Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-23-1764

Reviewer A

Thank you for allowing me the opportunity to review this manuscript. There are clearly un-identified factors that influence which patients will develop esophageal cancer. The statistical method utilized is difficult to follow. The statistically significance was very minimal for the renal function and could likely be impacted by confounders. The word confounders is used but not defined in the methods.

Comment 1: What were the "confounders" in this study? Were things known to affect renal function controlled for with this approach; age, co-morbidities (DM, HTN, etc), use of systemic therapies, etc.

Reply 1:

Thank you for your insightful queries regarding our manuscript. We are pleased to have the opportunity to clarify how we addressed confounding factors in our research, particularly concerning factors known to affect kidney function, such as age, hypertension (HTN), diabetes mellitus (DM), autoimmune diseases, genetic disorders, medications and toxins, infections, urinary tract obstructions, chronic kidney disease (CKD), lifestyle factors, obesity, and dehydration.

Our study employs Mendelian Randomization (MR) to investigate the causal relationship between kidney function and the risk of esophageal cancer (EC). The advantage of MR lies in its ability to mitigate the confounding factors that often plague observational studies. This is achieved by using genetic variants as instrumental variables, which are randomly assigned at conception and are generally not susceptible to the confounding factors that might influence exposure and outcome in observational studies. To ensure the robustness of our MR analysis, we adhered to the three core assumptions of Mendelian research: 1) The genetic variants (SNPs) used as instrumental variables have strong and reliable associations with key markers of kidney function (cystatin C, creatinine, urinary albumin, and glomerular filtration rate [GFR]). 2) The impact of these SNPs on EC risk is entirely mediated through their effect on kidney function, thus excluding direct effects on EC risk that do not operate through kidney function. 3) The selected SNPs are independent of known confounders, ensuring their associations with EC risk are not influenced by age, comorbidities, or medication use among other factors. Comprehensive MR methods, including MR-Egger and weighted median approaches among other sensitivity analyses, were employed to assess and mitigate potential biases, including any residual confounding.

Additionally, although MR is inherently designed to control for many confounding factors, our study also involved a thorough vetting of SNPs to minimize their association with known confounders. Any SNP associated with confounding factors in the PhenoScanner GWAS database (e.g., age, HTN, DM, autoimmune diseases, genetic disorders, medications and toxins, infections, urinary tract obstructions, CKD, lifestyle factors, obesity, dehydration, etc.) at a threshold of $P < 1\times10^{-5}$ was excluded from the analysis to ensure the validity of our instrumental variables.

Thus, as with most Mendelian Randomization studies currently, through inherent MR methodology and careful SNP selection, our research aims to control for known confounders

impacting kidney function, thereby providing more reliable evidence for the causal relationship between kidney function and EC risk.
Changes in the text: line114-119 in the resubmit manuscript.

Comment 2: I believe you are strongly overstating your conclusions. This data does not seem to support a "convincing" body of evidence that renal disease is a "risk factor" for the developing esophageal cancer. Or how do you make that leap? This paper seems to show that an elevated Creatinine (to what #?) is present in patients who already have esophageal cancer? Can you make more clearly state what is shown with the current data in your conclusions.

Reply 2:
Thank you for your comments and for emphasizing the need for a more targeted explanation of why our study findings suggest a causal relationship between kidney function and the risk of esophageal cancer (EC), rather than merely a correlation.

Our study is based on Mendelian randomization (MR), a method that uses genetic variants as instruments to infer causality between an exposure (in this case, kidney function) and an outcome (EC risk). This approach leverages the random allocation of genes from parents to offspring, akin to random assignment in controlled trials, thereby minimizing the confounding factors and reverse causation that typically limit observational studies. The genetic instruments in our study, namely single nucleotide polymorphisms (SNPs) associated with kidney function markers (such as cystatin C and creatinine), were stringently selected based on their association with the exposure rather than confounders of the exposure-outcome relationship. This selection process ensures that the observed relationship between genetic predictors of kidney function and EC risk is not confounded by other risk factors, thus supporting causal inference.Furthermore, the MR method inherently provides tests for causality through various approaches, including the inverse variance weighted (IVW) method, MR-Egger regression, and leave-one-out sensitivity analyses among others. Our findings indicate a significant association between genetically predicted levels of cystatin C and creatinine with EC risk, persisting across different MR methods, providing strong evidence against the presence of pleiotropy (where genetic variants affect the outcome through pathways other than the exposure).

Therefore, we are not overstating our results; Mendelian randomization is inherently a statistical method for studying causal relationships. The strengths of the MR method, combined with the stringent selection of genetic variables and the consistency of our results across various MR analyses, support a causal link between impaired kidney function and increased EC risk. Our study contributes to understanding the role of kidney function in cancer risk, emphasizing the need for further research to explore potential mechanisms and preventive strategies.

Reviewer B

The author's present an interesting study using mendelian randomization to assess the relationship between decreased renal function and the development of esophageal cancer. This is the first encounter I've had with a study using this technique and it is an interesting approach to assess population level data. From a clinicians standpoint however there are some major issues.

Comment 1: The odd's ratios of 1.0005 and 1.0007 are both incredibly small to the point that it makes one question the clinical significance.

Reply 1:

Thank you for your review of the details of our research and for your question regarding the clinical significance of the OR values. We acknowledge that the OR values we reported for the two biomarkers related to kidney function (cystatin C and creatinine), which are 1.0005 and 1.0016 respectively, are indeed small, potentially raising questions about their significance in clinical application. Here, we provide several clarifications and explanations in hopes of addressing your concerns:

1)Statistical Significance versus Clinical Significance: Although these OR values are very close to 1, their statistical significance (i.e., the 95% confidence intervals do not include 1) indicates that the associations exist. We recognize that such minor changes may not have significant clinical relevance at the individual level, but from a public health perspective, even very small changes in risk can have important implications, especially when it comes to prevalent risk factors and diseases.

2)Advantages of the Mendelian Randomization (MR) Approach: Our study utilized MR analysis, a method that employs genetic variants as instrumental variables to estimate causal effects, which can reduce confounding biases and reverse causality issues common in traditional observational studies. This proximity of the OR to 1 is a common occurrence in Mendelian studies. Our MR analysis provides evidence of a potential causal relationship between kidney function markers and the risk of esophageal cancer, reinforcing the scientific significance of our findings, even if the effect size is very small.

3)Biological Plausibility: Our findings are consistent with potential links between renal dysfunction and increased cancer risk observed in other studies. These associations support the hypothesis that there might be biological mechanisms linking kidney function markers with the development of esophageal cancer.

4)Basis for Future Research: While the effect sizes we reported are small, these findings lay the groundwork for future studies that could further explore the biological basis of the relationships between these kidney function markers and the risk of esophageal cancer, including potential interactions and other factors that may influence these relationships.

Comment 2,3: 2) There is no hypothesis for explanation for the why this association might exist for creatinine and cystitis sap individually 3) There is also no explanation why a relationship would be found for these two pathways but not GFR or urinary albumin. If the authors could make a plausible biochemical hypothesis for this, this would greatly strengthen the paper.

Reply 2,3:

Thank you for raising insightful questions regarding our manuscript. Your inquiries have prompted us to clarify and further elaborate on the biochemical hypothesis linking creatinine and cystatin C levels with the risk of esophageal cancer (EC), as well as the explanation for the lack of association with glomerular filtration rate (GFR) and urinary albumin. We are pleased to have the opportunity to strengthen our paper through a more detailed discussion of these key points.

1)Hypothesis of a Causal Link between Creatinine and Cystatin C with EC Risk:

Creatinine and cystatin C are considered markers of kidney function, reflecting the filtration efficiency of the kidneys. However, beyond their role in renal health, these biomarkers can also serve as indicators of systemic physiological and pathological processes potentially related to cancer risk. In our study, significant associations between the levels of creatinine and cystatin C with EC risk suggest a deeper biological link. Drawing on previous literature, our biochemical hypothesis for how impaired kidney function (indicated by elevated creatinine and cystatin C) could lead to esophageal carcinogenesis includes: a) Systemic inflammation: Kidney dysfunction can induce a pro-inflammatory state, characterized by increased levels of cytokines and inflammatory markers. Chronic inflammation is a well-documented factor in tumorigenesis, potentially promoting the occurrence and progression of cancers, including EC. b) Immune system dysfunction: Impaired kidney function might alter immune surveillance and response capabilities, reducing the efficiency of eliminating precancerous cells and cancer cells. c) Oxidative stress: Reduced renal clearance leading to the accumulation of waste products can cause oxidative stress, resulting in DNA damage and promoting genetic mutations, thereby leading to cancer development. These interconnected pathways suggest that elevated levels of creatinine and cystatin C, as manifestations of renal dysfunction, might indirectly increase EC risk through systemic effects on inflammation, immune function, and oxidative stress. Our findings underscore the complex interplay between kidney function markers and cancer risk, highlighting the need for further research to unravel the intricate mechanisms involved.

2)Explanation for the Lack of Causal Association between GFR and Urinary Albumin with EC:

In our study, no association was found between GFR or urinary albumin and EC risk, which may be due to the specificity of these markers in reflecting different aspects of renal and systemic health. While levels of creatinine and cystatin C are influenced by glomerular filtration and may be affected by muscle mass and other systemic factors, they might be more closely related to systemic pathologies affecting cancer risk. In contrast, GFR and urinary albumin, as comprehensive indicators of kidney function, indicate renal damage or stress and may not directly or sufficiently capture systemic pathophysiological changes related to cancer risk. This distinction suggests that the pathways linking renal dysfunction to EC risk may be more specifically related to the systemic effects reflected by creatinine and cystatin C, rather than the renal function state per se, as indicated by GFR and urinary albumin.

Changes in the text: line214-243 in the resubmit manuscript.

Comment 4: Differentiating between adenocarcinoma and squamous cell esophageal cancer in the analysis would be prudent as well as they are thought to have different developmental pathways.

Reply 4:

Thank you for your insightful comments on distinguishing between esophageal adenocarcinoma and squamous cell carcinoma in our analysis. We recognize the importance of differentiating these subtypes due to their distinct developmental pathways and potential impacts on the relationship between kidney function and esophageal cancer (EC) risk. In our study, we utilized publicly available genome-wide association study (GWAS) data, including single nucleotide polymorphisms (SNPs) related to EC from the UK Biobank and other datasets. A significant challenge we faced was the inability of GWAS data to differentiate between the pathological subtypes of EC. The SNPs extracted from these databases do not distinguish

between adenocarcinoma and squamous cell carcinoma, limiting our ability to analyze these subtypes separately within the Mendelian randomization (MR) analysis framework. Given this limitation, we analyzed EC as a single entity. We acknowledge this as a limitation of our study and an important consideration in interpreting our findings. A combined analysis approach was a practical solution that enabled us to explore the broader relationship between kidney function and EC risk. However, we understand the nuanced differences and divergent pathways in the development of adenocarcinoma and squamous cell carcinoma and their potential implications for research and clinical practice. We appreciate your emphasis on this aspect and have added a discussion point in our manuscript to clarify the limitations related to EC subtype-specific analysis due to the nature of the available GWAS data. All Mendelian randomization studies of esophageal cancer currently face this issue, but it should not be a limitation for conducting MR analyses on esophageal cancer relative to other cancer types. We look forward to further advancements in genetic epidemiology to enable more detailed subtype analyses in future studies.

Comment 5: Finally, while this is certainly an interesting analysis, I'm not sure JTD is the correct forum for this paper. Perhaps using this technique to assess multiple tumor types and submitting to a more general oncology or public health journal would make more sense

Reply 5: Thank you to the reviewer for their meticulous review and valuable suggestions on our manuscript. We do not deny the reviewer's viewpoint that this study might be more suited for a broad oncology or public health journal. However, we believe that our research holds particular significance and applicability for the readership of the Journal of Thoracic Disease (JTD) for the following reasons:

1) Focus on Esophageal Cancer: While our study employs a method that could be applicable to various types of tumors, our primary focus is on esophageal cancer, a significant thoracic disease that falls within the disease scope of JTD. Early detection and risk assessment of esophageal cancer are crucial for improving patient outcomes, and our study offers a novel perspective by predicting esophageal cancer risk through the evaluation of genetic markers of kidney function.

2) Innovation of the Study: We explored the potential causal relationship between kidney function and the risk of esophageal cancer using the Mendelian randomization method. This approach reduces the issues of confounding factors and reverse causation common in traditional observational studies, providing a new research tool and perspective for the thoracic field.

3) Relevance to JTD Readers: The readership of JTD may have a particular interest in research on new risk factors and pathological mechanisms of esophageal cancer. Our study not only enhances the understanding of the pathogenesis of esophageal cancer but may also offer insights for clinical practice, such as considering kidney function markers in risk assessment and early screening.