10 among women; AST: median, 25, IQR, 11 among men; median, 22, IQR, 9 among women; GGT: median, 33, IQR, 34 among men; median, 16, IQR, 12 among women), we repeated all analyses using sex-specific cut-off points for deciles of the baseline liver enzyme levels (U/L, ALT: ≤14, 15–16, 17–19, 20–21, 22–24, 25–27, 28–31, 32–37, 38–48, or ≥49 among men; ≤10, 11–12, 13–14, 15–16, 17, 18–19, 20–22, 23–26, 27–33, or ≥34 among women; AST: ≤17, 18–19, 20–21, 22–23, 24–25, 26–27, 28–29, 30–33, 34–40, or ≥41 among men; ≤15, 16–17, 18–19, 20, 21–22, 23, 24–25, 26–28, 29–33, or ≥34 among women; GGT: ≤15, 16–19, 20–23, 24–27, 28–32, 33–39, 40–48, 49–63, 64–95, or ≥96 among men; ≤9, 10–11, 12, 13–14, 15–16, 17–18, 19–21, 22–26, 27–35, or ≥36 among women) and changes in liver enzyme levels (U/L, ALT: ≤-19, -18 to -11, -10 to -7, -6 to -4, -3 to -1, 0–1, 2–4, 5–7, 8–14, or ≥15 among men; ≤-12, -11 to -7, -6 to -4, -3 to -2, -1 to 0, 1–2, 3–4, 5–7, 8–12, or ≥13 among women; AST: ≤-13, -12 to -8, -7 to -5, -4 to -3, -2 to -1, 0–1, 2–3, 4–6, 7–10, or ≥11 among men; ≤-10, -9 to -6, -5 to -3, -2, -1 to 0, 1–2, 3, 4–6, 7–10, or ≥11 among women; GGT: ≤-27, -26 to -13, -12 to -7, -6 to -3, -2 to 0, 1–3, 4–7, 8–13, 14–27, or ≥28 among men; ≤-10, -9 to -5, -4 to -3, -2 to -1, 0–1, 2, 3–4, 5–7, 8–13, or ≥14 among women), instead of the above mentioned sex-non-specific cut-off points, to confirm the robustness of the results.[7]"

5. I would be interested in knowing what levels of liver enzymes regardless of decile considered to be normal based on lab data used. This will help readers to understand study results in clinical context and assist them to draw their own conclusions.

Thank you for your insightful comment. As we responded to the earlier comment, the normal range of ALT is considered as 7–56 U/L, AST as 0–35 U/L, and GGT as 9¬–85 U/L.(1) Therefore, in the present study, the 1st and 10th decile of ALT, AST, and GGT can be considered to include the abnormal range, and the 2nd to 9th deciles of ALT, AST, and GGT can be considered to conform to

the normal range (Table 2). It is notable that, even in the normal range, lower or higher levels of ALT and AST and higher levels of GGT were found to be associated with higher mortality risk. We have revised the manuscript to describe this point clearly as follows:

In the RESULTS:

“When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile: HR = 2.05, 95% CI: 1.93, 2.18) (table 2).”

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“When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile, ≥72 U/L: HR = 2.05, 95% CI: 1.93, 2.18) (table 2). Because the normal range of ALT is generally considered as 7–56 U/L, AST as 0–35 U/L, and GGT as 9¬–85 U/L,[2] even within the normal range, lower or higher levels of ALT and AST and higher levels of GGT were associated with higher mortality risk (Table 2).”

In the DISCUSSION:

“The results of the present study, which suggest the presence of a monotonic non-linear association between the baseline GGT levels and all-cause mortality, may explain those earlier findings.”

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“The results of the present study, which suggest the presence of a monotonic non-linear association between the baseline GGT levels and all-cause mortality, may explain those earlier findings.

It has been reported that serum ALT, AST, and GGT levels are associated with a higher risk of type 2 diabetes mellitus and body mass index even within the normal ranges.[27] In the present study, liver enzyme levels within normal ranges were also found to be associated with mortality. Because the liver is a central organ of glucose and lipid metabolism and critical for maintaining health,[28] re-evaluation of the relevance of current standards is warranted.”

6. As indicated, use of lowest betas for each outcome of interest in the association analysis as the referent categories suggests these values correspond to decile 4 (ALT 17–18 U/L) and for AST (21 U/L) based on Table 2. Are these values falls under the normal cut off range for ALT and AST? If not, then what are the clinical implications and how are they significant in terms of mortality? Internationally, values of ALT (>40 U/L) and AST (>37 U/L) considered being normal, and these values seem to be quite low.

Thank you for your helpful comment, which has helped us to improve the clarity and readability of the manuscript. Normal range of ALT, AST, and GGT may vary by age, sex, laboratory, and other conditions. However, in general, the normal range of ALT is considered as 7–56 U/L and AST as 0–35 U/L.(1) Therefore, ALT decile 4 (17–18 U/L) and AST decile 4 (21 U/L), which were used as the reference categories, could be assessed to fall under the normal cut-off range. Because, in the present study, the 1st and 10th decile of ALT, AST, and GGT can be considered to include the abnormal range, and the 2nd to 9th deciles of ALT, AST, and GGT can be considered to conform to the normal range, the results of the present study suggest that, even in the normal range, lower or higher levels of ALT and AST and higher levels of GGT would be associated with higher mortality risk (Table 2). We have revised the manuscript as follows:

In the RESULTS:

“When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile: HR = 2.05, 95% CI: 1.93, 2.18) (table 2).”

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“When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile, ≥72 U/L: HR = 2.05, 95% CI: 1.93, 2.18) (table 2). Because the normal range of ALT is generally considered as 7–56 U/L, AST as 0–35 U/L, and GGT as 9¬–85 U/L,[2] even within the normal range, lower or higher levels of ALT and AST and higher levels of GGT were associated with higher mortality risk (Table 2).”

7. Non-monotonic dose response and their relationship to risk assessment is still of questionable relevance. Finding based on inconsistent reporting of liver enzyme levels by different laboratories and sensitivity and specificity of such diagnostics tests is more often questionable and should be included as one of the limitations of study.

Thank you for your insightful comment. We agree that the potential problem should be mentioned as a limitation of this study. Therefore, we have revised the manuscript according to your comment, as follows:

In the DISCUSSION:

“The present study has some limitations. First, although the NHIS-HEALS provides information on serum ALT, AST, and GGT levels, data from other standard liver panel tests, such as albumin, alkaline phosphatase, and conjugated and unconjugated bilirubin levels, are not available. This limits our ability to assess liver function comprehensively.”

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“The present study has some limitations. First, although the NHIS-HEALS provides information on serum ALT, AST, and GGT levels, data from other standard liver panel tests, such as albumin, alkaline phosphatase, and conjugated and unconjugated bilirubin levels, are not available. This limits our ability to assess liver function comprehensively. Second, although the national health screening program was performed at designated health care institutions meeting the quality standards, liver enzyme levels were analysed by different laboratories with a different sensitivity and specificity, leading to potential information bias.”

Reviewer #2

Comments to the Author

General Comment

This is an interesting report of a large survey. The manuscript may be improved based on my specific suggestions appended below.

Specific Comment

Major

1. The rationale of the study is not clear: further to stating that previous studies relating liver enzymes with clinical outcomes have been inconsistent, what did these Authors expect to find and why?

Thank you for your valuable comment, which has helped us to improve the clarity and readability of the manuscript. We agree that the rationale of the study was not clear in the original manuscript. Therefore, we have revised the manuscript explaining the rationale of the study more clearly in the Introduction section:

In the INTRODUCTION:

“Although liver enzyme levels have been associated with various health outcomes such as cardiovascular diseases,[4] type 2 diabetes mellitus,[5] and cancer,[6] the associations between liver enzyme levels and mortality remained unclear. Previous studies reported positive, null, and inverse associations between liver enzyme levels and mortality (reviewed by Kunstor et al.[3]). To explain this inconsistency, recent studies suggested non-monotonic dose-response associations between liver enzyme levels and mortality, although studies investigating these associations remain scarce.[3,7,8]”

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“Although liver enzyme levels have been associated with various health outcomes such as cardiovascular diseases,[4] type 2 diabetes mellitus,[5] and cancer,[6] the associations between liver enzyme levels and mortality in the general population remained unclear. Previous studies reported positive, null, and inverse associations between liver enzyme levels and mortality in the general population (reviewed by Kunstor et al.[3]). The association between liver enzyme levels and mortality might not be linear, and both higher and lower liver enzyme levels would be associated with higher mortality (i.e., non-monotonic dose-response association). Although we expected the non-monotonic dose-response associations between liver enzyme levels and mortality, this possibility has only been investigated in limited number of studies.[3,7,8]”

2. Non-monotonic dose-response associations. Please define this concept when used for the first time (now this is explained in the discussion only).

Thank you for your helpful comment, which has helped us to improve the clarity and readability of the manuscript. We agree that the manuscript would be improved with the concept of non-monotonic dose-response associations defined when used for the first time. Therefore, we have revised the manuscript as follows:

In the INTRODUCTION:

“Although liver enzyme levels have been associated with various health outcomes such as cardiovascular diseases,[4] type 2 diabetes mellitus,[5] and cancer,[6] the associations between liver enzyme levels and mortality remained unclear. Previous studies reported positive, null, and inverse associations between liver enzyme levels and mortality (reviewed by Kunstor et al.[3]). To explain this inconsistency, recent studies suggested non-monotonic dose-response associations between liver enzyme levels and mortality, although studies investigating these associations remain scarce.[3,7,8]”

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“Although liver enzyme levels have been associated with various health outcomes such as cardiovascular diseases,[4] type 2 diabetes mellitus,[5] and cancer,[6] the associations between liver enzyme levels and mortality in the general population remained unclear. Previous studies reported positive, null, and inverse associations between liver enzyme levels and mortality in the general population (reviewed by Kunstor et al.[3]). The association between liver enzyme levels and mortality might not be linear, and both higher and lower liver enzyme levels would be associated with higher mortality (i.e., non-monotonic dose-response association). Although we expected the non-monotonic dose-response associations between liver enzyme levels and mortality, this possibility has only been investigated in limited number of studies.[3,7,8]”

3. Both in the box and in the text address another limitation of the present submission, which fails to identify the mechanisms leading from deranged liver enzymes to events/mortality. A short section should discuss

- What the chief causes of raised liver enzymes probabilistically are in relation to the overall prevalence of various liver diseases in the general population (Dig Liver Dis. 2010;42:272-82).

- What relationship, if any, such causes of raised liver enzymes have on the risk of mortality in the most common liver disease, NAFLD (Dig Liver Dis. 2017;49:471-483; J Hepatol. 2016;65:589-600; Indian J Med Res. 2016;143:4-7).

Thank you for your insightful comments. We agree that another limitation of the present study is that we failed to identify the mechanisms underlying the association between deranged liver enzymes and mortality, as you indicated. We also agree that NAFLD should be further investigated in the future studies, as an underlying mechanism. Therefore, we have revised the manuscript as follows:

In the ARTICLE SUMMARY:

"● Information on albumin, alkaline phosphatase, and conjugated and unconjugated bilirubin levels was not available.

● Used claims data in which information on sociodemographic factors, lifestyles, past medical histories, and family histories is insufficient."

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"● Information on albumin, alkaline phosphatase, and conjugated and unconjugated bilirubin levels was not available, and information on sociodemographic factors, lifestyles, past medical histories, and family histories was insufficient.

● Could not identify the mechanisms underlying the association between liver enzyme levels and mortality."

In the DISCUSSION:

"Third, the present study used data that were not primarily collected for the purpose of research. Therefore, information on the sociodemographic factors, lifestyles, past medical histories, and family histories are insufficient, leading to the possibility of residual confounding."

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"Third, the present study used data that were not primarily collected for the purpose of research. Therefore, information on the sociodemographic factors, lifestyles, past medical histories, and family histories are insufficient, leading to the possibility of residual confounding. Fourth, although common liver disease, such as nonalcoholic fatty liver disease, could increase liver enzyme levels [29] and increase mortality [30–32] as well, specific mechanisms underlying the association between liver enzyme levels and mortality could not be identified thoroughly."

29 Loria P, Adinolfi LE, Bellentani S, et al. Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. Dig Liver Dis Off J Ital Soc Gastroenterol Ital Assoc Study Liver 2010;42:272–82. doi:10.1016/j.dld.2010.01.021

30 Italian Association for the Study of the Liver (AISF). AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions. Dig Liver Dis Off J Ital Soc Gastroenterol Ital Assoc Study Liver 2017;49:471–83. doi:10.1016/j.dld.2017.01.147

31 Targher G, Byrne CD, Lonardo A, et al. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. J Hepatol 2016;65:589–600. doi:10.1016/j.jhep.2016.05.013

32 Lonardo A, Romagnoli D. Gamma glutamyl transferase: A novel cardiovascular outfit for an old liver test. Indian J Med Res 2016;143:4–7. doi:10.4103/0971-5916.178574

Minor

4. Reference 1 appears to be inappropriate. Please consider Gastroenterology. 2002;123:1367-84.

Thank you for your helpful comment. We agree that the reference you introduce is more appropriate. Therefore, we replaced the reference as follows:

In the INTRODUCTION:

“Serum levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) are highly sensitive to liver dysfunction and damage.[1]

1 Reid AE. Nonalcoholic steatohepatitis. Gastroenterology 2001;121:710–23.

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“Serum levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) are highly sensitive to liver dysfunction and damage.[1]

1 Green RM, Flamm S. AGA technical review on the evaluation of liver chemistry tests. Gastroenterology 2002;123:1367–84.

Reviewer #3

Comments to the Author

This paper by Kim et al. is describing the associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale health screening cohort study. This paper is well written and their findings are of clinical relevance. Indeed, the liver as central organ of lipid and glucose metabolism is important for the whole organism (Bechmann L. J Hep. 2012).

1. The authors should discuss that also liver enzymes even in normal ranges have some metabolic alterations. And we may discuss about normal ranges! This should be at least discussed. Indeed, the liver enzymes are elevated in higher BMI groups, even in normal ranges! This is important (Kälsch J et al. Scientific reports 2015).

Thank you for your insightful comment. As you indicated, in the present study, liver enzyme levels were associated with mortality even within the normal ranges, suggesting re-assessment of current normal range limits. According to your comment, we have revised the manuscript as follows:

In the RESULTS:

“When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile: HR = 2.05, 95% CI: 1.93, 2.18) (table 2).”

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“When compared with the 1st decile of baseline GGT levels (≤11 U/L), the all-cause mortality risk was higher in higher baseline GGT deciles (10th decile: HR = 2.05, 95% CI: 1.93, 2.18) (table 2). Because

the normal range of ALT is generally considered as 7–56 U/L, AST as 0–35 U/L, and GGT as 9¬–85 U/L,[2] even within the normal range, lower or higher levels of ALT and AST and higher levels of GGT were associated with higher mortality risk (Table 2).”

In the DISCUSSION:

“The results of the present study, which suggest the presence of a monotonic non-linear association between the baseline GGT levels and all-cause mortality, may explain those earlier findings.”

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“The results of the present study, which suggest the presence of a monotonic non-linear association between the baseline GGT levels and all-cause mortality, may explain those earlier findings.

It has been reported that serum ALT, AST, and GGT levels are associated with higher risk of type 2 diabetes mellitus and body mass index even within the normal ranges.[27] In the present study, liver enzyme levels within normal ranges were also found to be associated with mortality. Because the liver is a central organ of glucose and lipid metabolism and critical for maintaining health,[28] re-evaluation of the relevance of current standards is warranted.”

27 Kälsch J, Bechmann LP, Heider D, et al. Normal liver enzymes are correlated with severity of metabolic syndrome in a large population based cohort. Sci Rep 2015;5:13058. doi:10.1038/srep13058

28 Bechmann LP, Hannivoort RA, Gerken G, et al. The interaction of hepatic lipid and glucose metabolism in liver diseases. J Hepatol 2012;56:952–64. doi:10.1016/j.jhep.2011.08.025

2. It has also been published that liver enzymes are associated with stenosis diameter in acute myocardial infarction (Baars T et al. Medicine 2015). This might also have some impact on mortality.

Thank you for your valuable comment. We agree that the reported association between liver enzyme levels and stenosis diameter in acute myocardial infarction can explain the results of the present study (especially regarding the association between liver enzyme levels and cardiovascular mortality). We have revised the manuscript according to your comment as follows:

In the DISCUSSION:

“A study using data from a prospective elderly cohort reported an association of higher AST levels with an increased risk of cancer mortality, as well as an association of higher GGT levels with an increased risk of cardiovascular disease mortality.[8]”

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“A study using data from a prospective elderly cohort reported an association of higher AST levels with an increased risk of cancer mortality, as well as an association of higher GGT levels with an increased risk of cardiovascular disease mortality.[8] Among patients with acute myocardial infarction who underwent percutaneous coronary intervention, ALT and AST levels were associated with stenosis diameter, an indicator of stenosis severity.[29]”

29 Baars T, Neumann U, Jinawy M, et al. In Acute Myocardial Infarction Liver Parameters Are Associated With Stenosis Diameter. Medicine (Baltimore) 2016;95:e2807. doi:10.1097/MD.0000000000002807

REFERENCE

1. Gowda S, Desai PB, Hull VV, Math AAK, Vernekar SN, Kulkarni SS. A review on laboratory liver function tests. Pan Afr Med J. 2009;3:17.

VERSION 2 – REVIEW

REVIEWER	Prashant Sakharkar Roosevelt University College of Pharmacy, USA
REVIEW RETURNED	21-Mar-2019

GENERAL COMMENTS	Dear Authors, Thank you for addressing critical comments and revising the manuscript with additional information. Manuscript is considerably improved with succinct and appropriate statistical data and additional results presented as supplementary material and it is easy to read.

REVIEWER	Amedeo LOnardo AOU - Modena, Italy
REVIEW RETURNED	06-Mar-2019

GENERAL COMMENTS	These Authors addressed most of concerns raised but this Reviewer.

REVIEWER	Canbay University Hospital Magdeburg
REVIEW RETURNED	06-Mar-2019

GENERAL COMMENTS	Well done!!