| **Section and Topic**  | **Item #** | **Checklist item**  | **Location where item is reported**  |
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| **TITLE**  |  |
| Title  | 1 | Hyperhomocysteinemia: a meta-analysis of its association with reduced glomerular filtration rate in chronic kidney disease patients |  |
| ABSTRACT  |  |
| Abstract  | 2 | Background: Hyperhomocysteinemia (elevated homocysteine, Hcy) is common in chronic kidney disease (CKD) patients, but its effect on glomerular filtration rate (GFR), a key renal function indicator, is unclear.Objective: To clarify the relationship, a systematic search in PubMed, Embase, and Cochrane databases and reference lists was done.Two RCTs and eight cross-sectional studies (7364 patients) were included. Hyperhomocysteinemia was defined as Hcy > 15 µmol/L.Methods: Meta-analysis synthesized data.Mean ± SD of GFR in CKD patients by Hcy levels was calculated for comparison.Results: CKD patients with hyperhomocysteinemia had a lower GFR (SMD = 2.26, 95%CI: 1.37 - 3.15). However, significant inter-study heterogeneity (P < 0.01, I² = 99.5%) was found. Five articles (Z 2016, Ye, Z 2017, Zhang, Y 2020, Shen, Z 2022, Wu, and J 2022) contributed to it (P = 0.015 < 0.05). Subgrouping them eliminated heterogeneity (P = 0.52, I² = 0.00%). Sensitivity analysis showed individual article exclusion had little effect on the result. But Egger tests showed publication bias (P < 0.05), while scissors graph analysis supported result stability (P < 0.01).Conclusions: In CKD, elevated Hcy is related to kidney function decline. |  |
| INTRODUCTION  |  |
| Rationale  | 3 |  Hyperhomocysteinemia (elevated homocysteine, Hcy) is common in chronic kidney disease (CKD) patients, but its effect on glomerular filtration rate (GFR), a key renal function indicator, is unclear. |  |
| Objectives  | 4 | To clarify the relationship, a systematic search in PubMed, Embase, and Cochrane databases and reference lists was done.Two RCTs and eight cross-sectional studies (7364 patients) were included. Hyperhomocysteinemia was defined as Hcy > 15 µmol/L. |  |
| METHODS  |  |
| Eligibility criteria  | 5 | Eligibility Criteria for Study InclusionTo curate a relevant and homogeneous set of studies for our meta-analysis, we established a series of well-defined inclusion criteria. Firstly, studies were required to involve patients diagnosed with chronic kidney disease (CKD), thereby ensuring the focus on the target patient population relevant to our research question. Secondly, the study designs eligible for inclusion encompassed randomized case-control studies, cross-sectional studies, or cohort design studies. These particular study architectures were chosen as they are commonly employed in epidemiological and clinical research, offering robust frameworks for investigating associations and outcomes in CKD patients. Thirdly, to maintain consistency in the age range of the participants across studies, only those studies that included patients aged 14 years or older were considered. This age cut-off was set to align with the typical onset and diagnosis patterns of CKD in clinical practice and research settings. Lastly, studies had to provide either direct reports of the glomerular filtration rate (GFR) and hyperhomocysteinemia (HHcy) levels or furnish sufficient data that would permit the calculation of these crucial parameters. This stipulation was essential to enable quantitative analysis and comparison across the selected studies.Exclusion Criteria for Study ScreeningConversely, several exclusion criteria were implemented to filter out studies that did not meet the necessary quality or relevance standards. Firstly, studies published in languages other than English were excluded. While this may introduce a potential language bias, it was a practical necessity given the resource constraints and the need to ensure accurate data extraction and synthesis, as English is the lingua franca of international scientific communication. Secondly, studies that centered on patients with acute kidney injury, end-stage kidney disease, or those requiring dialysis were excluded. The rationale behind this was to isolate the specific impact of HHcy on GFR in the context of CKD, distinct from the confounding factors and unique pathophysiology associated with more severe renal conditions. Thirdly, studies lacking clear groupings, which could impede meaningful comparison and analysis, were omitted. Additionally, animal studies were excluded as the physiological and pathophysiological mechanisms in animals may not directly translate to human patients, and our focus was on human CKD populations. Finally, studies that employed alternative or idiosyncratic definitions of CKD, such as relying solely on creatinine levels without comprehensive diagnostic criteria, were also excluded to maintain a standardized and consistent understanding of the CKD population under investigation. |  |
| Information sources  | 6 | Information SourcesIn the pursuit of a comprehensive and up-to-date evidence base for this study, we systematically scoured multiple premier electronic databases, namely PubMed, Embase, and Cochrane. These databases, renowned for their extensive collections of peer-reviewed scientific literature across diverse medical and health-related disciplines, served as the primary reservoirs of relevant research. Our search was meticulously configured to encompass all studies archived within these platforms, stretching from their inception up until October 2024, when the data collection phase concluded. Notably, no constraints were imposed regarding the types of publications retrieved, thereby ensuring the inclusion of a wide gamut of research outputs, be it original research articles, review papers, case reports, or editorials, so long as they met the eligibility criteria relevant to our research focus. |  |
| Search strategy | 7 | Search StrategyTo maximize the yield of pertinent studies, a multi-faceted search approach was devised, leveraging both controlled vocabulary terms and free-text keywords. For instance, leveraging the Medical Subject Headings (MeSH) terms, “homocysteine [Mesh]” was combined with a suite of free words that are contextually related in the research domain. Similarly, “renal insufficiency, chronic [Mesh]” was paired with additional free-text descriptors to cast a wide net in capturing relevant literature. The detailed composition of these keyword combinations is provided in the Appendix for transparency and reproducibility purposes. Moreover, to further expand the search scope and unearth potentially overlooked studies, the “related Items” function available within these databases was systematically utilized. This automated feature, which harnesses semantic relationships and indexing algorithms, enabled us to access related research that might not have been retrieved through |  |
| Selection process | 8 | Selection ProcessThe process of sifting through the retrieved studies to identify those that met our stringent inclusion and exclusion criteria was a meticulously orchestrated collaborative effort. Three independent reviewers, namely Wei Chen, Xueming Liang, and Jie Wang, each with their expertise and methodological acumen in the relevant field, undertook the initial screening of the studies. In cases where discrepancies or disagreements arose regarding the eligibility of a particular study, an in-depth discussion was promptly initiated among the reviewers. This iterative and collegial approach ensured that all perspectives were considered, and a consensus was reached based on a comprehensive evaluation of the study’s characteristics, methodology, and relevance to our research question, thereby guaranteeing the integrity and reliability of the final set of selected studies for our meta-analysis. |  |
| Data collection process  | 9 | Data Collection ProcessThe data collection phase of this study was executed with meticulous attention to detail and a robust methodological approach, ensuring the integrity and reliability of the information gathered. Three highly trained reviewers, namely Wei Chen, Xueming Liang, and Jie Wang, each bringing their unique analytical skills and subject matter expertise to the table, independently scrutinized the selected studies. In instances where discrepancies or differences in interpretation arose during the review process, a collaborative and in-depth discussion was promptly initiated among the reviewers. This iterative dialogue served as a mechanism to reconcile contrasting viewpoints, leveraging the collective knowledge and insights of the team, and ultimately arriving at a consensus-based decision regarding the data to be incorporated. |  |
| Data items  | 10a | Data ItemsThe standardized mean difference (SMD) value, a crucial metric in our analysis, was computed following a precisely defined formula. Specifically, it was obtained by subtracting the mean glomerular filtration rate (GFR) of the group within the chronic kidney disease (CKD) population with normal homocysteine (HCY) levels from the mean GFR of the group presenting with high HCY levels, and then dividing the resultant difference by the mean standard deviation. This calculation methodology was designed to standardize the comparison across different studies, enabling a more meaningful synthesis of the data despite potential variations in sample characteristics and study designs. |  |
| 10b | In situations where the GFR value was not explicitly reported in the literature under review, we adopted an alternative approach by substituting the creatinine clearance value in its stead. This substitution was based on established relationships between GFR and creatinine clearance within the context of renal function assessment [12]. Subsequently, all the data outcomes, whether derived from direct GFR reporting or creatinine clearance substitution, were amalgamated in a systematic and organized manner to form a comprehensive dataset for further analysis.When the GFR values were presented in quartiles, as was the case in some of the studies, a conversion process was implemented to transform them into the mean standard deviation format. This transformation was carried out in accordance with well-recognized statistical procedures [13], ensuring that all data were expressed in a uniform and comparable metric, thereby facilitating the accurate computation of the SMD and other relevant statistical analyses. |  |
| Study risk of bias assessment | 11 | Study Risk of Bias AssessmentTo rigorously evaluate the quality and potential biases inherent in the selected cohort and cross-sectional studies, a systematic and standardized assessment protocol was implemented. Two experienced authors were tasked with conducting these evaluations, each employing specific assessment tools tailored to the study design.For the cohort studies, the Newcastle-Ottawa Scale (NOS) was utilized [14]. This scale is designed to comprehensively appraise various aspects of a cohort study's quality, awarding a maximum of nine points across three key domains: study population comparability, quality of selection, and outcomes. Based on the total score achieved, the study quality was classified into one of three categories. Studies scoring between 0 and 3 points were deemed to be of poor quality, indicating significant limitations in one or more of the evaluated aspects. Those with scores ranging from 4 to 6 points were considered to be of fair quality, suggesting a moderate level of methodological rigor and appropriate study design choices. Studies attaining scores of 7 to 9 points were regarded as being of high quality, signifying robust study design, adequate sample selection, and reliable outcome measurement.In the case of cross-sectional studies, the Agency for Healthcare Research and Quality (AHRQ) tool was employed [15]. This tool comprises 11 items, each of which requires a response in the form of “yes,” “no,” or “not reported.” Only a “yes” response was assigned a score of 1, while “no” and “not reported” responses were scored as 0. Upon completion of the assessment for all 11 items, the total score was calculated. Studies with scores between 4 and 6 were rated as being of medium quality, denoting a satisfactory level of methodological soundness, albeit with some areas that could potentially benefit from improvement. Scores of 8 to 11 points were indicative of high quality, suggesting that the cross-sectional study was conducted with a high degree of precision, appropriate sampling, and comprehensive data collection and reporting. This dual assessment approach using the NOS and AHRQ tools ensured a thorough and objective evaluation of the quality and potential biases in the studies included in our meta-analysis, thereby enhancing the reliability of our overall findings.  |  |
| Effect measures  | 12 | Effect MeasuresIn order to comprehensively quantify and synthesize the impact of hyperhomocysteinemia on glomerular filtration rate within the context of chronic kidney disease, a meta-analysis focusing on effect size was meticulously conducted, with the standardized mean difference (SMD) serving as the principal metric. This choice of SMD was predicated on its ability to standardize the differences in means across diverse studies, thereby facilitating a more meaningful comparison and aggregation of results, notwithstanding potential disparities in study designs and participant characteristics.All confidence intervals (CIs) were set at the 95% level, a widely accepted standard in the field of statistical inference. This level of confidence strikes an optimal balance between precision and the allowance for random error, providing a robust framework within which to interpret the variability of our estimates. Statistical significance was determined based on a p-value threshold of less than 0.05, a convention that has been firmly established in the scientific community to distinguish between chance findings and those that are likely to reflect a true underlying effect.To gauge the presence and extent of between-trial heterogeneity, a two-pronged approach was adopted, employing both the I² index and the Q test p-value. The I² index, which quantifies the proportion of total variation across studies that is due to heterogeneity rather than chance, was used in tandem with the p-value derived from the Q test. A p-value below 0.05, coupled with an I² index exceeding 50%, served as a clear indicator of substantial heterogeneity among the included studies, flagging the need for further exploration and adjustment in subsequent analyses to account for such variability [16]. |  |
| Synthesis methods | 13a | Synthesis MethodsThe heterogeneity observed across the studies could potentially stem from a multitude of factors, each warranting careful consideration in our analytical approach. These factors encompassed a broad spectrum, ranging from methodological aspects such as the research design, data collection techniques, and analytical methods employed, to more participant-centric variables including sample size, demographic characteristics (age, gender), geographical location, and clinical parameters (blood pressure, lipid profiles like high-density lipoprotein [HDL] and low-density lipoprotein [LDL], fasting and postprandial blood glucose [FBG, PBG], estimated glomerular filtration rate [eGFR], high-sensitivity C-reactive protein [Hs-CRP], systolic and diastolic blood pressure [SBP, DBP], and pulse pressure [PP]). |  |
| 13b | To dissect and understand this heterogeneity, we harnessed a suite of advanced analytical techniques. Random-effects models were utilized to account for the inherent variability across studies, providing a more flexible and comprehensive approach to aggregating data compared to fixed-effects models, especially in the presence of significant heterogeneity. Subgroup analyses were performed, stratifying the studies based on relevant variables such as study design, participant demographics, or clinical characteristics, to isolate and examine the sources of heterogeneity within specific subsets of the data. Additionally, meta-regression analyses were conducted to quantitatively explore the relationships between potential sources of heterogeneity and the effect size estimates, thereby identifying the factors that most significantly contributed to the observed variability. |  |
| 13c | All data analysis and the generation of visual representations, including graphs and plots, were carried out using Stata version 12.0 for the Windows 8 operating system. This software, renowned for its robustness and versatility in handling complex statistical analyses, provided the necessary tools to execute our methodological framework with precision and reproducibility. |  |
| Reporting bias assessment | 14 | Reporting Bias AssessmentTo evaluate the potential presence of publication bias, a critical aspect in ensuring the integrity and representativeness of our meta-analysis, we employed two complementary methods: the construction of funnel plots and the application of Egger regression tests. Funnel plots, with their characteristic funnel-shaped visual representation, provide a graphical means of assessing the symmetry of the distribution of study effect sizes against sample size or precision. Asymmetry in the funnel plot can suggest the existence of publication bias, with smaller, less significant studies potentially being underrepresented or omitted.Complementing the visual inspection of funnel plots, the Egger regression test was conducted to statistically test for the presence of funnel plot asymmetry. This test, based on regression analysis, provides a quantitative measure of the likelihood of publication bias, enhancing the objectivity of our assessment and enabling a more nuanced understanding of the potential impact of selective reporting on our overall findings. |  |
| Certainty assessment | 15 | Certainty AssessmentTo appraise the certainty or credibility of the evidence base underpinning our outcomes, we implemented the trimming and filling methodology. This approach, designed to address potential publication bias and enhance the robustness of our conclusions, involves the systematic adjustment of the data set by either trimming or filling in studies based on the estimated impact of missing or underreported data. By accounting for potential biases in the available evidence, this technique provides a more accurate and reliable assessment of the true effect size, thereby strengthening the confidence in our overall findings and their implications for clinical practice and future research directions.  |  |
| RESULTS  |  |
| Study selection  | 16a | Figure 1 outlines the literature specific screening process. An electronic database search identified 2447 citations.After removing 722 duplicate literatures, 19 articleswere selected for full-text review to understand their relevance to this study after reading the literature abstracts.In the full-text review stage, 9 studies excluded becausedid not report details andlack necessary data. Finally, 10 studies were included in the systematicreview. The consistency between researchers during thefull-text review phase was excellent. |  |
| 16b |  |  |
| Study characteristics  | 17 | Study characteristicsThe characteristics of the 10 studies are shown in Table 1 [17–27]. A total of 7364 participants were included, The 10studies included 2 Randomized controlled trial and 8 Cross sectional study, 7 of which were conducted in chinese people, 2 from America, and 1 from Iranwith the subjects obtained from Patients with chronic kidney disease. According to NOS or AHRQ literature quality evaluation, the quality of 2 RCT studies and 7 cross-sectional studies were higher (NOS>6 or AHRQ≥8). 1 cross-sectional study was of moderate quality (AHRQ=7). (Table 1) |  |
| Risk of bias in studies  | 18 | Present assessments of risk of bias for each included study. |  |
| Results of individual studies  | 19 | For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots. |  |
| Results of syntheses | 20a | For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies. |  |
| 20b | Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect. |  |
| 20c | Present results of all investigations of possible causes of heterogeneity among study results. |  |
| 20d | Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results. |  |
| Reporting biases | 21 | Publication biasFunnel plot analysis qualitatively revealed the asymmetrical shape (Figure 5), suggesting that the association between homocysteine levels and GFR decline may be influenced by publication bias. Egger tests showed publication bias (P<0.05) (Figure 6). In order to determine whether the total effect results are stable, the scissors graph is used for analysis. Results show stable (P<0.01) (Figure 7). |  |
| Certainty of evidence  | 22 | Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed. |  |
| DISCUSSION  |  |
| Discussion  | 23a | DiscussionThe present meta-analysis was meticulously devised and executed with the primary aim of furnishing a comprehensive and exhaustive exploration of the nexus between hyperhomocysteinemia (HHcy) and the glomerular filtration rate (GFR) within the cohort of patients diagnosed with chronic kidney disease (CKD). The research landscape in this domain has hitherto been marred by equivocal findings, necessitating a systematic synthesis of extant evidence to distill the true nature of this relationship. A conspicuous and highly significant heterogeneity, manifested by an I² statistic of 99.5% along with a minuscule p-value (< 0.01), pervaded our study cohort. Such pronounced variability among the incorporated studies underscored the imperative to conduct sensitivity analysis and subgroup analysis, thereby enabling a more nuanced dissection of the relationship between homocysteine (Hcy) levels and CKD pathophysiology.The sensitivity analysis, a cornerstone of our methodological arsenal, unequivocally demonstrated that the exclusion of any single study from the aggregate dataset exerted a negligible impact on the overall relationship under scrutiny. This robustness implies that the observed association between HHcy and GFR transcends the idiosyncrasies of individual investigations and stands resilient across diverse data subsets, bolstering the reliability of our meta-analytic findings.Subgroup analysis, in turn, unearthed that cross-sectional studies, despite exhibiting an I² value of 0.00% yet accompanied by a significant p-value (< 0.05), constituted the principal wellspring of heterogeneity. This revelation not only sheds light on the underlying drivers of variability, attributable to disparities in study designs, participant demographics, and measurement modalities for Hcy and GFR, but also serves as a cautionary note when extrapolating results across different study archetypes.The amalgamated results, notwithstanding the heterogeneity, emphatically pointed towards a significant negative correlation between HHcy and GFR. In essence, our findings suggest that elevated Hcy levels portend a deleterious impact on renal function in CKD patients, with a high likelihood of precipitating a decline in GFR. This inference finds corroboration in several antecedent studies [17, 38 - 41], which have independently attested to Hcy's role as a prognosticator of kidney function deterioration, thereby fortifying the external validity of our meta-analysis and accentuating the clinical salience of vigilant monitoring and management of Hcy levels in CKD patients.Pathophysiological UnderpinningsThe pathophysiological mechanisms undergirding the observed relationship between HHcy and GFR are ostensibly multifaceted and intertwined [6, 42, 43]. HHcy has been shown to act as a potent inducer of endothelial dysfunction, a precursor to a cascade of deleterious events within the renal microenvironment. By instigating oxidative stress and inciting an inflammatory milieu, it sets in motion a series of direct and indirect insults to the glomerular and tubular architectures of the kidney. These insults culminate in structural alterations and functional impairments, ultimately leading to a reduction in GFR. Additionally, Hcy has been implicated in promoting the deposition of extracellular matrix components and fostering renal fibrosis over time, a process that inexorably erodes renal function and heralds the progression of CKD. |  |
| 23b | Nevertheless, our study is not without limitations. Firstly, the preponderant majority of the included studies were cross-sectional in nature, which inherently precludes the establishment of a causal relationship between Hcy and CKD. While associations have been robustly demonstrated, the directionality of causality remains shrouded in uncertainty, warranting longitudinal studies with well-defined temporal sequences to tease out the causative links. Secondly, the heterogeneity that pervaded our study, albeit dissected to some extent through subgroup analysis and meta-regression, persisted in residual form. This residual heterogeneity can be ascribed to a constellation of factors, including differences in study design, patient characteristics, measurement methodologies for Hcy and GFR, and treatment regimens across the included studies. Thirdly, the absence of gender-based comparisons represents a conspicuous lacuna in our analysis. Gender differences [44, 45] have been shown to play a pivotal role in many disease processes, including CKD, which exhibits a higher prevalence in men, potentially mediated by the direct and indirect effects of sex hormones. Sex hormones modulate the synthesis of cytokines, vasoactive factors, and growth factors, thereby exerting a profound influence on renal hemodynamics and disease progression, highlighting the need for future investigations to account for gender disparities. |  |
| 23c | Discuss any limitations of the review processes used. |  |
| 23d | In sum, our meta-analysis furnishes compelling evidence of an association between HHcy and a diminished GFR in CKD patients. These findings have far-reaching implications for clinical practice, underlining the indispensability of factoring in Hcy levels during the management and risk stratification of CKD patients. However, the road ahead is paved with opportunities for further research, which should be directed towards unraveling the pathophysiological intricacies and clinical ramifications of this relationship in greater detail, as well as devising more efficacious preventive and therapeutic strategies for CKD management. Such endeavors will not only enhance our understanding of this complex disease but also hold the promise of improving patient outcomes and alleviating the global burden of CKD. |  |
| OTHER INFORMATION |  |
| Registration and protocol | 24a |  the review was not registered. |  |
| 24b | the protocol was not prepared. |  |
| 24c |  |  |
| Support | 25 | This research was supported by the Natural Science Foundation of Hunan Province (2022JJ70004). |  |
| Competing interests | 26 | The authors declare that this study was conducted in the absence of any commercial or financial relationships that could serve as a potential conflict of interest. |  |
| Availability of data, code and other materials | 27 | The datasets used and/or analysed during the current study available from thecorresponding author on reasonable request. |  |

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