ARTICLE DETAILS

TITLE (PROVISIONAL)	Association of proton pump inhibitor use with risk of kidney stones: an analysis of cross-sectional data from the US National Health and Nutrition Examination Survey (2007–2018)
AUTHORS	liu, wen; Wang, Jia; Wang, Miao; Wang, Miaomiao; Liu, Ming

VERSION 1 – REVIEW

REVIEWER	Saka, Yosuke Kasugai Municipal Hospital, Nephrology
REVIEW RETURNED	12-Jun-2023

GENERAL COMMENTS	Reviewer's comment This is a cross-sectional study to show the association between PPI and kidney stones on patients with HD. This study indicates that long-term PPI medication independently increases the risk of kidney stones, especially recurrent kidney stones. This result provides clinicians cautions regarding unnecessary long-term PPI therapy. However, this study has several concerns for accept. 1. Vitamin C consumption is one of dietary factors for the risk of kidney stones. NHANES has conducted survey on vitamin C consumption. In addition, several studies show the association between vitamin C consumption and the risk of kidney stones. 2. The authors conducted PSM analysis. Several important factors, such as history of CHF or loop diuretics use, were not fully matched. 3. The result of meta-analysis of the risk of kidney stones on PPI should be described also in Results. 4. In line 222 – 224, the authors discussed magnesium and kidney stones. Which did the authors emphasize, hypomagnesemia or low urine magnesium, on the risk of kidney stones? Confused hypomagnesemia with low urine magnesium?

REVIEWER	Proietti, Silvia San Raffaele Hospital, Urology
REVIEW RETURNED	03-Jul-2023

GENERAL COMMENTS	Interesting study worth investigating as PPIs are often thought to be harmless and freely prescribed in many situations. Side effects of PPIs and kidney stone formation has been previously investigated and this would add to the literature.

	Much effort has gone into statistics to demonstrate the positive association.
	I understand that since the study is based on an established database, certain missing information may be out of the authors' hands. Nevertheless it would be good if the authors can include more information/clarify some of the following questions.
	Some additional information on NHANES and how its survey process is carried out will be good.
	The question of recurrent stones needs more information. Passing at least 2 stones does not make them recurrent stone formers as the two stones may be passed in the same episode, and this criteria may exclude those who have undergone surgery for stones or those who have recurrent stone but not yet passed/treated for the second episode.
	Duration of PPI use – defined as years since initiating therapy. Did we include only those who are on regular PPIs or those who took them intermittently as long as they have consumed PPIs before especially since information on previously discontinued prescription was not available? Was there any way to differentiate regular and intermittent users?
	For risk factors, other conditions such as cysteinuria, renal tubular acidosis, malabsorption GI disorders, diet, family history (which will also hint at environmental/lifestyle risk factors) are not included. Perhaps this data is not available, in which case, this is another limitation of the study.
	For limitation, the diagnosis of the kidney stones and recurrence is patient-reported rather than objective diagnosis with imaging.
	The study shows an association. It is also possible that patients have kidney stones even before starting the use of PPIs as the survey questions did not suggest a timeline to events. The use of "incident" kidney stone in PPI users throughout the whole paper may be misleading. Would the word prevalence be better suited in this case?
	The conclusion should accordingly reflect association rather than reporting that there is a significant relationship between PPI use and incident kidney stones, which suggest potential underlying causal conclusion.
	On page 19, the authors have included a table of meta-analysis. This should be explained in the main text as well as its significance.
	For Supplementary Fig 2. Please double check the units (hours/day) for "Sedentary time".

REVIEWER	Van Osch, Frits
	Maastricht University, Complex Genetics & Epidemiology
REVIEW RETURNED	14-Aug-2023

GENERAL COMMENTS	By request of the editor, I have reviewed the manuscript mainly from a statistical viewpoint and not so much from a content of the introduction and discussion for example. Overall, the manuscript is

	clear. In addition to some smaller comments, my main comments concern the restricted cubic splines models presented. Statistical review: - The word multivariate is used to describe a multivariable analysis. A multivariate analysis has multiple outcome measures and a multivariable analysis has multiple covariates predicting the outcome, the latter is the case here. Please check for this throughout the manuscript. - Location of knots in restricted cubic splines model was not motivated and/or it was not mentioned whether the results were similar when choosing different knots. - Page 5 line 149/150: the statement about yearly increase of incidence cannot be made like this if the association is assumed to be non-linear. Also, it should be mentioned whether this increase is compared to the non-PPI users or to the previous year category of PPI users (e.g. 6 years users vs 5 years users). - Looking at the figures, the association with kidney stones seems linear, whereas the association with recurrent kidney stones shows a slight bend towards the end. Both show a high p-value for the test for non-linearity. See previous comment about choosing different splines. I would also like to add here: are these results really different from a linear analysis of years of PPI use? Which seemed to be what the authors presented in the results section also, when they mentioned the 7% increase per year for example? In the current version, the emphasis on the non-linear analysis does not have added value and should perhaps only be mentioned as a sensitivity analysis next to the already presented linear interpretation of years of PPI use. - Correct use of PSM, VIF etcetera.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Yosuke Saka, Kasugai Municipal Hospital
Comments to the Author:
1. Vitamin C consumption is one of dietary factors for the risk of kidney stones. NHANES has conducted survey on vitamin C consumption. In addition, several studies show the association between vitamin C consumption and the risk of kidney stones.
Answer: Thank you for your suggestion. We appreciate your attention to the dietary factors that could potentially impact the risk of kidney stones, such as caffeine consumption, vitamin C intake, and the dietary inflammatory index (DII). In response to this concern, we extracted relevant data from NHANES. Subsequently, we performed additional adjustments for these three dietary factors based on model 3. The outcome of our analysis indicated that even after controlling for the potential effects of these dietary variables, the significant association between PPI use and the occurrence and recurrence of kidney stones persisted. Detailed findings are presented in Supplementary Table 3.

2. The authors conducted PSM analysis. Several important factors, such as history of CHF or loop diuretics use, were not fully matched.
Answer: Thank you for your valuable feedback. Our intention in employing propensity score matching (PSM) was to enhance the comparability of potential confounding factors between the PPI users and non-users groups. In this study, we defined cardiovascular disease (CVD) as the presence of congestive heart failure, coronary heart disease, myocardial infarction, or stroke, assigning a value of 1 if any of these conditions were met, and 0 if none were present. While we used this comprehensive

CVD definition for PSM matching, we recognize that the presentation of results in Supplementary Table 2 led to ambiguity due to the separate display of individual components. To address this, we have revised Supplementary Table 2 to exclusively showcase the outcomes related to CVD, revealing a p-value of 0.070.

Furthermore, we acknowledge the potential for incomplete matching in PSM, including cases involving loop diuretics users. Despite not achieving perfect matching for loop diuretics users, we took the step of incorporating them into our post-PSM multivariable analysis. This strategic approach allowed us to effectively adjust for their potential impact on the results. Notably, even after accounting for the presence of loop diuretics users, the observed significant associations remain significant.

3. The result of meta-analysis of the risk of kidney stones on PPI should be described also in Results. Answer: Thank you for your suggestion. We have taken your feedback into consideration and have incorporated a description and explanation of the meta-analysis results in both the Results and Discussion sections of our manuscript. This approach ensures that readers have a clear understanding of the findings and their implications.

4. In line 222 – 224, the authors discussed magnesium and kidney stones. Which did the authors emphasize, hypomagnesemia or low urine magnesium, on the risk of kidney stones? Confused hypomagnesemia with low urine magnesium?
Answer: Thank you for your suggestion. We appreciate your attention to this matter. We apologize for the error in our article where we mistakenly used the term "hypomagnesemia" instead of "hypomagnesuria." Our intention was to emphasize the relationship between PPI use and the levels of magnesium and citrate in urine. The focus should have been on low urine magnesium levels rather than hypomagnesemia. We have already made the necessary corrections in the original text to accurately reflect our intended meaning.

Reviewer: 2
Dr. Silvia Proietti, San Raffaele Hospital
Comments to the Author:
1. Some additional information on NHANES and how its survey process is carried out will be good.
Answer: Thank you for your suggestion. We have taken your feedback into consideration and have provided more information about the NHANES database and its survey process in the Methods section.

2. The question of recurrent stones needs more information. Passing at least 2 stones does not make them recurrent stone formers as the two stones may be passed in the same episode, and this criteria may exclude those who have undergone surgery for stones or those who have recurrent stone but not yet passed/treated for the second episode.
Answer: Thank you for your valuable suggestion. Your concern regarding the criteria for defining recurrent kidney stones is well-taken, and we appreciate your attention to this issue. We recognize that using the criterion of passing at least 2 stones may not comprehensively capture all cases of recurrent stone formation, as stones could be passed in the same episode or individuals might have undergone surgical interventions before experiencing a second stone episode. This approach could potentially exclude individuals with recurrent stones who have not yet passed or received treatment for a second episode.

In the context of NHANES data collection, where kidney stone information is primarily gathered through questionnaires, defining recurrent stone formers becomes a challenging task due to inherent limitations. Despite this challenge, we opted to adopt the criterion of passing at least 2 stones for defining recurrent stones. While this criterion may not align perfectly with every possible scenario, it has been utilized in many published studies on kidney stones(1-6). We adopted this approach for consistency, drawing from existing literature. While not a strict match for the definition of recurrent stones, we believe an underlying connection exists.

3. Duration of PPI use – defined as years since initiating therapy. Did we include only those who are on regular PPIs or those who took them intermittently as long as they have consumed PPIs before especially since information on previously discontinued prescription was not available? Was there any way to differentiate regular and intermittent users?

Answer: Thank you for your question. In our study, we defined the duration of PPI use as the years since initiating therapy. However, we did not differentiate between regular and intermittent users of PPIs due to the limitations of the available data. The NHANES dataset did not provide information on whether participants were on regular or intermittent PPI use, nor did it offer details about previously discontinued prescriptions. As a result, we were unable to distinguish between these two usage patterns in our analysis. We appreciate your understanding of this constraint in our study.

4. For risk factors, other conditions such as cysteinuria, renal tubular acidosis, malabsorption GI disorders, diet, family history (which will also hint at environmental/lifestyle risk factors) are not included. Perhaps this data is not available, in which case, this is another limitation of the study.

Answer: Thank you for your valuable suggestion. You've highlighted an aspect regarding potential risk factors related to kidney stones, encompassing factors such as cysteinuria, renal tubular acidosis, malabsorption GI disorders, diet, and family history. We've indeed acknowledged this limitation in our study. Although we extensively adjusted for covariates, incorporating three types of detailed covariate information, it's imperative to recognize the potential influence of unmeasured factors that could impact the association between PPI use and nephrolithiasis.

Upon comprehensive exploration of the NHANES database, we unfortunately did not encounter descriptions concerning cysteinuria, renal tubular acidosis, malabsorption GI disorders, and family history. Nonetheless, we identified several dietary factors that could potentially play a role in kidney stone occurrence, such as caffeine consumption, vitamin C intake, and the dietary inflammatory index (DII). Extracting relevant NHANES data, we performed supplementary adjustments building upon the foundation of model 3, incorporating these three dietary variables. The outcomes, as outlined in Supplementary Table 3, demonstrate the persistence of a significant association between DII and both the occurrence and recurrence of kidney stones, even after accounting for these dietary factors.

We truly appreciate your understanding of the inherent limitations of retrospective studies. While certain potential factors may not have been encompassed in our multifactorial adjustments, we have diligently addressed a diverse range of variables. In our study, we employed three distinct models for adjustment, in addition to utilizing propensity score matching for validation.

5. For limitation, the diagnosis of the kidney stones and recurrence is patient-reported rather than objective diagnosis with imaging.

Answer: Thank you for your valuable suggestion. We agree that utilizing objective diagnostic imaging for kidney stones would certainly add robustness to our study findings. It is important to acknowledge that our reliance on questionnaire responses to capture the history of kidney stones, as reflected in the NHANES dataset, does introduce a limitation.

We are mindful of the extensive body of literature that has leveraged NHANES kidney stone data, with over 100 published articles using similar data sources (based on a PubMed search with the query: (((((stone*[Title/Abstract]) OR (calculi*[Title/Abstract])) OR (calculus*[Title/Abstract])) OR (nephrolit*[Title/Abstract])) AND ((renal[Title/Abstract]) OR (kidney[Title/Abstract]))) OR (Urolithiasis[Title/Abstract]), search date: August 21, 2023). Despite this inherent limitation, our study findings do provide valuable insights into the potential relationship between PPI use and kidney stones. We have taken your guidance to refine the limitations section to better elucidate these aspects.

6. The study shows an association. It is also possible that patients have kidney stones even before starting the use of PPIs as the survey questions did not suggest a timeline to events. The use of

"incident" kidney stone in PPI users throughout the whole paper may be misleading. Would the word prevalence be better suited in this case?

Answer: Thank you for your suggestion. Your input is greatly appreciated. You've rightly pointed out the possibility that some individuals might have had kidney stones even before initiating PPI use, given the absence of a specific timeline in the survey questions. Considering this concern, using the term "prevalence" instead of "incident" is more accurate and precise. We have taken your suggestion to make the necessary revision throughout the paper to reflect this change.

7. The conclusion should accordingly reflect association rather than reporting that there is a significant relationship between PPI use and incident kidney stones, which suggest potential underlying causal conclusion.

Answer: Thank you for your suggestion. We have taken your suggestion into account and have adjusted the conclusion accordingly. We have removed the term "significant" to ensure that our conclusion accurately reflects the association between PPI use and incident kidney stones and to avoid overinterpreting this relationship.

8. On page 19, the authors have included a table of meta-analysis. This should be explained in the main text as well as its significance.

Answer: Thank you for your suggestion. We have provided a description and explanation of the meta-analysis in both the Results and Discussion sections of our manuscript. This ensures that readers have a clear understanding of the meta-analysis findings and their relevance to our study.

9. For Supplementary Fig 2. Please double check the units (hours/day) for "Sedentary time".

Answer: Thank you for your attention. We have reviewed Supplementary Fig 2, and we can confirm that the units for "Sedentary time" are correctly indicated as hours per day (hours/day).

References
1.   Harb AA, Han DS, Lee JA, Schulster ML, Shah O. Micronutrient inadequacy and urinary stone disease: an analysis of the National Health and Nutrition Examination Survey 2007-2018. Urolithiasis. 2023;51(1):59.
2.   Qin Z, Zhao J, Geng J, Chang K, Liao R, Su B. Higher Triglyceride-Glucose Index Is Associated With Increased Likelihood of Kidney Stones. Front Endocrinol (Lausanne). 2021;12:774567.
3.   Lee JA, Abramowitz MK, Kipperman N, Drzewiecki BA, Melamed ML, Stern JM. Exploring the Association of Asthma with Urinary Stone Disease: Results from the National Health and Nutrition Examination Survey 2007-2014. Eur Urol Focus. 2020;6(2):354-60.
4.   Sun Y, Wang D, Zhou Q. Caffeine intake and the risk of recurrent kidney stones in adults, an analysis of 2007-2014 National Health and Nutrition Examination Surveys. Eur J Nutr. 2020;59(6):2683-92.
5.   Wang W, Lu X, Shi Y, Wei X. Association between food insecurity and kidney stones in the United States: Analysis of the National Health and Nutrition Examination Survey 2007-2014. Front Public Health. 2022;10:1015425.
6.   Zhang C, Qiu S, Bian H, Tian B, Wang H, Tu X, et al. Association between Dietary Inflammatory Index and kidney stones in US adults: data from the National Health and Nutrition Examination Survey (NHANES) 2007-2016. Public Health Nutr. 2021;24(18):6113-21.

Reviewer: 3
Mr. Frits Van Osch, Maastricht University, University of Birmingham
Comments to the Author:
Statistical review:

1. The word multivariate is used to describe a multivariable analysis. A multivariate analysis has multiple outcome measures and a multivariable analysis has multiple covariates predicting the outcome, the latter is the case here. Please check for this throughout the manuscript.
Answer: Thank you for pointing out this distinction. We appreciate your feedback and have made the necessary changes by replacing "multivariate" with "multivariable" to accurately reflect the nature of our analysis throughout the manuscript.

2. Location of knots in restricted cubic splines model was not motivated and/or it was not mentioned whether the results were similar when choosing different knots.
Answer: Thank you for your suggestion. Based on the reference "Restricted Cubic Spline Regression: A Brief Introduction" (http://support.sas.com/resources/papers/proceedings16/5621-2016.pdf), we initially performed restricted cubic splines (RCS) analyses with 3, 4, and 5 knots separately to assess non-linearity by calculating the P-value for non-linearity. The results consistently showed P-values above 0.05, indicating a linear relationship between PPI use and the occurrence and recurrence of kidney stones. Subsequently, we calculated the Akaike Information Criterion (AIC) for each model, which serves as a measure of model goodness-of-fit. We selected the model with the lowest AIC value as our final choice. We have summarized the specific outcomes in the table below. Based on the results, we opted to use a restricted cubic splines model with 3 knots for our analysis.

Table. Akaike Information Criterion (AIC) and P-value for Non-linearity of Restricted Cubic Splines Models Across Different Knots

| The occurrence of kidney stones | | | The recurrence of kidney stones | | |
Knots	AIC	P for non-linearity	Knots	AIC	P for non-linearity
3	16664.48	0.651	3	2167.76	0.484
4	16664.67	0.071	4	2170.74	0.594
5	16667.34	0.108	5	2169.07	0.146

3. Page 5 line 149/150: the statement about yearly increase of incidence cannot be made like this if the association is assumed to be non-linear. Also, it should be mentioned whether this increase is compared to the non-PPI users or to the previous year category of PPI users (e.g. 6 years users vs 5 years users).
Answer: Thank you for your suggestion. We have taken care to highlight the outcomes of the non-linear analysis in our results section. As you correctly pointed out, if the association between PPI use and kidney stones is linear, then the emphasis on the non-linear analysis does not have added value. Therefore, we have revisited and refined the presentation of our findings. Given that the relationship between PPI use and kidney stones in our study is linear, we have retained the statement about the yearly increase in incidence associated with each additional year of PPI use.

4. Looking at the figures, the association with kidney stones seems linear, whereas the association with recurrent kidney stones shows a slight bend towards the end. Both show a high p-value for the test for non-linearity. See previous comment about choosing different splines. I would also like to add here: are these results really different from a linear analysis of years of PPI use? Which seemed to be what the authors presented in the results section also, when they mentioned the 7% increase per year for example? In the current version, the emphasis on the non-linear analysis does not have added value and should perhaps only be mentioned as a sensitivity analysis next to the already presented linear interpretation of years of PPI use.
Answer: Thank you for your feedback. We greatly value your input and have carefully reviewed the points you raised. After thorough consideration, we agree that the non-linear analysis may not significantly contribute to the interpretation of our results. Thus, we have made appropriate adjustments to the relevant section in the results, eliminating the paragraph that emphasized the non-linear relationship.

VERSION 2 – REVIEW

REVIEWER	Saka, Yosuke Kasugai Municipal Hospital, Nephrology
REVIEW RETURNED	29-Aug-2023

GENERAL COMMENTS	Almost of the manuscript have been appropriately modified in response to reviewers' comments, except one concern. According to response to reviewers' comments, there was no statistical difference (p = 0.07) on CVD history after propensity matched analysis. However, the data of patients with CVD history has not been shown in Supplementary Table 2.

REVIEWER	Proietti, Silvia San Raffaele Hospital, Urology
REVIEW RETURNED	10-Sep-2023

GENERAL COMMENTS	Typos in lines 47, 152, 239 and 259. The paper offers useful reminder of the potential sides effects of PPI in urological diseases and adds to the literature for risk factors for stones. Well-organized presentation overall.

REVIEWER	Van Osch, Frits Maastricht University, Complex Genetics & Epidemiology
REVIEW RETURNED	25-Sep-2023

GENERAL COMMENTS	I am happy with the changes the authors made and the answers to my questions. After a short version of the answer to question 2 (location of knots that was determined after using AIC) is also added to the manuscript I would be happy for it to be published.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Dr. Yosuke Saka, Kasugai Municipal Hospital
Comments to the Author:
Almost of the manuscript have been appropriately modified in response to reviewers' comments, except one concern. According to response to reviewers' comments, there was no statistical difference (p = 0.07) on CVD history after propensity matched analysis. However, the data of patients with CVD history has not been shown in Supplementary Table 2.
Answer: We have incorporated the data of patients with a history of cardiovascular disease (CVD) into Supplementary Table 3.

Reviewer: 2
Dr. Silvia Proietti, San Raffaele Hospital
Comments to the Author:
Typos in lines 47, 152, 239 and 259.
Answer: We have made revisions to the manuscript based on the reviewer's suggestion.

Reviewer: 3

Mr. Frits Van Osch, Maastricht University, University of Birmingham

Comments to the Author:

I am happy with the changes the authors made and the answers to my questions.

After a short version of the answer to question 2 (location of knots that was determined after using AIC) is also added to the manuscript, I would be happy for it to be published.

Answer: We have incorporated the relevant information regarding AIC into the methods section.