A meta analysis of randomized controlled clinical trials of role of folic acid in cardiovascular risks in chronic kidney disease patients

A shortened version of the paper's title: a meta-analysis of folate in patients with CKD

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Abstract: Backgrounds Previous studies have shown inconsistent results regarding the efficacy of homocysteine-lowering therapy with folic acid for reduction of cardiovascular risk, particularly in populations with chronic kidney disease (CKD). **Methods** We conducted a meta-analysis of clinical trials performed between January 1966 and December 2012 to assess the effects of folic acid supplementation in CKD populations. Data from 10 randomized controlled trials including 8879 patients with CKD were analysed. **Results** Different degrees of homocysteine reduction were achieved in all studies. A total of 1619 cardiovascular events were reported, and a beneficial trend but no statistical significance of homocysteine-lowering therapy with folic acid on reduction of cardiovascular events was shown (relative risk [RR], 0.93; 95% CI, 0.83 to 1.05; P=0.23). Subgroup analyses of cardiovascular events showed a statistical benefit in populations with end-stage renal disease and no folic acid fortification (RR, 0.82; 95% CI, 0.68 to 0.99; and RR, 0.74; 95% CI, 0.56 to 0.96, respectively), and a beneficial trend but no statistical significance was shown in populations with a high baseline homocysteine concentration, greater degree of homocysteine lowering, low baseline albumin concentration, and low incidence of diabetes mellitus. There were 1980 deaths, which was not significant (RR, 1.03; 95% CI, 0.96 to 1.10; P=0.46). Nonsignificant results were also observed for myocardial infarction, stroke events, and vascular mortality. **Conclusions** A beneficial trend but no statistical significance was observed for reduction of cardiovascular risks with folic acid supplementation in populations with CKD. Statistical benefits were demonstrated in some patients, which should encourage further discussion.

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Keywords: Cardiovascular disease; Chronic kidney disease; Folate; Homocysteine; Meta-analysis

Introduction

There is a markedly elevated risk of cardiovascular disease in patients with chronic kidney disease(CKD) compared with the general population, and prevention and treatment of cardiovascular diseases are major considerations in the management of individuals with CKD [(1,2)]. Studies suggest that traditional risk factors such as hypertension, hyperlipaemia, and hyperglycaem only account for about one- half of the risks of vascular diseases[(3)]. Homocysteine, a type of sulphur-containing amino acid, has been gradually identified as a new treatable risk factor as a result of its high prevalence and significant relationship to cardiovascular morbidity and mortality in patients with end-stage renal diseases(ESRD) [(1,4,5)]. Some inexpensive and easily accessible vitamins, (e.g., folic acid, vitamin B_{12} and vitamin B_6) have been reported to effectively decrease homocysteine concentrations in the general population and in patients with CKD [(6-9)]. Although the risk of cardiovascular disease is expected to be reduced by homocysteine-lowering therapy with folic acid, inconsistent results have been reported[(10-13)]. A meta-analysis of 7 studies reported a statistical benefit of folic acid intervention in populations with CKD, which seemed to bring about some support for this measure[(14)], but others observed insignificant results on subgroup analysis [(15).] We conducted a meta-analysis again to examine the effects of folic acid supplementation on cardiovascular risk in patients with CKD.

Methods

This systematic review was performed according to the Quality of Reporting of Meta-analyses guidelines [(16)]. Studies limited to patients with ESRD/CKD and randomized controlled trials published before December 2012 were included in the meta-analysis. All studies included folic acid supplementation (with or without additional vitaminB_6 and vitamin B_{12}) in the experimental group and placebo, very-low-dose folic acid, or usual care in the control group. Primary cardiovascular events (defined as myocardial infarction, stroke, other cardiovascular or cerebrovascular diseases, and cardiovascular death events), cardiovascular mortality, or all-cause mortality were observed as end points. The minimum follow-up

period was 6 months. All studies were assessed according to the principle of randomization, allocation concealment, completeness of follow-up, and intention-to-treat analysis.

We first identified 50 articles through a PubMed search with relevant text words and Medical Subject Headings that included all spellings of homocysteine, folate, vitamin B, cardiovascular disease, chronic kidney disease, renal dialysis and kidney transplantation. Most of the articles were eliminated for the following reasons after reviewing the abstracts: 9 articles had an unclear randomized design, 16 had inappropriate interventions or subjects, 10 lacked foregoing outcomes, and 4 had a follow-up period that was too short. One trial was eliminated after review of the full text showed that the dosage of folic acid in the control group was too large. As a result 10 trials were included in this meta-analysis (Figure 1).

Data from eligible trials were extracted in duplicate by 2 independent investigators according to intention-to-treat principles. Data on design characteristics, baseline information, laboratory indexes, prior disease histories of subjects, and raw counts of events in the included studies were extracted in detail. Only randomized data were collected if the trial was partly randomized. All disagreements were resolved by discussion or involvement of a third reviewer (V.P.) when necessary.

All data were managed using the Cochrane Collaboration's Review Manager software package (Rev-Man 5.1) through a random-effects model. Subgroup analyses were performed based on age, body mass index, laboratory values such as blood concentration of creatinine, albumin, cobalamine, cholesterol, and homocysteine, intervention measures such as dosage of folic acid, combination treatment of B vitamins, follow-up period, and degree of homocysteine lowering; and characteristics such as folic acid fortification and incidence of cardiovascular disease or diabetes mellitus. Relative risk (RR) with 95% CI was used for assessment of the association between folic acid supplementation and risk of vascular disease. Heterogeneity was assessed using chi-square test and I value. I^2 greater than 70% was regarded as unacceptable heterogeneity and less than 50% as acceptable. In order to detect and correct heterogeneity, we attempted to rectify incorrectly recorded data and exclude a few trials. Sensitivity analyses through removal of some small trials were used to reduce bias. Publication bias was assessed visually by funnel plot. For all analyses, $P<0.05$ was considered statistically significant except for heterogeneity analysis ($P>0.1$).

Results

Ten randomized controlled trials including 8879 patients with CKD were included in our meta-analysis. All studies revealed adequate sequence generation, and 8 studies showed the principle of double-blinding and allocation concealment as well. Different dosages of folic acid were adopted in these studies, ranging from 2.5mg to 40mg daily, and follow-up ranged from 12 to 60 months. Reductions of blood homocysteine concentration were achieved in the folic acid supplementation group in all studies, ranging from 2.2μmol/l to 30μmol/l. The detailed design characteristics, bias analysis, and baseline information for all 10 studies are presented in Table 1. Heterogeneity testing for overall analysis and stratified analysis showed no statistically significant difference (all $P>0.1$). Sensitivity analysis showed that RR and P values were not altered statistically even if a small trial was removed. There was no substantial asymmetrical appearance on funnel plot of overall analysis.

A total of 1619 cardiovascular events were reported in 9 studies with 6995 participants, and analysis showed a beneficial trend but no statistical significance of homocysteine-lowering therapy with folic acid on reduction of cardiovascular events (RR, 0.93; 95% CI, 0.83 to 1.05; $P=0.23$) (Figure 2). Among these cardiovascular events, 280 stroke events were reported in 8 studies with 8754 participants, accounting for 17.3% of total cardiovascular events, and 652 myocardial infarction events were reported in 7 studies with 8439 participants, accounting for 40.3% of total cardiovascular events. However, comparisons between the folic acid group and the placebo group in regard to these 2 events showed nonsignificant results (RR, 0.90; 95% CI, 0.71 to 1.13; $P=0.37$; and RR, 0.96; 95% CI, 0.83 to 1.11; $P=0.60$, respectively).

There were 1980 death events of any cause among 8439 participants in 7 studies. Six studies with 5968 participants reported 425 death events of cardiovascular disease, accounting for approximately 21.5% of total death events and 26.3% of total cardiovascular events. Identical insignificant results were obtained through pooled analyses (RR, 1.03; 95% CI, 0.96 to 1.10; and RR, 0.97; 95% CI 0.81 to 1.17, respectively) .

Subgroup analyses of primary cardiovascular events were conducted, and statistically beneficial effects were found in populations with no folic acid fortification and ESRD. Beneficial trends but no statistical significance was observed in populations with a baseline homocysteine level greater than 30 μmol/l, a reduction in homocysteine level of 10–20 μmol/l, a baseline albumin level less than 4g/l, and a proportion of patients with diabetes mellitus less than 40%. However, no significant results were obtained in subgroups based on baseline characteristics such as age; body mass index; blood concentration of cobalamine, creatinine, and cholesterol; and proportion of cardiovascular disease. Identical nonsignificant results

were observed in subgroups based on dosage of folic acid, follow-up period, and combination treatment with B vitamins (Figure 3).

Discussion

Effects of folic acid in different studies and populations

Although a meta-analysis published in 2011 that pooled 7 studies demonstrated a beneficial effect of homocysteine-lowering therapy with folic acid on reducing cardiovascular risk in populations with CKD, a recent meta-analysis that pooled 11 studies found nonsignificant results [14, 17]. Previous subgroup analyses in other meta-analyses also reported nonsignificant results of folic acid supplementation on cardiovascular events in patients with CKD even if a significant result was found in the general population [15]. Recently, several new studies were completed and new data were obtained [20,22], so we reviewed the literature and systematically analysed these studies again. Although the studies included in the meta-analysis all demonstrated nonsignificant results of homocysteine-lowering therapy with folic acid on the risk of cardiovascular disease, a slightly beneficial tendency was shown in most studies [18, 19, 21, 25-27] and on pooled analysis. An 11% lower risk of coronary artery disease and 19% lower risk of stroke have been observed with folic acid supplementation in the general population [28]. However, our analyses of patients with CKD failed to show significant results of folic acid intervention on stroke or myocardial infarction events. It is well known that higher homocysteine concentrations and higher morbidity of atherosclerotic diseases often coexist in patients with CKD, possibly these characteristics result in different levels of efficacy for interventions in the general population and patients with CKD, which suggests that different intervention measures may be needed in these groups.

Influence of different intervention measures

The superiority of supplementation with 0.8mg/d folic acid has been shown in previous studies, and this is reported to be the most suitable and effective dosage for lowering homocysteine concentration and reducing cardiovascular events in the general population [29]. Folic acid supplementation with 2 to 15mg/d is recommended due to refractory lower levels in patients with CKD [30, 31]. Most studies in our analysis adopted a higher dosage of folic acid, for example, 2.5mg in the HOPE-2 Renal study, 5mg in the Judith Heinz trial, 15mg in the Marco Righetti trial, and 40mg in the HOST trial. However, not only the results of each trial but also pooled stratified analysis according to the dosage of folic acid failed to show statistical significance in regard to cardiovascular disease. The intervention dosage may not be the key point, or a larger dosage may be needed.

Another factor is the period of follow-up. Although more than 3 years of follow-up showed a statistical benefit in cardiovascular disease, especially stroke events [15, 32], and a period of more than 2 years was reported to be effective in a meta-analysis of patients with CKD[14], our meta-analysis failed to show statistical significance in regard to follow-up time.

An additional factor is whether combination treatment with B vitamins is more effective than folic acid supplementation alone. Previous studies demonstrated the superiority of combined treatment with B vitamins compared with folic acid alone in the general population [15], but a nonsignificant result was observed in regard to combination treatment or folic acid supplementation in our meta-analysis.

Hopeful results were found in regard to the degree of reduction in homocysteine concentration. Our analysis demonstrated the benefit of folic acid supplementation in patients with CKD with an obvious decrease in homocysteine concentration. Prior studies also reported similar results[14,15]. Considering the previously mentioned findings some questions should be asked. What dosage of folic acid is useful for patients with CKD? What is an effective intervention period? What degree of reduction in homocysteine concentration should be considered as the standard to evaluate intervention efficacy? Further studies are needed on decreased homocysteine concentration with folic acid supplementation and cardiovascular risk in patients with CKD.

Influence of baseline characteristics

Previous studies and our meta-analysis showed the beneficial effect of reducing homocysteine concentration with folic acid on cardiovascular risk in populations without folic acid fortification [14, 15, 33]. It has been reported that lower baseline homocysteine concentration and insensitivity to folic acid often occur in populations with folate fortification whereas adverse conditions occur in populations without folate fortification[34,35]. This could potentially explain the reduced cardiovascular risk with folic acid supplementation in populations without folate fortification. Another definite benefit of folic acid has been observed in patients with ESRD, studies have shown that plasma homocysteine levels increases as estimated glomerular filtration rate declines in approximately 36-89% of patients with CKD, depending on severity[36,37], and 85-100% of those with ESRD[38]. A beneficial trend of folic acid intervention in populations with a higher homocysteine level was also shown in the meta-analysis. We may be able to interpret this to be the cause of the benefit of folic acid in patients with ESRD.

There are other interestingly beneficial trends with low blood concentration of albumin or a low proportion of subjects with diabetes mellitus. It is well known that most homocysteine (approximately 75%) is

combined with albumin and only a small amount (approximately 25%) exists in free status in blood. A low blood concentration of albumin is more often found in patients with renal diseases, but whether this situation would increase free homocysteine concentrations is indefinite. Currently, most studies concerning homocysteine levels have primarily focused on total homocysteine levels and not on free homocysteine or free/bound ratios. After all, the percentage of free homocysteine in blood is very small. It is important to note that only free (unbound) homocysteine is filtered and metabolized by the kidneys. Whether a stronger effect of folic acid supplementation is found in patients with CKD with more dissociative homocysteine is also unknown and requires further studies. Diabetes mellitus often generates and aggravates renal damage and subsequent metabolic disorders, and it also has a more complicated vascular pathophysiological mechanism and is estimated to be less affected by an intervention for a single risk factor. Moreover, for each 5 μmol/l increase in serum total homocysteine concentration, the risk of 5-year mortality rises by 17% in non-diabetics subjects and 60% in diabetic subjects [(39)], so a lower proportion of diabetes mellitus in subjects is favourable.

Several insignificant results

The failure of age, body mass index, cholesterol concentration, and creatinine concentration to have an effect on reduction of homocysteine concentration by folic acid intervention on cardiovascular risk is regarded as appreciable. It has been proven that advanced age, overweight and hyperlipidaemia are traditional risks of vascular diseases. Nonsignificant results seem to support the opinion that a synergistic effect of these traditional risk factors and homocysteine on cardiovascular events does not exist. The efficacy of decreasing high homocysteine levels by folic acid supplementation for reduction of cardiovascular risk should be assessed objectively because vascular diseases are the result of multiple factors.

Conclusions

A beneficial trend but no statistically significant results were observed for reduction of cardiovascular risk with folic acid supplementation in populations with CKD. Statistical benefits were demonstrated in patients with certain baseline characters and certain intervention effects, which should encourage hope and further discussion.

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Table 1 design characteristics and baseline data of 10studies

studies	VIANNA AC (18)	AFAST (19)	DIVINe (20)	ELIZABETH.M (21)	FAVORIT (22)	Hope-2 renal study (23)	HOST (24)	Judith Heinz (25)	Marco Righetti 2003(26)	Marco Righetti 2006(27)
adequate sequence generation	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
allocation concealment	yes	yes	yes	yes	yes	yes	yes	yes	no	no
blinding	yes	yes	yes	yes	yes	yes	yes	yes	no	no
intention to treat	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
intervention	5mg FA	15mg FA	2.5 mg FA 25 mg B_6 1 mg B_{12}	5mg FA/ *15mg FA 12.5 mg B_6 0.006 mg B_{12}	5mg FA 50mg B_6 1 mg B_{12}	2.5 mg FA 50mg B_6 1 mg B_{12}	40 mg FA 100 mg B_6 2mg B_{12}	2.5 mg FA 20 mg B_6 0.05mg B_{12}	5mg FA *15mg FA	5 mg FA 250 mg B_6 0.5mg B_{12}
control	placebo	placebo	placebo	1mg FA 12.5 mg B_6 0.006mg B_{12}	1.4mg B_6 0.002mg B_{12}	placebo	placebo	0.1mg FA 1.0mg B_6 0.004mgB_{12}	placebo	usual care
FA fortification	no	partly	yes	yes	partly	partly	yes	no	no	no
follow up time(mo)	24	43	31.9	24	48	60	38	25	12	29
total subjects	186	315	238	510	4110	619	2056	650	81	114
age	49.3	56	60.7	59.8 *59.5	52	72.2	65.4	61	63 *65	63.9
BMI		26	32.6	25.27 *26.08	29	29	27.9	27	NR	NR
Hcy(μmol/L)	23.5	27	14.7	30.62 *33.52	15.9	15.9	22.5	30	47.2 *53.2	37.2
Hcylevel reduction (μmol/L)	13	3.1	2.2	4.3 *10.2	4.6	4	6.3	10.4	23 *30	14.6
creatinine (μmol/)	NR	NR	NR	677.95 *664.99	145	130	NR	823	NR	907.5
albumin (g/dL)	3.75	NR	NR	4.0 *3.97	NR	NR	4.0	3.9	3.9 *4.1	4.0
cobalamine(pmol/L)	365	NR	412	514.51 *518.16	NR	332.3	NR	334	363 *431	444
cholesterol(mmol/L)	4.63	5.1	4.74	4.66 *4.52	4.8	4.89	4.31	4.9	5.41 *4.90	4.84
Prior CVD	23.7%	42.2%	31.1%	43.9%	20%	86.8%	80.5%	48%	35.7%	57.9%
Prior DM	22.6%	23.2%	100%	32.3%	40.5%	43.5%	54.9%	40.3%	12.5%	19.3%

*means different intervention group in one trial; FA means folic acid; NR means no reported; CVD means cardiovascular diseases; DM means diabetes mellitus

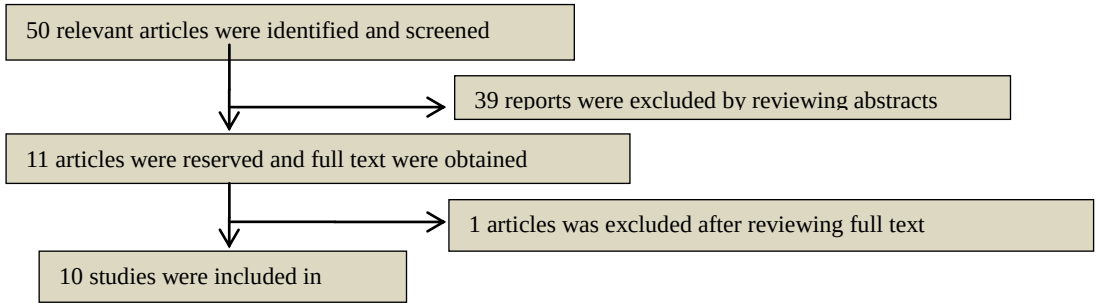

Figure 1 Process of study selection

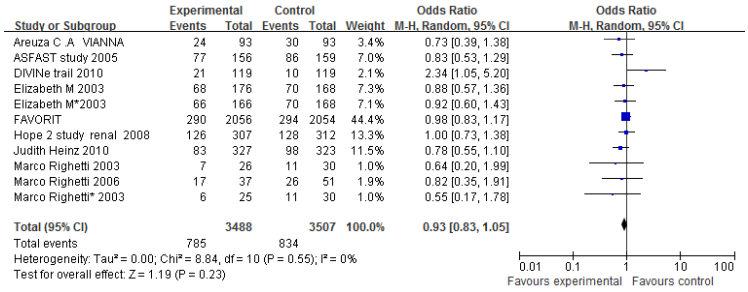

Figure 2. RR (risk ratio) with 95% CI estimates for primary CVD events (folic acid vs control). M-H indicates Mantel-Haenszel methods.

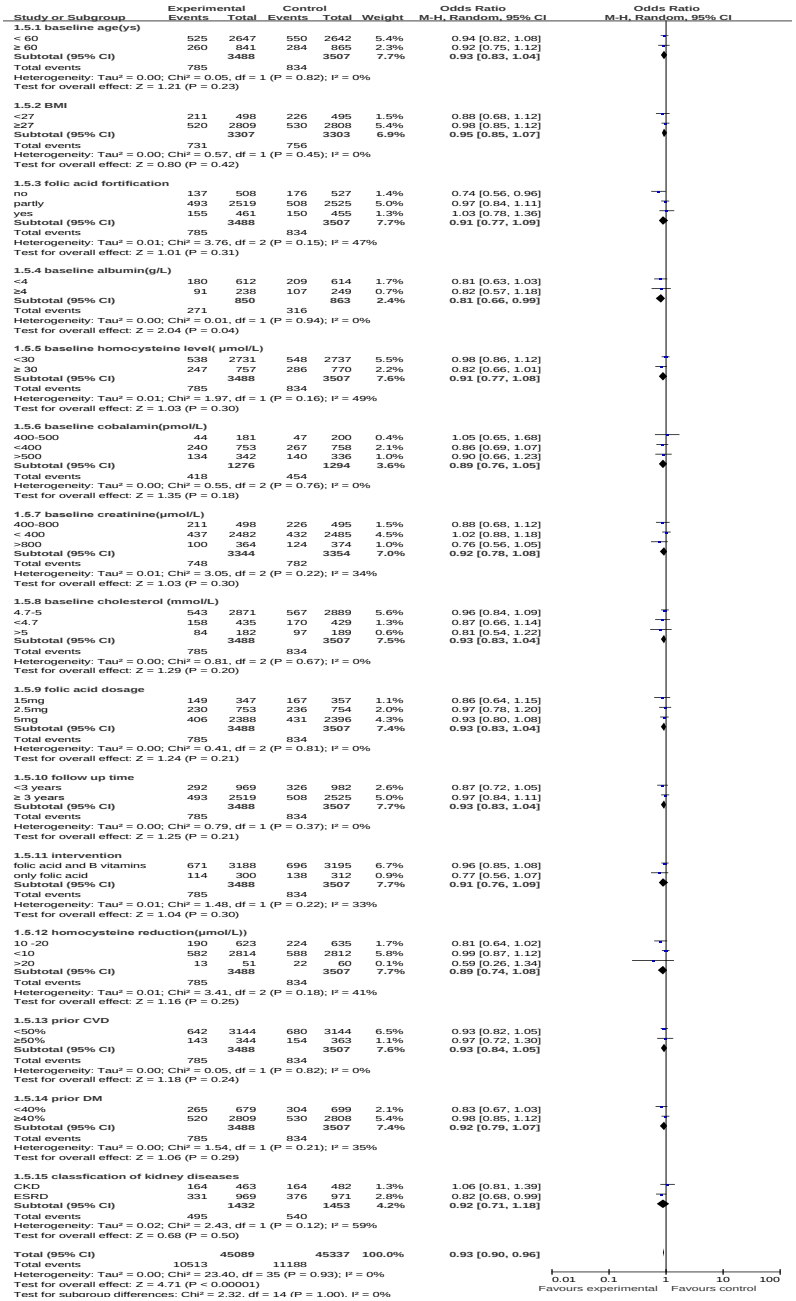

Figure 5.RR (risk ratio) with 95% CI estimates for subgroup analysis about primary cardiovascular events (folic acid vs control). M-H, Mantel-Haenszel methods.