Systematic Review and Meta-Analysis

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Effects of phenytoin on serum levels of homocysteine, vitamin B12, folate in patients with epilepsy

A systematic review and meta-analysis (PRISMA-compliant article)

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Abstract

Background: To determine the influence of phenytoin (PHT) monotherapy on the serum levels of homocysteine (Hcy), folate and vitamin B12 in patients with epilepsy.

Methods: Literature retrieval was performed through PubMed, Web of Science, Embase, Cochrane Library, Chinese Wanfang Data, China National Knowledge Infrastructure (CNKI), Chinese Biomedical Database databases as of the end of March 2018. Pooled weighted mean difference (WMD) and 95% CIs were calculated using a random effect model.

Results: A total of ten eligible studies were identified. The result revealed that the serum level of homocysteine in PHT-treated patients with epilepsy was significantly higher than that in control group (WMD = 8.47, 95% CI: 6.74 to 10.20, $P < .001$). In addition, the serum levels of folate (WMD = −3.51, 95% CI: −4.20 to −2.83, $P < .001$) and vitamin B12 (WMD = −62.23, 95% CI: −83.27 to −41.19, $P < .001$) were decreased significantly compared with the control group.

Conclusions: Our meta-analysis indicates that PHT monotherapy is associated with the increase in the serum homocysteine levels and decreased levels of folate and vitamin B12, and hyperhomocysteinaemia may contribute to the acceleration of the atherosclerotic process. Therefore, the patients under these medications should be monitored plasma homocysteine.

Abbreviations: AEDs = antiepileptic drugs, CI = confidence interval, Hcy = homocysteine, MTHFR = 5,10-methylenetetrahydrofolate reductase, NOS = Newcastle–Ottawa scale, PHT = phenytoin, PRISMA = Preferred Reporting Items for Systematic Review and Meta-Analyses, SD = standard deviation, WMD = weighted mean difference.

Keywords: epilepsy, homocysteine, meta-analysis, phenytoin

1. Introduction

Epilepsy, one of common neurological disorders, affects approximately 500 million people worldwide. The estimated proportion of active epileptics is up to 8% unexpectedly. Meanwhile, epilepsy, a chronic and dynamic condition which requires long-term treatment with anticonvulsants, exists in developing countries commonly.[1] Despite the availability of long term or lifelong antiepileptic drugs (AEDs) therapy, more than 30% of patients do not have seizures remission.[2] In addition, long-term drug therapy may increase the risk of cardiovascular diseases, such as atherosclerosis.[3,4] Previous research has shown that the development of atherosclerosis is often concomitant with the change of total cholesterol, serum levels of homocysteine, and C-reactive protein.[5] Furthermore, numerous studies also suggested that AEDs therapy could increase serum levels of homocysteine and C-reactive protein.[6,7] The elevated serum homocysteine is a crucial risk factor for atherosclerosis, cerebrovascular diseases and fetal malformations.[8,9] The pathological process of the above diseases often involves 5,10-methylenetetrahydrofolate reductase (MTHFR) gene polymorphism and deficiencies of folic acid, vitamin B12, and pyridoxal.[10,11]

Phenytoin (PHT), as older-generation AEDs, remains one of the most commonly prescription in clinic. Several studies revealed that PHT could reduce serum levels of folate and vitamin B12,[11,12] while folic acid and vitamin B12 are cofactors to support the converting of homocysteine to methionine.[13,14] Thus, long-term use of PHT may lead to the emergence of hyperhomocysteinaemia.

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Decades ago, some research revealed that enzyme-inducing AEDs includes carbamazepine and PHT, which may promote the progression of atherosclerosis in patients with epilepsy due to the increase of serum homocysteine level.[11,15] However, other case–control studies have not shown any change of serum homocysteine level in patients who take PHT medications.[16] Considering the inconsistent results and the insufficiency of sample size in some research, a comprehensive systematic review and meta-analysis of case–control is urgently needed

In this context, the purpose of this systematic review and meta-analysis is to evaluate whether PHT treatment could lead to the elevation of plasma homocysteine, folate, and vitamin B12 levels in patients with epilepsy.

2. Materials and methods

Our study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement.[17] No ethical approval was required because this paper was based on the previous articles.

2.1. Search strategy

PubMed, Web of Science, Embase, Cochrane Library, Chinese Wanfang Data, China National Knowledge Infrastructure (CNKI), and Chinese Biomedical Database were comprehensively searched as of the end of March 2018 using the following terms: epilepsy, homocysteine, vitamin B12, folic acid, and phenytoin. The reference lists of relevant literature also manually searched for identify additional articles. The language was limited to English and Chinese. The search terms are listed in Table 1.

2.2. Selection criteria

Studies were considered eligible and included in this review if they met the following Population/Intervention/Comparison/Outcome(s) (PICO) criteria (Table 2).

Articles were excluded if: relevant data were not reported; vitamin or folate supplementation; reviews, case report, abstract or animal studies; duplicated data.

2.3. Data extraction

Two authors independently extracted eligible data according to a standard protocol, and discrepancies were resolved by the third author. The extracted data included first author, year of publication, country, study sample size, age, gender, mean and standard deviation (SD) of serum homocysteine, vitamin B12 and folate in cases and controls.

2.4. Quality assessment

The Newcastle–Ottawa scale (NOS) was used to assess the methodological quality of the included studies.[18] It contains 3 major NOS components: the selection of the study group, the comparability of the groups, and the ascertainment of the outcome. Those scored over 6 points were regarded as high-quality studies.

2.5. Statistical analysis

Statistical analysis was performed using Stata 12.0 (Stata Corp, College Station, TX). Weighted mean difference (WMD) with 95% confidence intervals (95% CIs) for continuous outcomes (homocysteine, vitamin B12, and folate) was used to estimate the pooled effects. A P-value $< .05$ was considered statistically significant. The Cochran Q test and an I^2 statistic were used to quantify the heterogeneity of included studies. An I^2 value $\geq$ 50% or chi-squared value $< .05$ indicated significant heterogeneity and the random effects model was used. Otherwise, the fixed effects model was used. The sensitivity analysis was performed to determine the pooled results stability. Subgroup analysis was performed based on age and country of participants, to explore possible of heterogeneity. Publication bias was assessed by Begg's

Table 1

Search strategy in PubMed.

Num	Concept	Keywords
1	Epilepsy	"Epilepsy"[MeSH Terms] OR "epilepsy"[All Fields] OR Epilep*[Title/Abstract]
2	Phenytoin	"Phenytoin"[MeSH Terms] OR "phenytoin"[All Fields] OR "phenytoin" [Title/Abstract]
3	Homocysteine	"Homocysteine"[Title/Abstract] OR "Hcy" [Title/Abstract] OR" Hcy" [All Fields] OR "homocysteine"[MeSH Terms] OR "homocysteine"[All Fields]
4	Vitamin b12	"Vitamin b 12"[MeSH Terms] OR "vitamin b 12"[All Fields] OR ("vitamin"[All Fields] AND "b12"[All Fields]) OR "vitamin B12"[All Fields]
5	Folic acid	"Folic acid"[MeSH Terms] OR ("folic"[All Fields] AND "acid"[All Fields]) OR "folic acid"[All Fields] OR ("vitamin"[All Fields] AND "b9"[All Fields]) OR "vitamin b9"[All Fields]
6		"#3 OR #4 OR #5"
7		"#1 AND #2 AND #6"

Table 2

Selection criteria.

	PICO strategy
Population	Studies recruiting patients with epilepsy, and healthy people or not receive anti-epileptic drugs patients with epilepsy as control group.
Intervention or Exposure	Investigations examining the influence of PHT monotherapy on the serum levels of homocysteine, vitamin B12 or folate in patients with epilepsy.
Comparison	Groups were compared between those that patients with epilepsy who receive PHT monotherapy versus those that healthy people, or groups were compared between those that not receive anti-epileptic drugs patients with epilepsy.
Outcome (s)	Serum levels of homocysteine, vitamin B12 or folate.
Design	Case–control studies
Language	English or Chinese language publications.

and Egger's test. To investigate the influence of potential moderators, meta-regression analysis was conducted with the following predictors: year of publication, and Asians vs Caucasians.

3. Result

A total of 326 relevant studies were identified by literature search, of which 32 articles were excluded due to duplication. Following abstract and title screening, the inclusion criteria was not met by 273 studies. Ultimately, 10 eligible studies were included in this meta-analysis (Fig. 1).[11,12,15,16,19,20,21,22,23,24]

3.1. Study characteristics

The baseline characteristics of the ten eligible studies are shown in Table 3. The publication years of the articles were ranged from 1999 to 2017. Ten studies including 382 case and 921 controls subjects. Three studies were conducted in China.[15,19,20] Three studies were performed in India.[21,22,23] One study was conducted in each of Turkey,[16] USA,[12] Korea,[24] and Germany.[11] Among included studies, 6 papers[15,16,19–21,22] reported the serum levels of all 3 parameters. One of them had levels of folate and vitamin B12[11] and the 2 studies had the level of Hcy,[12,23] and the last one had solely level of Hcy and folate.[24]

3.2. Quality assessment of included studies

Methodological quality of included studies was assessed by 2 independent investigators, using the NOS scale (Table 4). The full score is 9 in NOS system. All studies reported that diagnoses of cases and controls were based on criteria and clinical records, and thus all studies were assigned points for "adequate definition of cases" and "definition of controls." Overall, scores of included studies got scores higher than 6, ranking as high quality.

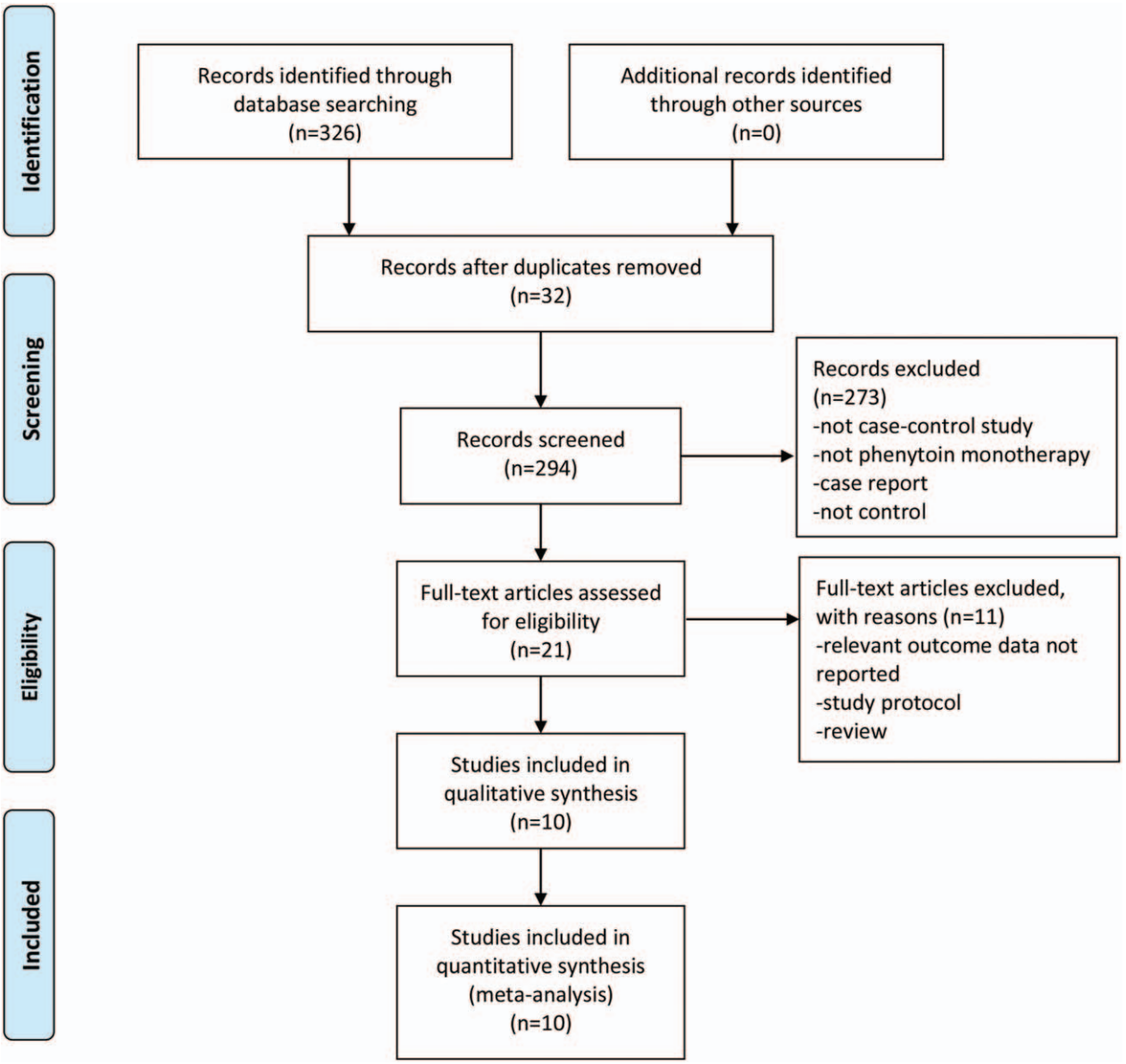

Figure 1. Flow diagram of included studies.

Table 3

Summary of studies included in the meta-analysis.

Study	Year	Country	Group	Sample size	Age	Hcy, μmol/L	Folate, ng/mL	Vit B12, pg/mL
Sener	2006	Turkey	Case	16	>18	17.2±9.2	4.1±1.6	349±194
			Con a	11	32±6	11.5±11.4	8.8±3.6	315±136
			Con b	18	30±12	9.8±5.9	6.4±2.8	338±186
Mintzer	2009	USA	Case	12	>18	13.1±6.6	–	–
			Con	16	38.7	11.5±5.3	–	–
Wang	2007	China	Case	30	>18	23.4±7.5	6.91±3.7	477±146.6
			Con a	40	23.7	10.2±4.3	11.3±3.1	502±178.6
			Con b	30	19.8	11.5±3.7	10.8±4.0	510±166.5
Chuang	2012	China	Case	39	37.8±8.9	14.50±5.60	14.12±9.10	—
			Con	60	34.5±10.5	9.41±2.65	24.58±12.14	—
Linnebank	2010	Germany	Case	15	—	—	4.0±1.6	412±338
			Con a	200	—	—	6.3±3.7	366±160
			Con b	170			6.6±3.7	364±
Shan	2014	China	Case	30	>18	21.39±7.24	6.83±1.76	486±100
			Con a	30	23.98±11.47	10.47±2.43	10.82±2.56	617±109
			Con b	30	24.51±9.49	11.28±2.57	9.82±2.05	591±126
Munisamy	2015	India	Case c	50	>18	25.2±3.6	2.2±1.2	142±21.6
			Case d	50	>18	20.7±4.6	2.7±1.1	168±21.7
			Con	100	24	12.5±3.7	6.9±1.9	215±16.1
Chandrasekaran	2016	India	Case	62	8	20.3±11.5	10.5±8.5	365±155
			Con	50	6	9.1±3	12.6±4.8	474±332
Palanisamy a	2013	India	Case	24	19~50	17.78±2.80	—	—
			Con	13	19~50	11.80±4.01	—	—
Palanisamy b	2013	India	Case	36	19~50	16.23±3.14	—	—
			Con	50	19~50	11.06±3.44	—	—
Yoo	1999	Korea	Case	18	28.5±7.6	14.6±1.6	7.9±4.0	
			Con	103	28.1±9.8	7.9±1.2	9.0±4.1	

Hcy = Homocysteine, Vit B12 = Vitamin B12, a = non-anti-epileptic drug group, b = healthy control, c = epilepsy with toxicity, d = epilepsy with non-toxicity.

Table 4

Newcastle–Ottawa scale quality assessment of included studies.

	Selection				Comparability		Exposure			
Study	Adequate Definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Control for additional factor	Ascertainment of exposure	Same method to ascertain	Nonresponse rate	Total
Sener	1	1	1	1	1	0	1	1	0	7
Mintzer	1	1	1	1	1	0	1	1	0	7
Wang	1	1	1	1	1	0	1	1	0	7
Chuang	1	1	1	1	1	0	1	1	0	7
Linnebank	1	1	1	1	1	0	1	1	0	7
Shang	1	1	1	1	1	0	1	1	0	7
Munisamy	1	1	1	1	1	0	1	1	0	7
Chandrasekaran	1	1	1	1	1	0	1	1	0	7
Palanisamy	1	1	1	1	1	0	1	1	0	7
Yoo	1	1	1	1	1	0	1	1	0	7

4. Meta-analysis

4.1. Homocysteine

Nine studies[12,15,16,19–24] were included in this meta-analysis. However, a significant heterogeneity ($I^2 = 90.1\%$, $P < .05$) was found in the homocysteine analysis. Therefore, the random effects model was used. The result showed that, compared with control group, the serum homocysteine level in patients with epilepsy treated with PHT monotherapy was significantly increased (WMD = 8.47, 95% CI: 6.74–10.20, $P < .001$) (Fig. 2). The result of Egger's and Begg's test showed no publication bias (Egger's $P = .578$, Begg's $P = .352$) (Fig. 3). However, considering significantly heterogeneity in the result and sensitivity analysis was conducted after removing Munisamy toxicity et al ($I^2 = 82.1\%$, $P < .05$), and the results were still found to be significant (WMD = 8.06, 95% CI: 6.61–9.52, $P < .001$). Meta-regression indicated no significant association of effect size estimate with year of publication ($t = .67$, $P = .518$), and significant association of effect size estimate with ethnicity ($t = -2.51$, $P = .027$) (Table 5).

4.2. Folate

Eight studies[11,15,16,19–21,22,24] included in this meta-analysis. The random effects model was used due to significant heterogeneity. The result showed that the serum folate level

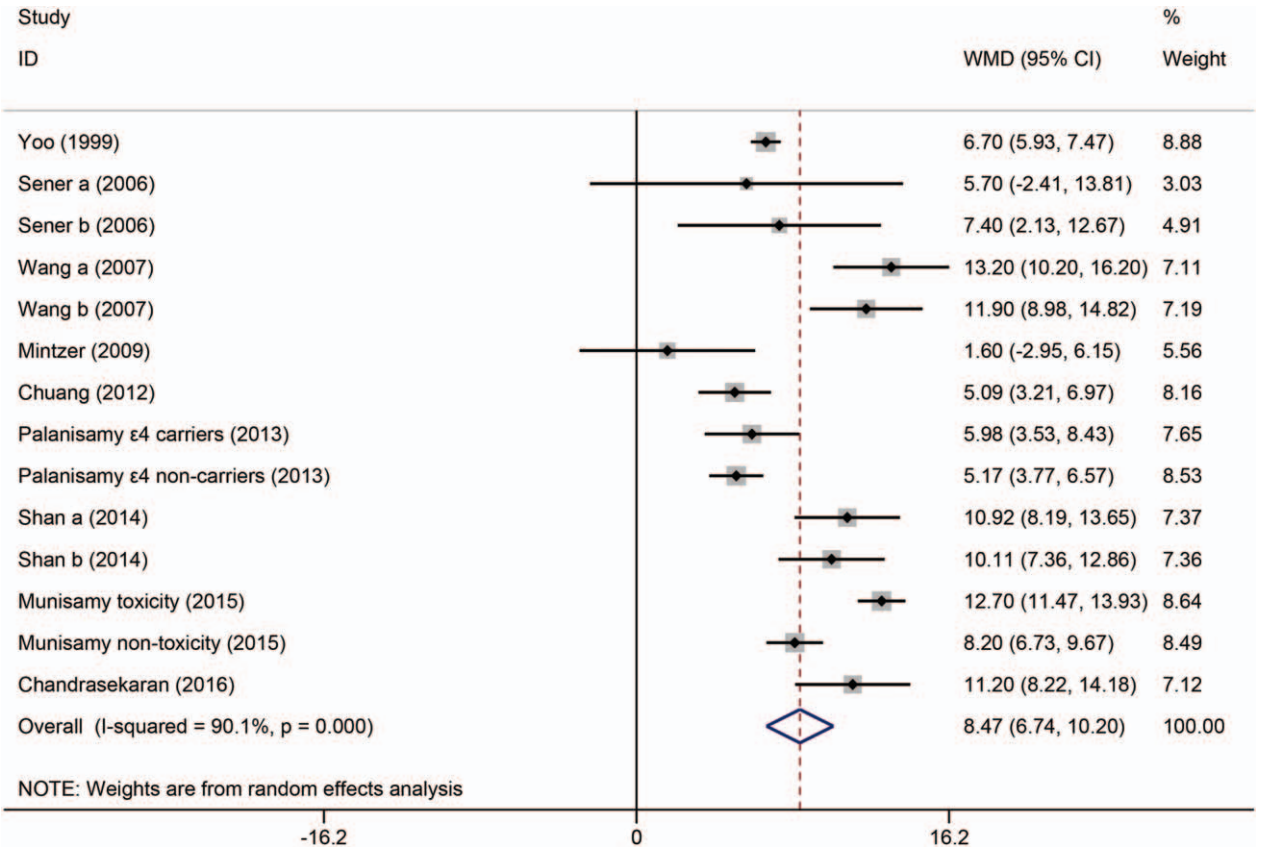

Figure 2. Pooled estimate of weighted mean differences and 95% CI of serum homocysteine levels in patients with epilepsy who received phenytoin monotherapy. (A), Non-anti-epileptic drug group; (B), Healthy control.

was reduced significantly in the PHT-treated patients (WMD = −3.51, 95% CI: −4.20 to −2.83, $P < .001$) (Fig. 4). The result of the Egger's test showed no publication bias (Egger's $P = .315$, Begg's $P = .714$) (Fig. 5). A sensitivity analysis was performed after removing Munisamy toxicity et al. ($I^2 = 73.3\%$, $P < .05$), and the results were still found to be significant (WMD = −3.36,

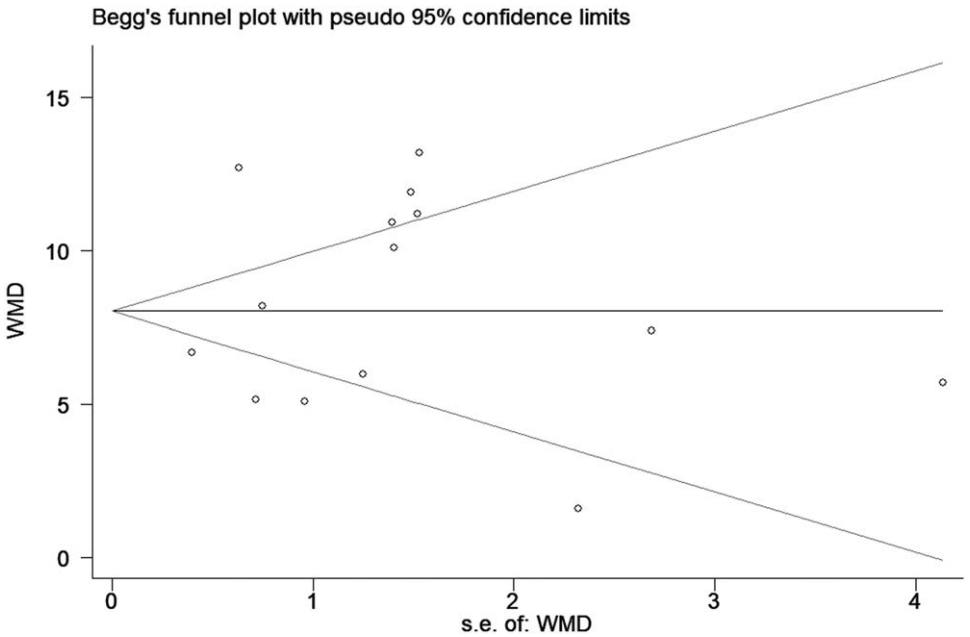

Figure 3. Funnel Plot of serum homocysteine levels.

Table 5

Meta-regression results.

	Coefficient	Standard Error	*T* value	*P* value	95% Confidence interval
Homocysteine					
Ethnicity	−3.878	1.542	−2.51	.027	−7.238~−0.51
Year of publication	0.128	0.193	0.670	.518	−0.291~0.548
Folate					
Ethnicity	1.101	0.913	1.210	.253	−0.909~3.112
Year of publication	−0.108	0.092	−1.170	.265	−0.311~0.095
Vitamin B12					
Ethnicity	35.706	30.469	1.170	.271	−33.219~104.632
Year of publication	−8.533	3.866	−2.210	.055	−17.279~0.213

95% CI: −4.09 to −2.64, $P<.001$). Meta-regression indicated no significant association of effect size estimate with ethnicity ($t=1.21$, $P=.253$) or year of publication ($t=-1.17$, $P=.265$) (Table 5).

4.3. Vitamin B12

Six studies[11,16,19–21,22] were included in this meta-analysis. There was a significant heterogeneity among those studies. The pooled estimate of 6 studies suggested that the serum vitamin B12 level was decreased significantly in the PHT-treated patients (WMD = −62.23, 95% CI: −83.27 to −41.19, $P<.001$) (Fig. 6). The result of Egger's test showed no publication bias (Egger's $P=.780$, Begg's $P=.036$) (Fig. 7). A sensitivity analysis was conducted after removing Munisamy nontoxicity et al ($I^2=47.5\%$, $P=.046$), and the results were still found to be significant (WMD = −72.93, 95% CI: −79.50 to −66.35, $P<.001$). Meta-regression indicated no significant association of effect size estimate with ethnicity ($t=1.17$, $P=.271$) or year of publication ($t=-2.21$, $P=.055$) (Table 5).

5. Subgroup analysis

Further subgroup analysis was carried out to explore the sources of heterogeneity among the studies (Table 6). In subgroup analysis by age, there was significant difference in all 3 parameters ($P<.005$), in spite of the heterogeneity as ever. In subgroup analysis of country, the increased Hcy was seen in China, Turkey, Korea, and India statistically, while no difference in the United States. In addition, a significant difference of

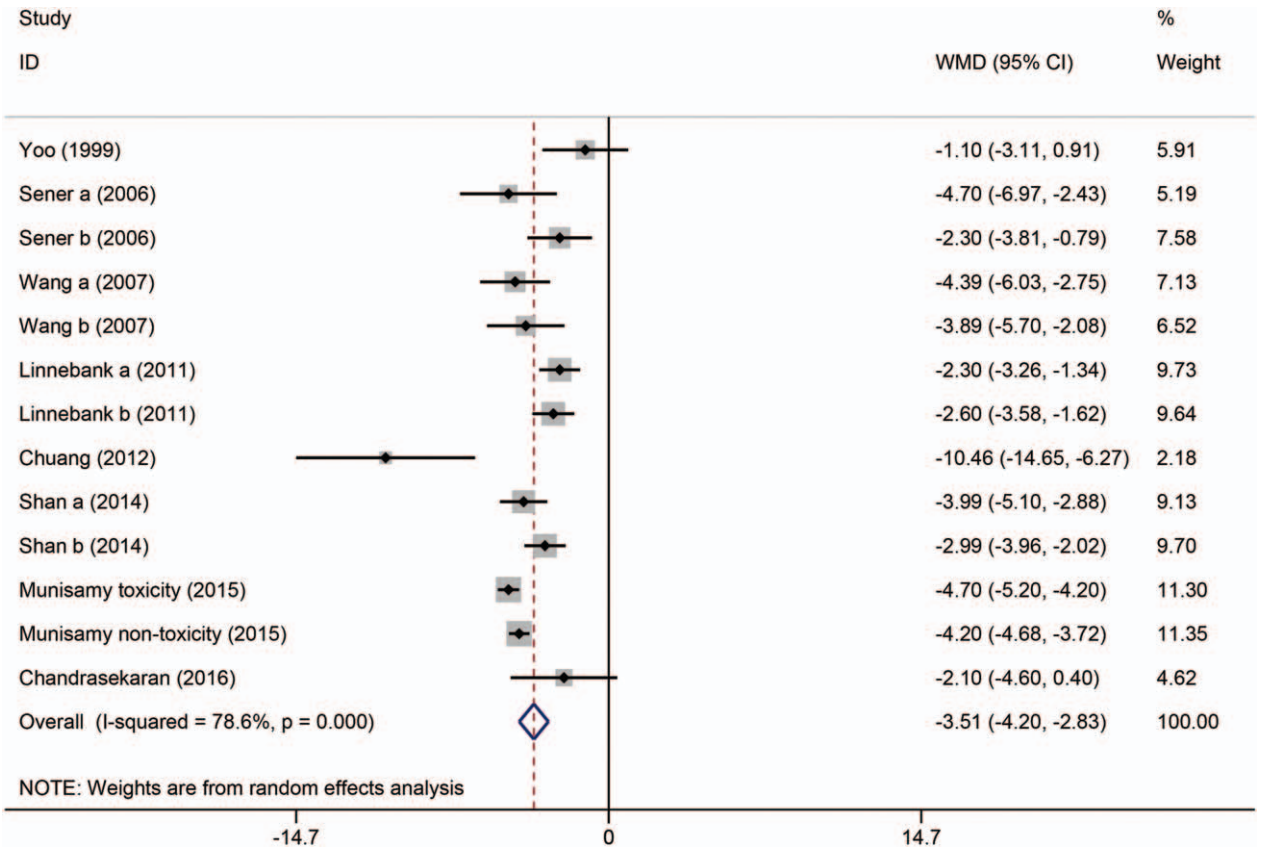

Figure 4. Pooled estimate of weighted mean differences and 95% CI of serum folate levels in patients with epilepsy who received phenytoin monotherapy. (A) Non-anti-epileptic drug group; (B) Healthy control.

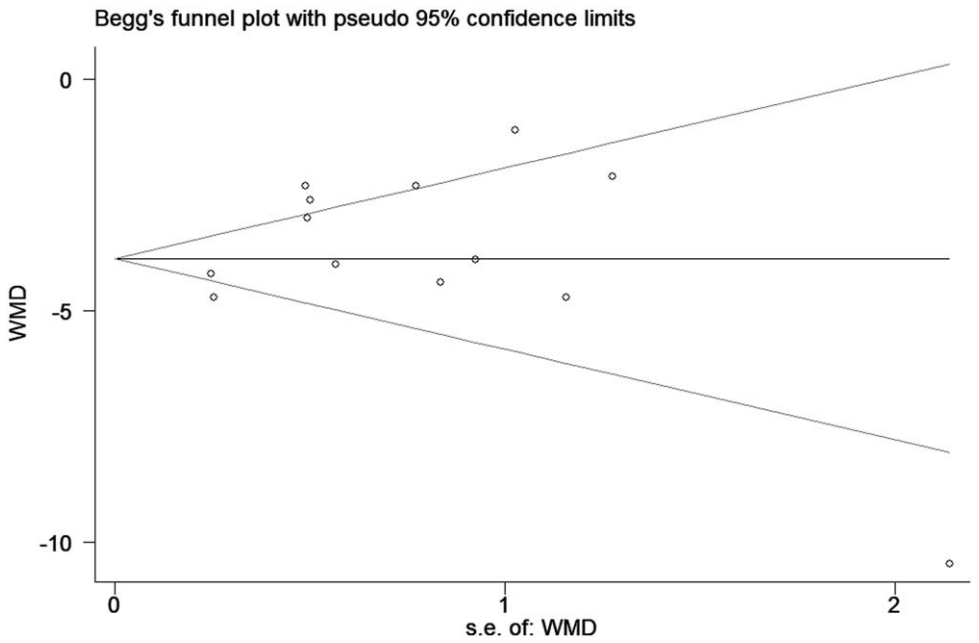

Figure 5. Funnel Plot of serum folate levels.

vitamin B12 was observed in India and China. Meanwhile, the serum level of folate was significantly decreased in all countries except Korea. Sensitivity analysis was conducted to test the robustness of our results, which showed that the result was not substantially altered regardless of the deletion of any single study.

6. Discussion

To our knowledge, this is the first meta-analysis including 8 studies, and the effect of PHT on the serum level of homocysteine, vitamin B12, and folate in patients with epilepsy were assessed. The results of this systematic review

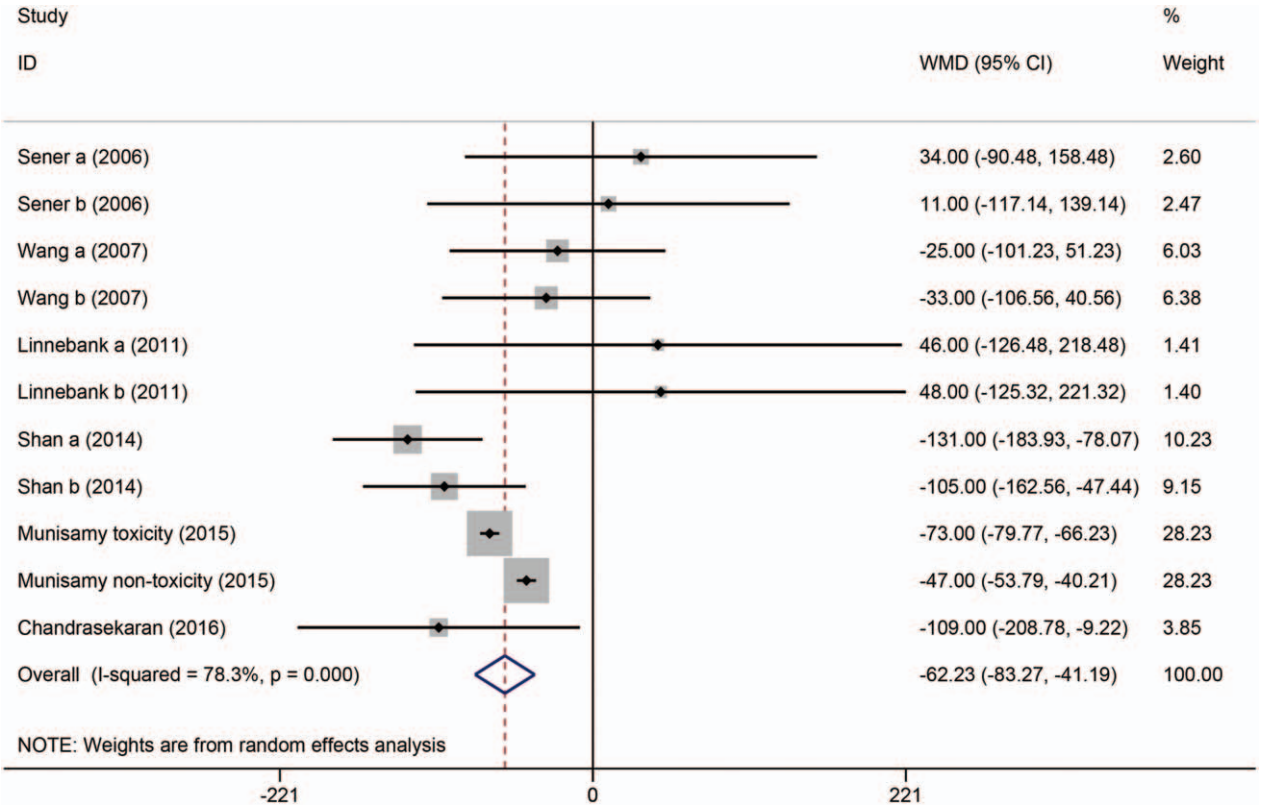

Figure 6. Pooled estimate of weighted mean differences and 95% CI of serum vitamin B12 levels in patients with epilepsy who received phenytoin monotherapy. (A) Non-anti-epileptic drug group; (B) Healthy control.

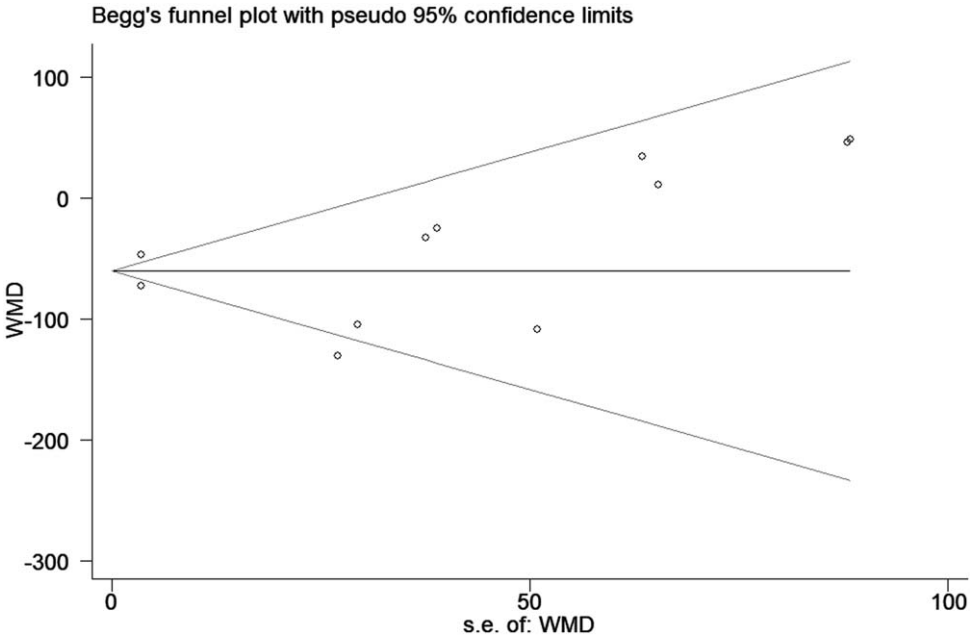

Figure 7. Funnel Plot of serum vitamin B12 levels.

and meta-analysis suggested that compared with control, PHT use has been correlated with a significantly increase in the serum level of homocysteine and reduced the serum levels of vitamin B12 and folate in patients with epilepsy. To find out the sources of heterogeneity, we conduct subgroup analysis, sensitivity analysis and meta-regression. Unfortunately, the results were no substantial modification, meaning. We speculate that heterogeneity may be derived from the sample size was small, different therapeutic doses, and so on. Rather, no significant difference was observed in Egger's test and Begg's test, suggesting that the publication bias was low in this meta-analysis.

Table 6

Subgroup analysis.

Subgroups	Number of studies	WMD (95%CI)	I^2 (%)	*P* value
Homocysteine				
Country				
USA	1	1.60 (−2.95, 6.15)	—	.491
China	3	10.14 (6.93, 13.34)	86.8	<.001
Turkey	1	6.20 (4.05, 8.34)	0	<.001
India	3	9.27 (5.55, 12.99)	95.5	<.001
Korea	1	6.70 (5.93, 7.47)	—	<.001
Age				
<18	1	11.20 (8.22, 14.18)	—	<.001
≥18	8	8.26 (6.46,10.06)	90.6	<.001
Folate				
China	3	−4.24 (−5.49, −2.98)	69.2	<.001
Turkey	1	−3.35 (−5.68, −1.01)	66.4	.005
Germany	1	−2.45 (−3.13, −1.76)	0	<.001
India	2	−4.29 (−4.97, −3.61)	62.3	<.001
Korea	1	−1.10 (−3.11, 0.91)	—	.284
Age				
<18	1	−2.1 (−4.60, 0.40)	—	.100
≥18	7	−3.58 (−4.28, 2.88)	75.5	<.001
Vitamin B12				
China	2	−79.09 (−130.24, −27.94)	60.7	.002
Turkey	1	22.83 (−66.45, 112.12)	0	.616
Germany	1	47.00 (−75.26,169.25)	0	.451
India	2	−62.66 (−87.32, −38.00)	93.1	<.001
Age				
<18	1	−109.00 (−208.78, −9.22)	—	.032
≥18	5	−60.33 (−81.85, −38.82)	80.1	<.001

CI = confidence interval, WMD = weighted mean difference.

The causal relationship between increased serum level of homocysteine and seizures remains controversial. Available evidence suggests that homocysteine is a potent agonist for the *N*-methyl-D-aspartate (NMDA) receptor, which are linked with epileptogenesis.[25] Furthermore, Schwarz and Zhou[26] reported that 20% of patients with homocystinuria have seizures and concomitant with high serum concentration of homocysteine. Besides, increased homocysteine level and reduced folate associated with AEDs treatment aggravate seizures and neuronal damage, which contributes to the brain atrophy observed in patients with epilepsy.[27] In addition, Ono et al[28] revealed that seizures could not be controlled effectively once the serum level of homocysteine is above 20 mmol/L. Moreover, high serum level of homocysteine may underlie the atherosclerosis in patients with epilepsy.

Metabolic disturbance of homocysteine, folate and vitamin B12 may be typical manifestation in patients using PHT. First of all, abnormally high level of serum homocysteine may be a risk factor for cerebrovascular events or ischemic heart disease.[4] Second, increased homocysteine and low folate status may boost the pathogenesis of AEDs side effects, such as impaired cognitive function and fetal malformations.[29] Third, the raise of the serum homocysteine level may also be involved in poor seizure control in patients with epileptic.[16]

Although several studies revealed that PHT use was associated with low folate status, the exact mechanism underlying the effect of PHT on homocysteine metabolism has not yet been clarified. Previous research showed that phenytoin, as enzyme inducers, can directly regulate the activity of different liver enzymes.[30] Liver enzyme induction may cause depletion of the cofactors, including folic acid and vitamin B12. Numerous studies revealed that folic acid and vitamin B12 are considered as main probable regulatory factors for increasing homocysteine. Deficiency of folate and vitamin B12 may lead to miscellaneous types of diseases, such as anemia, cognitive decline and impairment, osteoporosis, cancer, psychiatric disease, and congenital malformations.

Homocysteine is the intermediate degenerated by one-carbon metabolism (OCM), and elevated serum homocysteine would be detrimental to the vascular structure and function.[31] Meanwhile, hyperhomocysteinaemia is a dominant risk factor for atherosclerotic vascular diseases such as stroke and myocardiac infarction.[15] The mean common carotid artery intima media thickness (CCA IMT) is regarded as a marker to stratify the risk of atherosclerosis. Previous studies indicated that phenytoin monotherapy correlated significantly with elevated CCA IMT and homocysteine concentrations.[15] Furthermore, some research demonstrated that long-term phenytoin monotherapy is associated with an increase in risk of atherosclerotic vascular diseases in patients with epilepsy.[15,21,22]

Based on subgroup analysis of ethnicity for homocysteine, we found that it was no difference in Turkey and the United States. Hyperhomocysteinaemia is frequently generated by not only folic acid or vitamin B12 deficiency, but also by genetic polymorphisms. Previous studies depicted that MTHFR C677T mutation was a determinant of hyperhomocysteinaemia in patients with epilepsy receiving AEDs. The 677C→T is the most common MTHFR polymorphism, and in the homozygous state, the enzymatic activity decrease by 50% to 60% and serum folate level is reduced.[32] Xuan et al[33] indicated that the prevalence of the 677 T allele ranged from 9.34% to 40.53% in different ethnic groups, with South Asians having the lowest prevalence and the highest for East Asians. In addition, several studies reported that patients with MTHFR TT genotypes receiving AED monotherapy showed significantly lower folate and vitamin B12 levels compared with controls.[34,35] However, whether heterogeneity influenced by the MTHFR polymorphism needs further exploration. Recent studies revealed that participants with the MTHFR TT genotype exhibited a lower 5-year decrease in Hcy concentration following a B-vitamin supplementation than did participants with the CC or CT genotype.[36] Therefore, it is necessary for patients with epilepsy to maintain homocysteine homeostasis requiring sufficient amount of folic acid and vitamin B12.

Some limitations should be noted in this study. First, significant heterogeneity was found in this meta-analysis. Second, the number of studies is relatively small. Third, the dosage of PHT monotherapy was incomplete information due to lack of reports in several included studies. Finally, all of the studies published in English and Chinese may product potential bias. Based on those limitations, future clinical studies should focus on the investigation of larger and more representative sample, employ the optimal treatment dosage, record relevant demographic that may affect the serum level of homocysteine, vitamin B12, folate after PHT treatment (e.g., gender, age, and ethnicity) and segregate patients into sub-cohorts based on these factors to allow factor-based analysis.

In conclusion, this meta-analysis shows that PHT monotherapy is associated with an increase in serum homocysteine level and reduced in serum vitamin B12 and folate levels in patients with epilepsy. Hyperhomocysteinaemia is an independent risk factor for thrombosis and atherosclerosis. Therefore, folic acid and vitamin B12 supplementation may be a simple cost-effective measurement to reduce homocysteine level in PHT-treated patients with epilepsy. In addition, further research is needed to explore the optimal treatment protocols in patients with epilepsy to maximize the efficiency and to minimize the risks of PHT treatment.

Author contributions

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