

## Review Article

# Homocysteine-Lowering Interventions in Chronic Kidney Disease

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## INTRODUCTION

The prevalence of cardiovascular disorders is higher in patients suffering from chronic kidney diseases (CKD) in comparison with the general population, and such disorders are considered as the leading cause of death.<sup>[1]</sup> For example, deaths from cardiovascular problems are 10–100 times higher in dialysis patients compared to the population that is matched in terms of age and gender.<sup>[2]</sup> Therefore, the prevention and treatment of cardiovascular diseases (CVDs) and an understanding of its factors in individuals with CKD are essential.<sup>[3,4]</sup> According to previous evidence,<sup>[5,6]</sup> the high prevalence of CVD among CKD patients is due to known traditional risk factors (e.g., hypertension, dyslipidemia, and diabetes mellitus) and other related factors (e.g., malnutrition or systemic inflammation increases cardiovascular morbidity and mortality in this group of patients).

## ABSTRACT

The incidence of cardiovascular events and mortality is higher in patients with chronic kidney disease (CKD) compared to the general population. Homocysteine (Hcy) appears to be an independent risk factor for cardiovascular diseases in general populations and patients with CKD. Further, hyperhomocysteinemia can cause endothelial damage and increase the activity and production of coagulation factors, and its prevalence among patients with end-stage renal disease is approximately 85%–100%. Most treatments, which lower Hcy levels and have been considered in previous studies, include folic acid, B vitamins, omega-3 fatty acids, and N-acetylcysteine. However, the effect of therapies that can decrease Hcy levels and thus cardiovascular events in these patients is still unclear. The results are conflicting and require further investigation. To guide treatment decisions and improve patient outcomes, multiple databases were searched, including Web of Science, PubMed, and Medline to summarize the available evidence (i.e., clinical trial and meta-analyses) on Hcy-lowering interventions and cardiovascular events.

**KEYWORDS:** *Chronic kidney disease, homocysteine, hyperhomocysteinemia*

Homocysteine (Hcy) is a nonessential sulfur-containing amino acid that is produced in the metabolic cycle of Hcy-methionine and plays a crucial role in this metabolic pathway by interfering with folic acid and B12.<sup>[7]</sup> Hcy appears to cause endothelial damage and increase the activity and production of coagulation factors.<sup>[8]</sup> There is ample evidence that hyperhomocysteinemia impairs endothelial function, increases the oxidation of low-density lipids, and stimulates the inflammation and proliferation of smooth muscle cells.<sup>[9]</sup>

Epidemiological studies have shown that elevated Hcy levels increase the risk of cardiovascular events in the general population and people with CKD.<sup>[10-12]</sup> A decrease of 25% in Hcy levels in the general population reduces

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the risk of coronary artery disease (CAD) and stroke risk by 11% and 19%, respectively.<sup>[13]</sup> Decreased glomerular filtration in CKD increases Hcy levels in most patients depending on the severity of renal impairments. Given that studies have demonstrated that Hcy levels decrease after kidney transplantation, it is suggested that renal mechanisms are relatively responsible for the increase in Hcy levels in CKD patients.<sup>[14-18]</sup>

Therefore, due to the direct relationship between Hcy and cardiovascular events, therapies reducing Hcy levels may decrease the CVD burden. According to previous evidence, most treatments lowering Hcy levels are folic acid, B vitamins, omega-3 fatty acids, and N-acetylcysteine. Although patients with CKD have hyperhomocysteinemia, the impact of Hcy reductions on cardiovascular mortality in these patients is still unknown. Thus, conflicting results exist in this regard, requiring further evaluations.<sup>[8,19-22]</sup>

The effectiveness of any intervention should be thoroughly evaluated and clarified due to the high burden of CVD in CKD patients. Accordingly, this review aimed to discuss the clinical studies conducted to reduce the Hcy level in CKD patients and their benefits and harms to guide decisions and improve patient outcomes.

## METHODS

Materials for this review were obtained by searching ISI Web of Science, PubMed/Medline, Scopus, Cochrane central register of controlled trials, and Cochrane database of systematic reviews. Key words used as search terms were "chronic kidney disease", "homocysteine", and "hyperhomocysteinemia". This search was performed without time limitation. Major well-designed studies which used Hcy-lowering effect as a main surrogate end point were included.

## RESULTS

### The Relationship between Homocysteine Levels and Renal Function

Patients with CKD have higher levels of Hcy compared to the general population,<sup>[19,23]</sup> and the prevalence of hyperhomocysteinemia among patients with end-stage renal disease (ESRD) is about 85%–100%.<sup>[24]</sup> The important role of the kidney in the excretion and metabolism of Hcy is well known.<sup>[25]</sup> There is an inverse relationship between the estimated glomerular filtration rate (eGFR) and the plasma concentrations of Hcy.<sup>[26]</sup> Nevertheless, according to a previous study,<sup>[25]</sup> Hcy has a high plasma protein binding that allows it to remain mainly in the circulation during filtration (although its free form has a low molecular weight and can easily cross ultrafiltration barriers). On the other hand, it seems

that the impaired Hcy metabolism in the kidneys has a more critical effect on increasing Hcy levels instead of reducing its excretion. Elevated uremic toxins, acidosis, and systemic inflammation may all lead to metabolic disorders.<sup>[27]</sup> The metabolic pathways of transsulfuration and remethylation, causing the effective removal of Hcy in the body, are particularly affected by kidney diseases.<sup>[28,29]</sup>

### Homocysteine and Cardiovascular Diseases

The association between Hcy and atherosclerotic vascular diseases and states of increased coagulation has been suggested as a risk factor for CVD since the early 1990s.<sup>[30]</sup> Elevated Hcy levels appear to be an independent risk factor for atherosclerosis.<sup>[31]</sup> Furthermore, Hcy can damage endothelial cells and increase CVD by creating several different mechanisms. Some of the hypothetical mechanisms of these effects include decreased bioavailability of nitric oxide, a potent vasodilator,<sup>[32]</sup> oxidative damage and cytotoxicity, increased proliferation of vascular smooth muscle cells, increased collagen synthesis,<sup>[33]</sup> and thrombosis.<sup>[34]</sup> It also increases cholesterol synthesis by increasing HMG Co-A 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase activity.<sup>[35]</sup>

Research<sup>[34]</sup> has reported a clear link between serum Hcy and the risk of CVD (CAD, heart disease, cerebrovascular diseases, and peripheral vascular diseases). The relationship between serum Hcy levels and the severity of CAD was investigated in a study of 70 patients who were scheduled for coronary angiography. The results showed that fasting serum Hcy levels were significantly higher in patients with CAD in comparison with patients without CAD ( $P < 0.001$ ). Moreover, the Hcy level was significantly associated with increased CAD severity ( $P < 0.001$ ). The findings also revealed that endothelial dysfunction due to increased Hcy is the most common and acceptable mechanism for increasing the risk of CAD.<sup>[36]</sup> Other observational studies represented that the elevated blood Hcy level is a risk factor for cardiovascular disorders.<sup>[32]</sup> The results of another *post hoc* analysis study demonstrated that high Hcy levels in patients with CKD were associated with increased CAD risk.<sup>[37]</sup> Based on the long-term cardiovascular risk assessment (for 29 months) in 175 dialysis patients, a 10  $\mu\text{mol/L}$  increase in the Hcy level was associated with a 20% increase in cardiovascular problems.<sup>[38]</sup>

Therefore, it seems that lowering Hcy levels may limit or prevent cardiovascular events.<sup>[39]</sup> For example, in a meta-analysis of 30 different studies, a 25% reduction in Hcy levels was related to 11% and 19% reductions in ischemic heart disease and stroke, respectively.<sup>[13]</sup> However, in a systematic review of

15 randomized controlled trials (RCT) in 71,422 individuals, no difference was reported regarding the effects of Hcy-lowering interventions on reducing deaths or complications from myocardial infarction. In terms of stroke, this study showed a slight difference in the effect of Hcy-lowering interventions alone or compared with the placebo.<sup>[40]</sup> Nevertheless, it is yet unclear whether and how Hcy-reducing treatments may reduce CVD risk, and many researchers doubt such a link between Hcy and CVD.<sup>[39-41]</sup> Accordingly, further studies are needed to determine the exact association between decreased Hcy levels and CVD.

### Effect of Homocysteine-Lowering Interventions in Chronic Kidney Diseases Patients

#### Folic acid

Folic acid is a water-soluble vitamin that is naturally found in plants with dark green leaves. Humans do not synthesize folate, thus daily needs are met through folic acid supplements or foods that are rich in this vitamin.<sup>[42]</sup> The role of folic acid is well known. More precisely, it is not only a significant factor in Hcy metabolism but its disturbance in homeostasis may be directly related to cardiovascular risk and CKD progression.<sup>[43,44]</sup> The maximum reduction in plasma Hcy concentrations can be achieved with a minimum dose (0.8 mg/day) of folic acid.<sup>[45]</sup> Numerous studies have evaluated the effects of lowering the concentration of Hcy with folic acid and its consequences on the progression of CKD or CVD events. In a 1-year randomized study, 81 hemodialysis patients were divided into three groups of control, folic acid 5, and 15 mg. The results revealed that although folic acid could significantly reduce Hcy concentrations, 88% of patients still had higher-than-normal Hcy concentrations, and higher doses of folic acid were not superior to lower doses. According to the results of this study, although lowering Hcy levels can reduce cardiovascular events, conclusions about this issue cannot be cited due to the limited number of participants and the short duration of the study.<sup>[46]</sup> In another RCT, hemodialysis patients treated with 10 mg of folic acid were categorized into dose reduction (5 mg folic acid) and dose increase (15 mg folic acid) groups. The findings of this study indicated that increasing the dose of folic acid causes a significant decrease in Hcy concentrations. However, it still could not make the concentration of Hcy normal.<sup>[47]</sup> Similarly, Tamadon *et al.* found that although folic acid significantly reduced Hcy concentrations, there was no difference between low-and high-dose treatment.<sup>[48]</sup> One reason for the nonnormalization of Hcy concentrations with folic acid can be related to the short duration of studies because the maximum clinical effects of folic acid can be obtained after six months.<sup>[49,50]</sup> Another trial examined the difference between folic acid

and folic acid in hemodialysis patients. Folic acid is a shortened form of folic acid that acts similarly to folic acid as a vitamin. The results of the above-mentioned study showed that both folic and folic acids cause a significant reduction in Hcy concentrations and can be safely used interchangeably in these patients.<sup>[51]</sup> Likewise, Delfino *et al.* concluded that folic acid therapy lowers Hcy concentrations while improving plasma antioxidant capacity, and therefore, may effectively reduce cardiovascular death.<sup>[50]</sup> Conversely, further studies found no link between a decrease in Hcy and a reduction in cardiovascular events. In a three-arm trial in 510 chronic hemodialysis patients, Wrona *et al.* reported no difference in death and cardiovascular events with folic acid at doses of 1, 5, or 15 mg/day.<sup>[52]</sup> In an atherosclerosis and folic acid supplementation trial, 315 CKD patients (with eGFR <25 ml/min) were treated with 15 mg/day folic acid or a placebo. After an average follow-up of 3.6 years, the results were not significant in providing the beneficial effects of folic acid regarding reducing cardiovascular mortality.<sup>[53]</sup> The results of a 2-year, double-blind, randomized, placebo-controlled trial on 186 patients with ESRD demonstrated that 10 mg of folic acid (three times a week) after each dialysis session could normalize Hcy concentrations in 92.3% of patients and cause a significant reduction in intima-media thickness. However, it caused no changes in cardiovascular events compared to the control group.<sup>[54]</sup> Contrarily, Righetti *et al.* concluded that folate treatment could decrease cardiovascular events in dialysis patients by reducing Hcy.<sup>[55]</sup> A summary of all the mentioned studies in this review is provided in Table 1.

Due to the inconsistent results and differences in the protocols of studies, the number of samples, the dose of folic acid, and the measured outcomes, in a meta-analysis of seven clinical trials, Qin *et al.* studied the reducing effects of Hcy on folic acid and cardiovascular events. Based on their report, folic acid treatment could reduce CVD risk by 15% in 3886 ESRD patients, especially if treated for a long time while not receiving folate supplements before starting the treatment.<sup>[2]</sup> Nevertheless, another meta-analysis of 11 trials for a total of 10,951 CKD patients showed that doses of folic acid, which decreased serum Hcy, had no effect on reducing cardiovascular events and should not be used for this purpose.<sup>[56]</sup> The controversy in the obtained results of these two meta-analyses was due to their differences in the criteria for entering clinical studies, the study population, and their methodological differences. Accordingly, additional studies are required to reveal the exact association between Hcy reduction and cardiovascular events in CKD patients.

**Table 1. Selected clinical studies of homocysteine-lowering interventions in CKD patients**

Study	Design/Intervention	Participants, <i>n</i>	End point	Findings
Thambyrajah <i>et al.</i> , 2000	Double blind RCT: folic acid 5 mg versus placebo	100 predialysis patients	Hcy level, endothelial function	Significant reduction in Hcy, No differences in endothelial function
Righetti <i>et al.</i> , 2003	RCT: folic acid 5, 15 mg versus control	81 hemodialysis patients	Hcy level, CV events	Significant reduction in Hcy, No differences in CV events
Wrone <i>et al.</i> , 2004	Three arms, double blind RCT: folic acid 1 mg or 5 mg or 15 mg	510 hemodialysis patients	Hcy level, CV events	Significant reduction in Hcy, No differences in CV events
Zoungas <i>et al.</i> , 2006	Double blind RCT (ASFAST): folic acid 15 mg versus placebo	315 CKD (eGFR <25 mL/min) patients	Hcy level, CV events	Significant reduction in Hcy, No differences in CV events
Righetti <i>et al.</i> , 2006	Open prospective trial: folic acid 5 mg versus control	114 hemodialysis patients	Hcy level, CV events	Folic acid decreases CV events
Delfino <i>et al.</i> , 2007	Double blinded RCT: folic acid 10 mg versus placebo	46 hemodialysis patients	Hcy level, plasma antioxidant capacity	Significant reduction in Hcy, improved the total plasma antioxidant capacity
Vianna <i>et al.</i> , 2007	Double blind RCT: folic acid 5 mg versus placebo	97 hemodialysis patients	Hcy level, CV events	Significant reduction in Hcy, significant decrease in intima-media thickness, No differences in CV events
Ossareh <i>et al.</i> , 2010	RCT: folic acid 5 mg versus 15 mg	80 hemodialysis patients	Hcy level	Significant reduction in Hcy with 15 mg folic acid
Soleimani <i>et al.</i> , 2011	RCT: 15 mg of folic acid versus 15 mg of oral folinic acid	60 hemodialysis patients	Hcy level	Folic and folinic acid decreased the blood homocysteine level with no meaningful difference
Tamadon <i>et al.</i> , 2011	RCT: 2, 5, 10, and 15 mg of folic acid	31 hemodialysis patients	Hcy level	Different doses of folic acid were not significantly different
Dierkes <i>et al.</i> , 2000	RCT: folic acid 800 µg+ vitamin B12 6 µg+ vitamin B6 10 mg versus folic acid 160 µg+ vitamin B6 10 mg versus placebo	61 hemodialysis patients	Hcy level	Significant reduction in Hcy with preparation including 800 µg folic acid
Manns <i>et al.</i> , 2001	Double blind RCT: folic acid 1 mg+ vitamin B12 1 mg versus control	81 hemodialysis patients	Hcy level	Significant reduction in Hcy
Álvarez <i>et al.</i> , 2005	Double blind RCT: folic acid 5 mg+ vitamin B6 10 mg + vitamin B12 0.4 mg versus folic acid 15 mg+ vitamin B6 100 mg+ vitamin B12 1 mg	60 hemodialysis patients	Hcy level	No differences in two vitamin regimen
Tungkasereerak <i>et al.</i> , 2006	Double blind RCT: folic acid 15 mg + vitamin B6 50 mg + vitamin B12 1 mg versus control (folic acid 5 mg)	54 hemodialysis patients	Hcy level, Intima-media thickness	Significant reduction in Hcy, No differences in Intima-media thickness
Jamison <i>et al.</i> , 2007	Double blind RCT: folic acid 40 mg+ vitamin B6 100 mg+ vitamin B12 2 mg versus control	2056 CKD (eGFR ≤30 mL/min) patients	Hcy level, CV events	Significant reduction in Hcy, No differences in CV events
Mann <i>et al.</i> , 2008	Double blind RCT: folic acid 2.5 mg + vitamin B6 50 mg + Vitamin B12 1 mg versus control	619 CKD (eGFR <60 mL/min) patients	Hcy level, CV events	Significant reduction in Hcy, No differences in CV events
Azadibakhsh <i>et al.</i> , 2009	Double blind RCT: folic acid 5 mg+ vitamin B12 1 mg versus folic acid 15 mg+ vitamin B12 1 mg	36 hemodialysis patients	Hcy level	Significant reduction in Hcy with versus folic acid 15 mg+ vitamin B12 1 mg
Heinz <i>et al.</i> , 2010	RCT: folic acid 5 mg+ vitamin B6 20 mg + Vitamin B12 50 µg versus control	650 hemodialysis patients	Hcy level, CV events	Significant reduction in Hcy, No differences in CV events
Tayebi <i>et al.</i> , 2016	Double blinded RCT: vitamin B12 100 µg versus control	140 hemodialysis patients	Hcy level, plasma	Significant reduction in Hcy

Beavers <i>et al.</i> , 2008	Double blind RCT: omega-3 6 g per day versus control	69 hemodialysis patients	Hcy level	No significant reduction in Hcy
Rasmussen <i>et al.</i> , 2010	Double blind RCT: omega-3 1.7 g per day versus placebo	206 hemodialysis patients	Hcy level, lipid profile	No significant reduction in Hcy
Khosroshahi <i>et al.</i> , 2013	RCT: omega-3 3 g per day versus placebo	100 hemodialysis patients	Hcy level	Significant reduction in Hcy
Ziaie <i>et al.</i> , 2020	single group clinical trial: omega-3 1 g per day versus placebo	19 peritoneal dialysis patients	Hcy level, inflammatory markers and lipid profile	CRP, HS-CRP, and Hcy levels increased insignificantly, No significant effect on lipid profile
Scholze, <i>et al.</i> , 2003	RCT: NAC 5 g IV during hemodialysis session versus placebo	20 hemodialysis patients	Hcy level	Significant reduction in Hcy after dialysis
Thaha <i>et al.</i> , 2006	RCT: NAC 5 g IV during hemodialysis session versus placebo	60 hemodialysis patients	Hcy level	Significant reduction in Hcy after dialysis
Nascimento <i>et al.</i> , 2010	RCT: NAC 1200 mg/day versus placebo	30 peritoneal dialysis patients	Hcy level	No significant reduction in Hcy
Renke <i>et al.</i> , 2010	RCT: NAC 1200 mg/day versus placebo	20 patients with normal or slightly decreased kidney function (eGFR 61-163 ml/min)	Hcy level, Blood pressure	No significant reduction in Hcy and blood pressure
Urquhart, <i>et al.</i> , 2007	RCT: 5.0 mg/kg of intravenous mesna or placebo at 2 separate dialysis sessions 1 week apart	5 hemodialysis patients	Hcy level	Significant reduction in Hcy after dialysis
Urquhart <i>et al.</i> , 2008	Double blinded RCT: Mesna 5 mg/kg predialysis session versus placebo	48 hemodialysis patients	Hcy level	No significant reduction in Hcy
Cutler <i>et al.</i> , 2009	RCT: Mesna 12 mg/kg predialysis session versus placebo	8 hemodialysis patients	Hcy level	No significant reduction in Hcy
Pakfetrat <i>et al.</i> , 2013	RCT: zinc supplement 50 mg/day versus placebo	100 hemodialysis patients	Hcy level	Significant reduction in Hcy

CKD: chronic kidney disease, Hcy: homocysteine, CV: cardiovascular, RCT: randomized controlled trial, eGFR: estimated glomerular filtration rate, CRP: C-reactive protein, HS-CRP: high-sensitivity C-reactive protein, NAC: N-acetyl cysteine

### Folic acid plus B vitamins

In addition to folate, B vitamins (including B12 and B6) are essential determinants of Hcy metabolism and act as a cofactor to convert Hcy to cysteine and methionine.<sup>[57-59]</sup> Some studies have shown the potential of supplements containing these vitamins to reduce circulating Hcy. The treatment of B vitamins to reduce Hcy and cardiovascular events, the risks attributed to Hcy, has been investigated. The results of these mixed experiments and their collective interpretation are still controversial due to design heterogeneity, study limitations, and other considerations.<sup>[60-62]</sup>

Although several studies on healthy populations and patients have evaluated the effect of different doses of folic acid supplementations in reducing Hcy, a few of them have considered the independent effects of Vitamin B12.<sup>[57]</sup> A recent clinical trial has represented that Vitamin B12 alone can significantly reduce Hcy in hemodialysis patients.<sup>[63]</sup> However, most clinical studies

have examined the effects of combined treatment with folic acid and B vitamins. In a parallel clinical study involving three treatment groups, Dierkes *et al.* found that a multivitamin supplement containing at least 800 µg of folic acid could significantly decrease Hcy in ESRD patients by nearly 50%. It should be noted that two groups of patients received two different multivitamin formulations, and the third group received no multivitamins (the placebo group). In contrast, supplements containing only 160 µg of folic acid failed to reduce Hcy compared to the placebo.<sup>[32]</sup> In a randomized, double-blind trial on 81 hemodialysis patients, the addition of 1 mg of oral Vitamin B12 to patients, who did not meet Vitamin B12 deficiency by laboratory standards and received 1 mg of folic acid, reduced total Hcy. However, only 13.6% of patients had normal Hcy levels at the end.<sup>[35]</sup> Similar results were reported in another study.<sup>[64]</sup> The difference between the efficacy of multivitamins containing folic acid, Vitamin B12, and Vitamin B6 in the daily dose and multivitamin

supplementations in the super physiological daily dose in hemodialysis patients was compared in 43 patients. The results showed that a water-soluble multivitamin combination with two different doses effectively lowered Hcy levels although Hcy reached normal levels only in a small percentage of patients. Accordingly, the decrease rate in the Hcy level is directly related to the basal level of Hcy and folic acid.<sup>[65]</sup>

In another randomized, double-blind trial of 54 chronic hemodialysis patients, 6 months of treatment consisting of 15 mg of folic acid daily, 50 mg of Vitamin B6, and 1 mg of vitamin B12 compared with 5 mg of folic acid significantly decreased the serum level of Hcy. Intima-media thickness was also measured before and after the intervention. Although there was no significant difference in intima-media thickness between the two groups, the treatment group showed a tendency to have less thickness in the sixth month.<sup>[66]</sup>

In a large-scale double-blind, RCT, Jamison *et al.* treated 2056 CKD patients (with eGFR  $\leq$ 30 ml/min) with high doses of supplements (including 40 mg folic acid, 100 mg Vitamin B6, and 2 mg Vitamin B12) and followed up patients for all causes of mortality for 3.2 years. Their results indicated that treatment with high doses of folic acid and B vitamins did not improve survival or reduce the incidence of vascular diseases. These negative outcomes may be due to the higher burden of CVD and less than optimal compliance with treatment.<sup>[67]</sup>

In addition, Mann *et al.* designed an RCT of 619 chronic hemodialysis patients for 5 years with 2.5 mg of folic acid, 1 mg of vitamin B12, and 50 mg of Vitamin B6 versus a placebo. Although treatment with B vitamins decreased Hcy levels in participants with CKD, there was no difference in the risk of cardiovascular events between the two groups.<sup>[68]</sup> The results of a multicenter analysis of 650 hemodialysis patients also demonstrated that active treatment with folic acid, Vitamin B12, and Vitamin B6 did not significantly reduce overall mortality and cardiovascular risk in patients with ESRD.<sup>[69]</sup>

Due to the high importance of interventions that can decrease mortality or cardiovascular events in CKD patients and the conflicting results of clinical studies, several meta-analyses have examined the cardiovascular benefits of Hcy-lowering therapies. Likewise, Heinz *et al.* conducted a meta-analysis on a total of 23 retrospective and observational studies regarding the effect of Hcy on overall mortality and CVD risk and analyses of five RCTs to evaluate the reduction of cardiovascular events and mortality with B vitamin treatment. Based on their findings, Hcy levels are generally a risk factor for

cardiovascular events and overall mortality, and taking Vitamin B supplements significantly reduces CVD while not decreasing the overall risk of mortality.<sup>[21]</sup> It should be noted that one of the limitations of this study was the small number of analyzed RCTs. In addition, some of these RCTs involved a small number of patients, or the study protocol was not of randomized or placebo-controlled type. Another meta-analysis of 4836 patients with CKD assessed the relationship between Hcy-lowering therapy and CVD. According to the results of this review, Hcy-lowering treatment was not associated with a significant reduction in the risk of CVD, stroke, and all-cause mortality.<sup>[70]</sup> A meta-analysis of 14 studies represented that Hcy-lowering therapy based on Vitamin B supplements was related to a significant decline in stroke risk in patients with CKD.<sup>[71]</sup> A recent meta-analysis examined the benefits and harms of folic acid treatment, Vitamin B6, and Vitamin B12, on all-cause mortality, and cardiovascular events in chronic hemodialysis patients. The results of the analysis of six studies on 2452 hemodialysis patients showed no significant difference in mortality and cardiovascular events in patients treated with Hcy-lowering supplements.<sup>[8]</sup>

In general, although the available evidence strongly supports the reduction of Hcy levels with medical treatments (i.e., folic acid, Vitamin B12, and Vitamin B6), the benefits of these vitamins in reducing mortality and cardiovascular events in patients with CKD and ESRD are not yet known in most cases.

### Omega-3 fatty acids

In addition to Hcy-reducing treatment with folic acid and B vitamins, omega-3 unsaturated fatty acids have been considered as a group of nutrients with a potential protective effect on the cardiovascular system.<sup>[72]</sup> Data from various epidemiological studies suggest that omega-3 fatty acid supplementation effectively reduces the risk of CVD.<sup>[73-76]</sup> Based on some reports, omega-3 fatty acids can also lower cholesterol, triglycerides, and inflammation.<sup>[77,78]</sup> Although several RCTs have suggested that omega-3 supplementations may decrease CVD risk factors,<sup>[79-82]</sup> other studies have shown no beneficial effect regarding reducing cardiovascular events.<sup>[83-86]</sup>

Some clinical trials investigated the association between Hcy and omega-3 fatty acid intake.<sup>[87]</sup> Plasma Hcy levels were reported to be inversely related to omega-3 supplementation.<sup>[84]</sup> The mechanism of action of omega-3 in lowering Hcy levels is not fully understood yet. However, it has been indicated that omega-3 fatty acids may regulate the gene expression of enzymes or the activity of enzymes involved in

plasma Hcy metabolism.<sup>[88]</sup> Therefore, a limited number of studies have evaluated the impact of omega-3 fatty acids on serum Hcy levels in patients with ESRD at different doses and durations. Khosroshahi *et al.* treated 100 hemodialysis patients with 3 grams of omega-3 daily or a placebo for two months and assessed Hcy levels before and after the intervention. They showed a significant reduction in Hcy levels with short-term administration of omega-3 fatty acids in hemodialysis patients.<sup>[83]</sup> Conversely, other clinical trials failed to represent Hcy-lowering effects with omega-3 fatty acids. In a study by Rasmussen *et al.* on 206 patients with documented CVD undergoing hemodialysis in Denmark, it was found that omega-3 (1.7 grams per day for 6 months) had no significant effect on patients' Hcy levels. Nonetheless, there was an inverse relationship between plasma Hcy and docosahexaenoic acid contents in serum phospholipids.<sup>[84]</sup> A similar study was performed on 69 hemodialysis patients, and it was reported that omega-3 (6 grams/day for up to 6 months) did not reduce Hcy levels in ESRD patients.<sup>[89]</sup> In an open-label, single-group clinical trial on peritoneal dialysis patients, 21 cases were treated with 1 g/day of omega-3, followed by assessing inflammatory markers including Hcy and lipid profiles. After three months of follow-up, the results revealed that omega-3 supplementation had no significant effect on Hcy levels.<sup>[90]</sup>

Moreover, Xu *et al.* studied the effects of omega-3 on serum lipids and vascular inflammation in ESRD patients. The results of data analysis from a total of 20 RCTs including 1461 patients demonstrated that omega-3 supplementation significantly decreased triglycerides, low-density lipoprotein cholesterol, and C-reactive protein while having no significant impact on other inflammatory markers including Hcy.<sup>[88]</sup>

There are currently a limited number of studies regarding the impact of omega-3 on lowering Hcy levels in ESRD patients. Due to the conflicting findings of the dose or duration of the required treatment and the underlying mechanism, the reported results are inconclusive. Therefore, further studies are necessary for evaluating its effects on Hcy and other inflammatory markers and cardiovascular outcomes in this group of patients.

### N-acetyl cysteine

NAC, a sulfur-containing and thiol-containing amino acid, is the primary precursor to the antioxidant glutathione<sup>[91,92]</sup> and may reduce Hcy-induced oxidative stress through its antioxidant properties. In addition to these properties, NAC, when orally or intravenously administered to healthy individuals, can displace circulating thiols from its protein binding site by

modifying the binding of thiol components, increasing Hcy urination<sup>[93,94]</sup> and decreasing total plasma Hcy levels.<sup>[94,95]</sup>

In a placebo-controlled crossover study on 20 ESRD patients, Scholze *et al.* found that intravenous administration of NAC during dialysis could significantly increase Hcy clearance through dialysis and thus reduce serum Hcy levels. They also showed that NAC could improve pulse pressure and endothelial function by reducing Hcy and its antioxidant effects.<sup>[96]</sup> Similar results were reported in another study.<sup>[97]</sup> However, in a placebo-controlled study of 30 peritoneal dialysis patients, the oral administration of NAC over 8 weeks failed to significantly decrease plasma Hcy concentrations. However, this short-term oral NAC treatment significantly reduced circulating interleukin 6 levels and suppressed the inflammatory response.<sup>[98]</sup> The effect of oral NAC (1200 mg) with the renin-angiotensin system blockade treatment on blood pressure and cardiovascular markers (including Hcy) was compared in nondiabetic patients with normal or slightly decreased renal function (eGFR 61–163 ml/min). Based on the results, NAC did not affect blood pressure and Hcy in nondiabetic patients with CKD.<sup>[99]</sup>

Overall, although NAC appears to have beneficial effects on reducing the risk of cardiovascular events in hemodialysis patients with its antioxidant effects (13 of 2010 trials), its effects on Hcy levels and cardiovascular outcomes are still unclear and thus are not currently recommended for this purpose in CKD patients.

### Mesna

Similar to NAC, mesna (sodium-2-mercaptoethanesulfonic acid) has been used as a thiol exchange agent in combination with hemodialysis to reduce Hcy levels. *In vitro*, mesna released 36.5% of the protein-bound Hcy within 30 min. *In vivo*, intravenous bolus 5 mg/kg pre-dialysis significantly decreased Hcy after dialysis.<sup>[100]</sup> However, the administration of mesna (5 mg/kg three times a week) before dialysis for 8 weeks did not significantly reduce Hcy concentrations.<sup>[101]</sup> Due to the safety and limited side effects of mesna, increasing the dose to 12 mg/kg was investigated, and the results showed that increasing its dose could not lower Hcy concentrations.<sup>[102]</sup> Although mesna could reduce Hcy levels after dialysis, its long-term treatment could not represent positive effects in this regard.

### Other Supplements

Although no study has so far determined the role of zinc in reducing Hcy, a double-blind, RCT evaluated the

effect of zinc supplementation on serum Hcy levels in ESRD patients. The administration of zinc for 6 weeks in 100 hemodialysis patients could significantly decrease Hcy compared to the placebo.<sup>[103]</sup> However, as far as it is known, no further studies have been performed on ESRD patients.

## DISCUSSION AND CONCLUSION

Cardiovascular mortality rates are high in ESRD patients, and high Hcy levels in these patients have been studied as an important therapeutic target. Routine Hcy measurement is not currently recommended in this respect. In addition, reviewing the published evidence suggests that the treatment of hyperhomocysteinemia may not currently be a reasonable therapeutic goal for reducing cardiovascular risk in CKD patients. The question of whether Hcy-lowering therapies reduce the risk of these cardiovascular complications is still of increasing concern in the general population and the ESRD population. Different responses to Hcy-reducing therapies may be influenced by differences in gender, genotype, and nutritional status of these populations. Data on the optimal dose of supplements, the exact target of Hcy, and the duration of treatment are still limited for examining their effectiveness.

Overall, it seems reasonable to consider folic acid with/without supplementation with B vitamins after the careful assessment of folate status as adjunctive therapy in patients with CKD. Agents such as NAC and mesna may displace Hcy from the protein binding site, thus facilitating its removal during dialysis. Nevertheless, their use has not been effective in the continued consumption. Although the exact dose and frequency of use have not been determined, omega-3 and other related antioxidants are also interesting agents that have the potential to lower Hcy levels. Further, high doses of omega-3 can be safely used in CKD patients.

Robust interventional studies are still necessary for determining whether plasma Hcy depletion has beneficial effects on CKD patients. Finally, the difference in effectiveness between various Hcy-lowering treatments has not been compared in the literature.

## AUTHORS' CONTRIBUTION

S. Badri, S. Vahdat, S. Seirafian, and M. Pourfarzam developed the idea of research and criticized the findings. T. Gholipur, and S. Ataei searched and recruited the studies. All authors contributed in manuscript preparation and revision.

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## Conflicts of interest

There are no conflicts of interest.

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