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Associations of pregnancy-associated plasma protein-A level with essential hypertension and hypertensive disorders in pregnancy in Chinese population: a meta-analysis of 20 research studies involving 3332 individuals

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Abstract:

Objective: The aim of this study was to explore the associations between serum pregnancy-associated plasma protein-A (PAPP-A) level and essential hypertension (EH) and hypertensive disorders in pregnancy (HDP) in Chinese population.

Methods: PubMed, Embase, Cochrane Library, Chinese Biomedical Database (CBM), Wanfang databases and China National Knowledge Infrastructure (CNKI) were utilized to identify available articles. The standardized mean difference (SMD) with 95% confidence intervals (CI) was used to estimate the size of the effect. The subgroup analyses and meta-regression analysis were performed to identify the sources of heterogeneity among studies. Sensitivity analysis was used to assess the stability of result. The publication bias between studies was examined by Begg's funnel plots and Egger's test.

Results: A total of 20 studies involving 1493 patients and 1839 controls were included for the current meta-analysis. For EH group, the PAPP-A level was significantly higher in EH patients than in controls (SMD=1.960, 95% CI=1.305-2.615, P<0.001) and the significant associations were observed in all the subgroups. For HDP group, the PAPP-A level was significantly higher in HDP patients than in healthy pregnant women (SMD=2.249; 95% CI 1.324-3.173, P<0.001). The positive association between PAPP-A level and the risk of HDP was consistently observed in all of the subgroups except in the subgroup with low NOS score.

Conclusion: The present meta-analysis suggested that the elevated PAPP-A level may be associated with the susceptibility to EH and HDP.

Strengths and limitations of this study

1. To our knowledge, the present study is the first meta-analysis focused on the associations of PAPP-A level with EH and HDP in Chinese population.

2. The results suggested that PAPP-A level were higher in patients with EH or HDP than in healthy controls.

3. Multicentre, multi-ethnicity and larger scale studies are needed to clarify the actual pathogenic mechanism of PAPP-A underlying EH and HDP.

Keywords: pregnancy-associated plasma protein-A; essential hypertension; hypertensive disorders in pregnancy; Chinese

INTRODUCTION

As a growing public health problem in developing countries, essential hypertension (EH is considered as the major risk factor for cardiovascular diseases, such as coronary heart disease, ischemic stroke, heart failure, etc. In the last decade, the prevalence of EH in China has increased significantly.[1] In general, the etiology and the development of EH are affected by genetic and environmental factors.[2, 3] Recently, numerous studies have shown that EH is closely related to inflammation and inflammation plays an important role in the pathogenesis and the maintenance of EH.[4]

Hypertensive disorders in pregnancy (HDP) remain as a major cause of both fetal and maternal mortality.[5] In general, HDP has five subtypes: 1) gestational hypertension, 2) preeclampsia, 3) eclampsia, 4) chronic hypertension in pregnancy, and 5) preeclampsia superimposed on chronic hypertension. The true etiology and the pathophysiology of HDP remain unclear. The genetic susceptibility and other biophysiological factors (such as endothelial dysfunction, immune imbalance, trophoblast cell ischemia, oxidative stress, etc.) may also contribute to the pathogenesis.

Pregnancy-associated plasma protein-A (PAPP-A), first identified by Lin et al.[6] in 1974, belongs to the matrix metalloproteinase family. PAPP-A is synthesized and secreted not only by placental trophoblast cells, but also by fibroblast cells, vascular smooth muscle cells, mesenchymal cells, etc. In the last decade, the role of PAPP-A in the diagnosis and prognosis of coronary heart disease has been investigated in depth.[7, 8]

Several recent studies have indicated that an increased serum PAPP-A level may be a biomarker of EH and HDP based on its important effect on the proliferation of vascular smooth muscle cells and on the reconstruction of extracellular matrix.[9-27] But some results are inconsistent.[28, 29] At the same time, the sample size of individual study was relatively small. Therefore, it is important to perform a meta-analysis of all available data to assess the value of peripheral blood PAPP-A levels for the diagnosis of EH and HDP.

METHODS

Studies selection

The current study was conducted according to the Meta-analysis of Observational Studies in Epidemiology group (MOOSe) [30] and the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines (PRISMA).[31]

The studies, published prior to December 1st, 2014, without language restriction, were searched from the following electronic databases: PubMed, Embase, Cochrane Library, Chinese Biomedical Database (CBM), Wanfang databases and China National Knowledge Infrastructure (CNKI). The following keywords and Medical Subject Headings (MeSH) terms were used: (''PAPP-A'' or ''pregnancy associated plasma protein-A'') and (''hypertension'' or ''essential hypertension'' or ''high blood pressure'' or ''hypertension in pregnancy'' or ''hypertensive disorders in pregnancy'' or ''pregnancy-induced hypertension syndrome'' or ''hypertensive disorder complicating pregnancy'' or ''preeclampsia'' or ''eclampsia'' or ''gestational hypertension''). The manual search was conducted by checking the references of the relevant studies to reduce the omissions.

Inclusion criteria

The included studies must meet all the following criteria: (1) the study design must be a case-control or a cohort study; (2) the study must be used to evaluate the relationships between PAPP-A and EH or HDP; (3) the patients must conform to the diagnostic criteria of EH or HDP; (4) the study must be conducted in Chinese population; (5) the specimen must be serum or plasma, rather than placental tissue; (6) the mean and standard deviation must be clear in the case and control groups. If the results of one population were published in multiple studies, we select the most comprehensive study. If we have some doubts about the data, we will contact the corresponding author by email.

Data extraction

Two investigators (Cai and Zhang) independently evaluated the eligibility of all retrieved studies and extracted the original information, including the first author's name, year of publication, average age, type of disease, geographic distribution, sample size of cases and controls, peripheral blood PAPP-A level, detecting methods, specimen type. Any disagreement was resolved by discussion or in consultation with another author (Shi).

Quality assessment

A quality score was used to assess the quality of the studies according to the Newcastle-Ottawa Scale (NOS) criteria,[32] including subject selection (0-4), comparability (0-2) and exposure (0-3). The quality of the paper was considered as high if the NOS scores were more than 7.[33]

Statistics and analyses

Because the unit weights of PAPP-A level in each study were not consistent, the standardized mean difference (SMD) with 95% confidence intervals (CI) was used to estimate the size of the effect in present study.

Heterogeneity across studies was calculated by using Cochran's Chi-square based Q-test. At the same time, it was also detected by using the I^2 test. If the heterogeneity was statistically significant (P<0.01, I^2>50%), the random-effects model (a Dersimonian-Laird method) was used. Otherwise, the fixed-effects model (a Mantel-Haenszel method) was applied.[34] In order to identify the sources of heterogeneity, the subgroup analyses were performed. The studies were stratified by sample size (>100 or ≤100), specimen type (serum or plasma), detecting method (ELISA or Chemiluminescence), NOS score (≥7 or <7), type of disease (EH or HDP) and geographical location (North China or South China). The meta-regression analysis was also performed to identify the sources of heterogeneity among studies. To assess the stability of result, sensitivity analysis was used by calculating the pooled effect again when omitting single study one by one.

The publication bias was assessed via Begg's funnel plot and Egger's regression test. If the funnel plot was asymmetry, the trim-and-fill method was used to identify and correct the publication bias.

The STATA version 12.0 (StataCorp LP, College Station, Texas 77845 USA) was used for the statistical analysis. A P value <0.05 was considered statistically significant.

RESULTS

Study selection

A total of 214 potentially relevant articles were identified, by using the above search strategy. 173 articles were excluded after the title and abstract review. Another 21 studies were excluded, after we reviewed the full article texts. Finally, a total of 20 studies involving 1493 patients and 1839 controls met the inclusion criteria and were included for the current meta-analysis. The detailed process of literature search and study selection is described in figure 1.

Study characteristics

Among the 20 studies, six studies involving 395 cases and 233 controls were focused on the relationship between the level of PAPP-A and EH , and fourteen studies involving 1098 cases and 1606 controls were focused on HDP. The characteristics of the 20 studies are summarized in table 1. All of the 20 studies were published in Chinese. Publication years of the eligible studies ranged from 2005 to 2014. The sample size of each study varied from 80 to 814. Nineteen studies were conducted in Han population and one study was conducted in Kazakhs population. Seventeen studies used serum specimens to examine the PAPP-A level, and three studies used plasma. Nineteen studies used ELISA as the PAPP-A level detecting method, except Zhu's study.[27] The NOS scores in seventeen of the 20 studies were above seven.

Association between the PAPP-A level and EH

The pooled SMD was calculated by random-effects model because there was signficant heterogeneity between studies (I^2=89.7%, P<0.001). As shown in figure 2, the pooled analysis indicated that the PAPP-A level was significantly higher in EH patients than in controls (SMD=1.960, 95% CI=1.305-2.615, P<0.001).

In the stratified analyses based on sample size, specimen type, detecting methods, NOS score and geographical location, significant associations were observed in all the subgroups (table 2).

The stability of the study was detected by sensitivity analysis, through re-meta-analysis by excluding individual study each time. As shown in figure 3, no individual study significantly affects the pooled SMD.

The publication bias was assessed by Begg's funnel plot and Egger's test. The funnel plot

was symmetric and the Egger's test also revealed the absence of publication bias (P=0.450) (figure 4).

Association between the PAPP-A level and HDP

The obvious heterogeneity was found (I^2= 98.6%, P<0.001), so the pooled SMD was also calculated by random-effects model. As shown in figure 5, the results have demonstrated that the PAPP-A level was significantly higher in HDP patients than in healthy pregnant women (SMD=2.249; 95% CI=1.324-3.173, P<0.001).

The stratified analyses were conducted based on sample size, detecting methods, NOS score and geographical location. The positive association between PAPP-A level and the risk of HDP was consistently observed in all of the subgroups except in the subgroup with low NOS score (table 2).

The sensitivity analysis suggested that only the study of Hu Y [28] appeared to have a large influence, but the result did not change after exclusion of this study, which suggested that the results have sufficient statistical power (figure 6).

Due to the significant heterogeneity between studies, besides the subgroup analyses, a meta-regression was also performed to further identify the potential source of heterogeneity. Sample size, detecting methods, NOS score and geographical location, were included in regression analysis. The result indicated that only geographical location might partially contribute to the heterogeneity ($P_{\text{meta-regression}}$= 0.025) and can explain 30.61% of the heterogeneity. Other factors, such as NOS score ($P_{\text{meta-regression}}$= 0.812), sample size ($P_{\text{meta-regression}}$= 0.507) and detecting methods ($P_{\text{meta-regression}}$= 0.605), might not be the sources of heterogeneity.

The obvious asymmetry of the funnel plot was visualized (Egger's test P=0.000) (figure 7). The trim-and-fill method was used to identify and correct the publication bias.[35] The number of missing studies was estimated by the linear method. After two iterations, the value of diff came to zero and the number of estimated deficient study was zero, which indicated that result was stable.

DISCUSSION

To our knowledge, the present study is the first meta-analysis focused on the associations of PAPP-A level with EH and HDP. We found that PAPP-A level were higher in patients with EH or HDP than in healthy controls.

In 2011, a large-scale samples with multi-variables research, involving 112,386 pregnant women, was conducted to estimate the prevalence of HDP in China.[36] This epidemiological investigation revealed that the prevalence of HDP in China was about 5.22%, although smaller than other research of 8-10%.[37] Although numerous studies have been conducted, the precise mechanism underlying the pathogenesis of EH and HDP remains poorly understood.

PAPP-A, about 750~820 kDa in size, plays an important role in the proliferation of vascular smooth muscle cells and on the reconstruction of extracellular matrix. Recently, a growing number of studies have suggested a significantly elevated serum level of PAPP-A might be involved in the development of EH and HDP by the following mechanisms: 1) PAPP-A may increase the IGF-1 level, which can promote the proliferation of vascular smooth muscle cells and the remodeling of extracellular matrix; 2) The elevated IGF-I level also increases the degradation of the NO synthetase, which can lead to the sustained contraction of vessel wall; 3) PAPP-A can promote the release of inflammatory cytokines, which can increase the arterial endothelial injury; 4) In HDP patients, hypoxia causes the trophoblast cells to secret more PAPP-A, which can augment the placenta local oxidative stress and aggravate the placenta hypoxia.

Although most of the studies reached the positive conclusions, the results were not consistent and the sample sizes in each study were relatively small. To resolve this problem, we conducted the current meta-analysis to examine the associations of PAPP-A level with risk of EH and HDP.

In the present meta-analysis, 20 studies met the inclusion criteria. Six of them were focused on EH and 14 of them focused on HDP. According to the results, we found that the PAPP-A level was significantly higher in EH (SMD=1.960, 95% CI=1.305-2.615, P<0.001) and HDP patients (SMD=2.249; 95% CI 1.324-3.173, P<0.001) than in controls.

A significant heterogeneity was found among studies. A meta-regression was performed to identify the sources of heterogeneity in HDP group. We found that only the geographical

location can explain 30.61% heterogeneity. In the previous studies, the prevalence of EH and HDP in Chinese population represents a characteristic of geographical location, which is higher in North China than in South China. Although the exact mechanism was not clear, the low temperature-induced vasoconstriction might trigger the onset of these diseases. Our meta-analysis suggested that geographical location might be a source of the heterogeneity. Subgroup analyses were also performed in EH and HDP groups. In the stratified analyses were conducted according to sample size, detecting methods, NOS score and geographical location. The positive associations between PAPP-A level, and EH and HDP were consistently observed in all of the subgroups except in the subgroup with the low NOS score. We also preformed the sensitivity analysis to assess the influence of individual studies on the pooled SMD. No single study can influence the stability of the whole study.

Although our meta-analysis is a practical way to generate a more powerful result with less random error than each individual study, our study has some limitations. First of all, the sample size of the studies included in this meta-analysis was relatively small. Secondly, all subjects in studies came from hospital, which may lead to the selection bias. Thirdly, researches have revealed that the PAPP-A levels varies among different subtypes of the disease. The PAPP-A level is even different between in early-onset preeclampsia and in late-onset preeclampsia. [28] At the same time, it is increasingly accepted that early-onset preeclampsia and the late-onset preeclampsia have the different pathogenic mechanisms.[38] However, some studies included in our study were not divided into subgroups and the blood collection time was not described, which could contribute to the heterogeneity. Fourthly, the obvious publication bias was found in HDP group: it is more difficult to publish negative results and the study population was also limited in the Chinese population. Although obvious publication bias exists, sensitivity analysis of trim-and-fill method showed the result was reliable. Finally, a major confounding factor was the heterogeneity among studies. In EH group, due to the number of study was smaller than ten, the meta-regression method was not repeated to further identify the source of heterogeneity. In the HDP group, although the meta-regression was performed, only part of the heterogeneity could be explained by geographical location.

CONCLUSIONS

In conclusion, our meta-analysis suggested that the PAPP-A level was significantly higher in EH and HDP patients than in controls. However, multicentre, multi-ethnicity, and larger scale studies are needed to clarify the actual pathogenic mechanism of PAPP-A underlying EH and HDP.

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The authors declare that they have no competing interests.

Supplementary file: PRISMA-2009-Checklist-MS

Author Contributions

Conceived and designed the experiments: GJC, BFZ. Performed the experiments: GJC, BFZ, WJW, LPY, GWS, SLX, XLF. Analyzed the data: GJC, BFZ, XLF. Contributed reagents/materials/analysis tools: GJC, BFZ. Wrote the paper: GJC, BFZ, XLF.

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Table 1. Characteristics of 20 studies on PAPP-A and risk of EH and HDP

Author	Year	Type of disease	Geographic distribution	Age (case/control)	Case			Control			Detecting method	Unit	Specimen type	NOS
					N	M	SD	N	M	SD				
Li CF [9]	2005	HDP	Shanxi	26.5±4.3/ -	60	58.85	21.05	60	36.8	10.4	ELISA	IU/L	serum	8
Xia JX [10]	2005	HDP	Guangdong	27.85±5.20/ 27.50±5.47	95	46.36	14.83	103	23.6	6.1	ELISA	mIU/ml	serum	8
Wang LY [11]	2007	EH	Jilin	-/ 60±12	70	3	0.6	56	1.5	0.5	ELISA	mU/L	serum	6
Zhou Z [12]	2007	HDP	Jiangsu	5.8±4.8/ 26.5±4.3	109	57.84	12.25	105	29.4	9.65	ELISA	mg/l	serum	8
Fan JF [13]	2009	EH	Anhui	64.19±13.5/ 61.78±16.9	84	8.66	3.26	22	5.17	3.15	ELISA	mIU/L	serum	6
Liu JG [22]	2009	HDP	Hunan	29.7±5.7/ 30.2±4.1	40	2.61	0.96	40	1.83	0.75	ELISA	ug/l	serum	8
Pan QR [14]	2010	HDP	Jiangsu	26.8±4.7/ 26.3±3.8	92	58.34	12.19	35	28.4	8.57	ELISA	U/L	serum	8
Wang J [15]	2010	EH	Anhui	64±12/ 61±16	80	11.91	3.42	15	7.1	1.9	ELISA	mU/L	serum	6
Zhang JY [16]	2010	HDP	Hebei	23-36/ 22-35	115	181	32	127	69	5	ELISA	mU/ml	serum	6
Meng ZH [17]	2011	EH	Xinjiang	56.6±11.7/ 56.4±13.2	80	6.04	1.07	30	3.9	0.64	ELISA	mg/l	plasma	9
Xu XL [18]	2011	HDP	Zhejiang	25.7±5.7/ -	70	58.8	12.47	70	30.5	10.5	ELISA	IU/L	serum	8
Zhang CY [19]	2011	EH	Shandong	61±9/ 52±13	39	21.22	4.12	64	7.97	4.36	ELISA	mU/L	plasma	5
Cui FL [20]	2012	HDP	Yunnan	27.3±2.4/ 27.1±2.6	72	51.2	10.4	75	32.7	9.6	ELISA	IU/L	serum	8
Gao L [21]	2012	HDP	Sichuan	32.49±2.1/ 29.15±2.3	120	73.39	5.33	40	38.2	4.56	ELISA	U/L	serum	8
Hu Y [28]	2012	HDP	Henan	-/ -	88	19.98	5.36	726	22.5	1.62	ELISA	U/L	serum	5
Peng HY [23]	2012	HDP	Guangdong	8.6±7.7/ 28.8±7.6	45	56.7	12.6	45	29.1	12.2	ELISA	mU/L	serum	8
Zhong CX [24]	2012	HDP	Guangdong	27±4.2/ 28.2±4.0	50	2.6	0.94	50	1.84	0.73	ELISA	ug/l	serum	7
Liu Z [25]	2014	HDP	Jilin	28.27±3.00/ 27.13±2.80	50	62.7	11.7	50	28.2	13.1	ELISA	mIU/L	serum	8
Zhang L [26]	2014	EH	Guangdong	37±8/ 35±9	42	65.7	16.5	46	48.8	10.4	ELISA	ug/l	plasma	8
Zhu F [27]	2014	HDP	Jiangsu	27.5±5.3/ 26.2±4.5	92	63.06	21.98	80	40.3	11.5	Chemiluminescence	U/L	serum	7

HDP, hypertensive disorders in pregnancy; EH, essential hypertension; N, number; M, mean, SD, standard deviation; ELISA, enzyme linked immunosorbnent assay; NOS, Newcastle-Ottawa Scale;

Table 2. Effect estimates in subgroup analyses

Subgroup according to	EH				HDP			
	N	SMD (95%CI)	I^2 (%)	$P_{heterogeneity}$	N	SMD (95%CI)	I^2 (%)	$P_{heterogeneity}$
Total	6	1.960(1.305-2.615)	89.7	<0.0001	14	2.249 (1.324-3.173)	98.6	<0.0001
NOS score								
≥7	2	1.711(0.770-2.652)	86.7	0.006	12	2.270(1.694-2.846)	95.3	<0.0001
<7	4	2.086(1.140-3.033)	92.0	<0.0001	2	1.962(-4.005-7.929)	99.8	<0.0001
Specimen type								
Serum	3	1.755(0.752-2.757)	91.0	0.001	14	2.249 (1.324-3.173)	98.6	<0.0001
Plasma	3	2.167(1.117-3.217)	92.0	<0.0001	0	-	-	
Sample size								
>100	3	1.988(1.042-2.935)	90.8	<0.0001	10	2.472(1.254-3.691)	99.0	<0.0001
≤100	3	1.935(0.807-3.061)	92.3	<0.0001	4	1.690(0.783-2.597)	92.9	<0.0001
Geographical location								
North China	3	2.649(2.155-3.142)	62.2	0.071	10	3.969(1.720-6.218)	98.6	<0.0001
South China	3	1.242(0.950-1.533)	0	0.582	4	1.574(0.649-2.500)	98.4	<0.0001
Detecting methods								
ELISA	6	1.960(1.305-2.615)	89.7	<0.0001	13	2.327(1.307-3.346)	98.7	<0.0001
Chemiluminescence	0	-	-	-	1	1.272(0.943-1.601)	0	

HDP, hypertensive disorders in pregnancy; EH, essential hypertension; N, number; SMD, standardized mean difference; CI, confidence intervals; ELISA, enzyme linked immunosorbnent assay; NOS, Newcastle-Ottawa Scale;

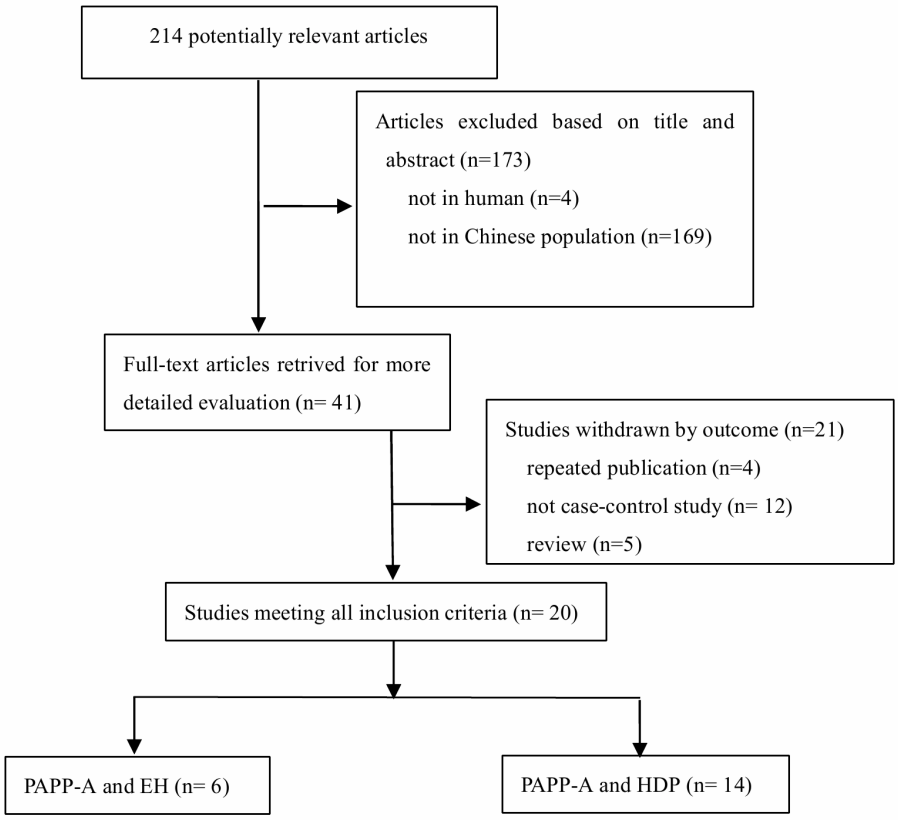

Figure 1. Flow diagram of article selection process

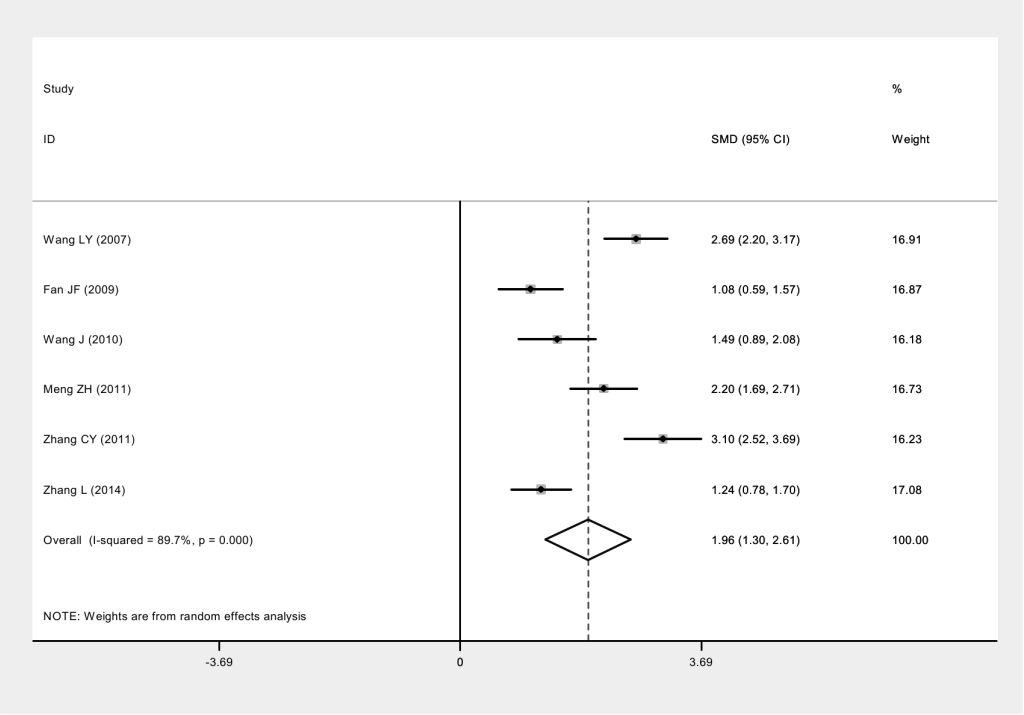

Figure 2. Forest plot of relationship between the level of PAPP-A and EH

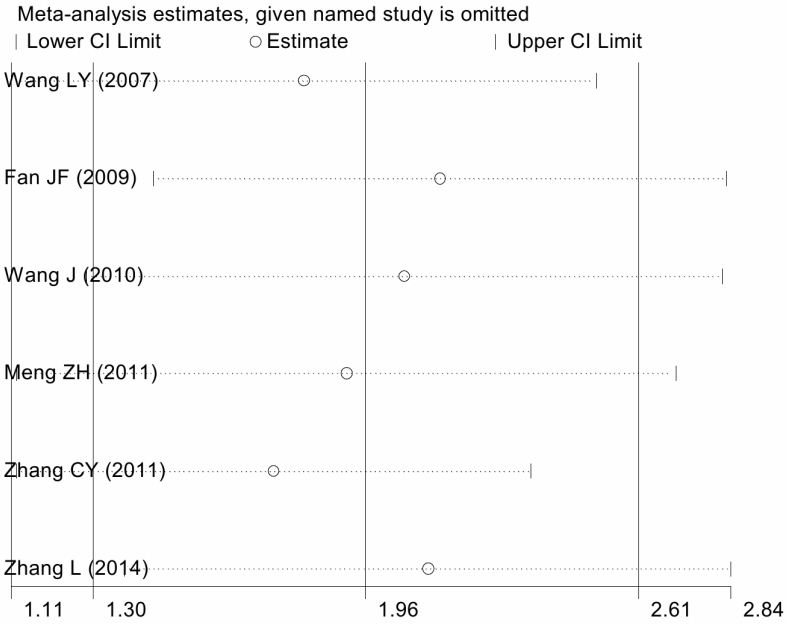

Figure 3. Analysis of influence of individual study on the pooled estimate in EH group

(Open circle indicate the pooled SMD. Horizontal lines represent the 95% confidence intervals)

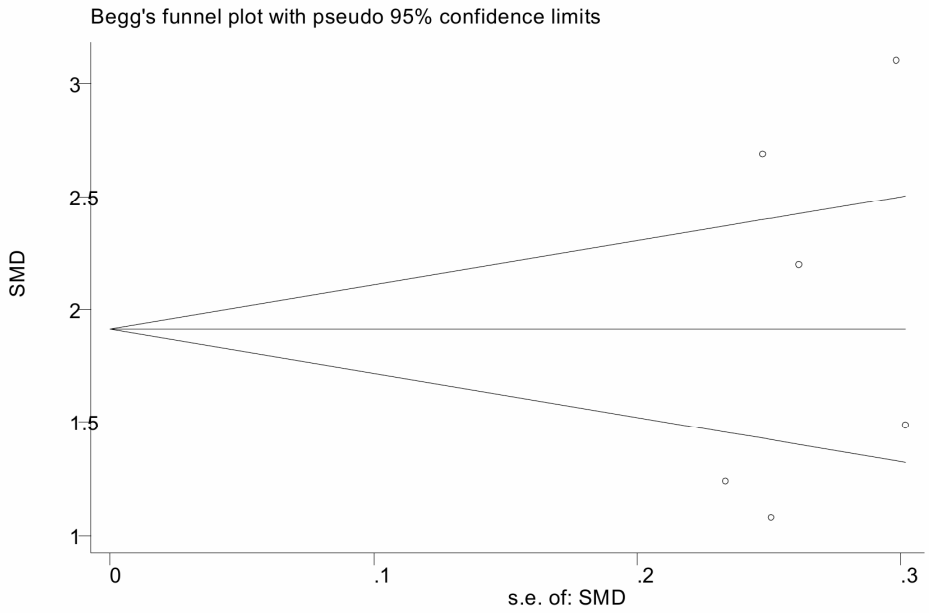

Figure 4. Funnel plot of PAPP-A associated with EH.

(Each point represents a separate study for the indicated association)

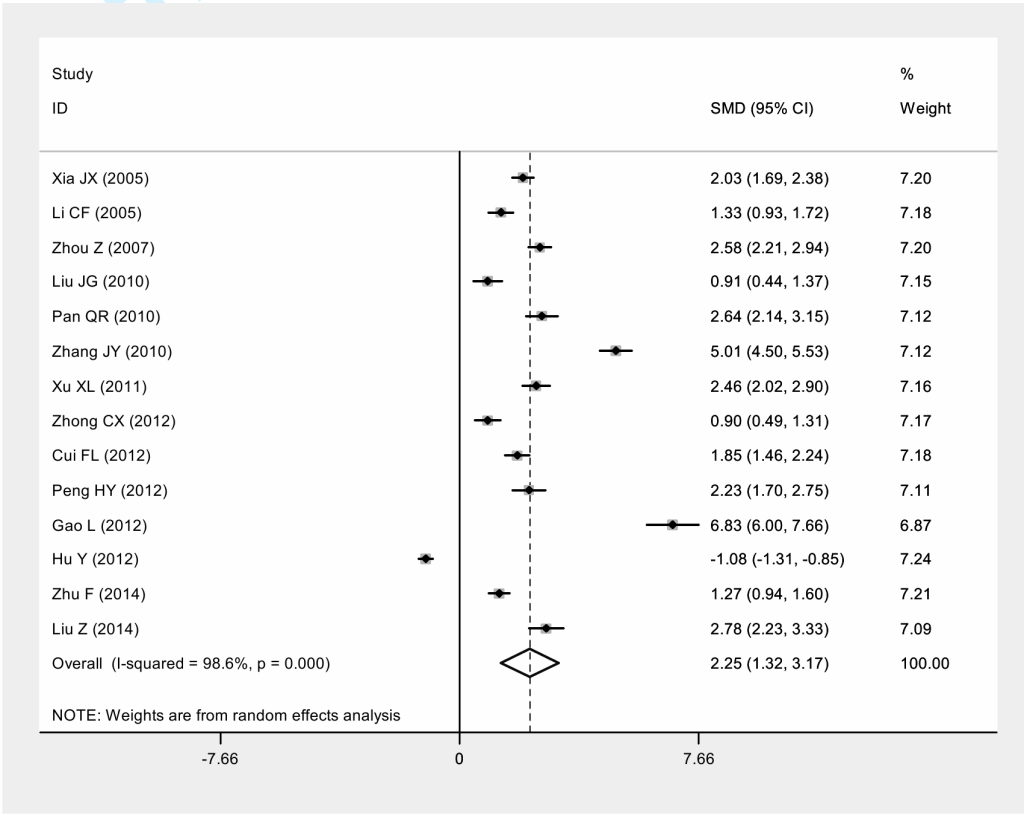

Figure 5. Forest plot of relationship between the level of PAPP-A and HDP

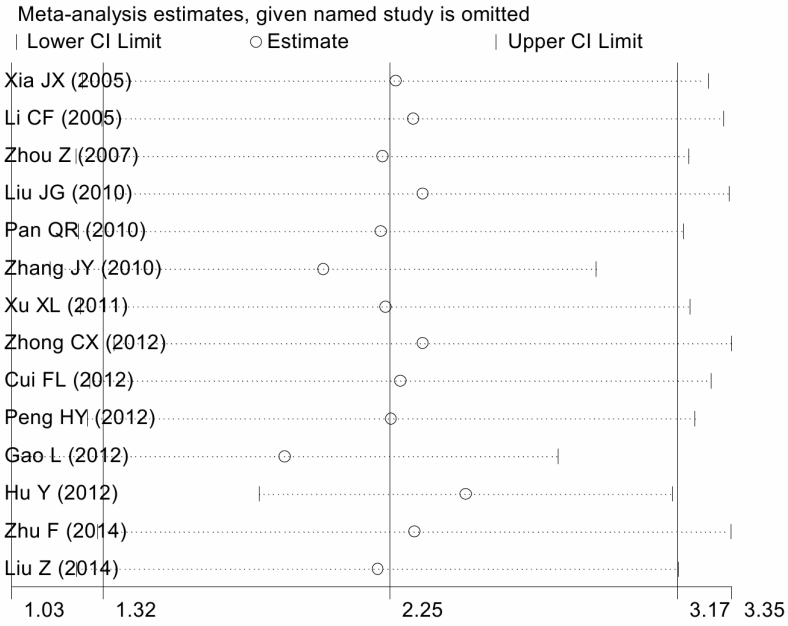

Figure 6. Analysis of influence of individual study on the pooled estimate in HDP group

(Open circle indicate the pooled SMD. Horizontal lines represent the 95% confidence intervals)

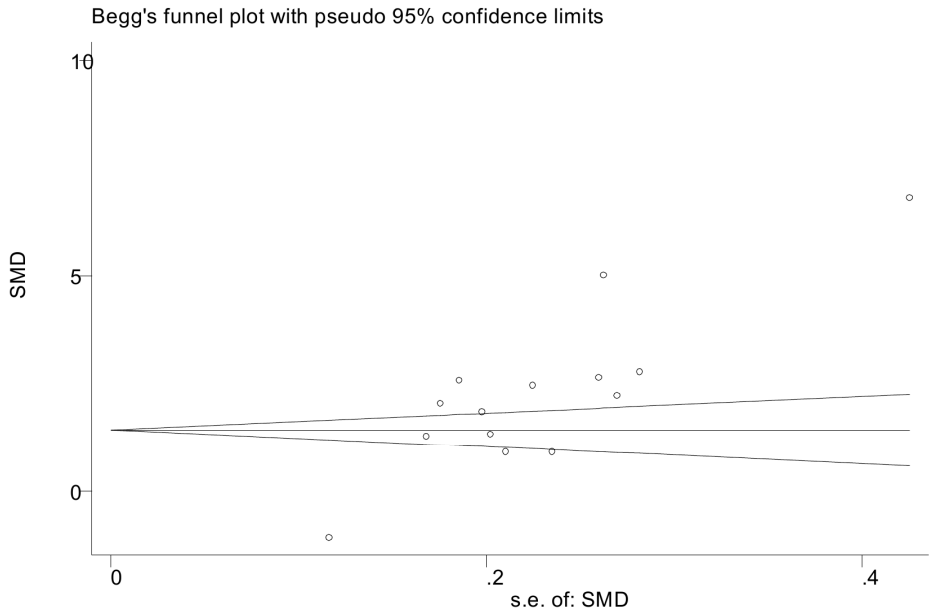

Figure 7. Funnel plot of PAPP-A associated with HDP.

Each point represents a separate study for the indicated association.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	4
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6-7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	6-7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	6-7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	6-7
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	6-7
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8-9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	10

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

Associations of pregnancy-associated plasma protein-A level with essential hypertension and hypertensive disorders in pregnancy in Chinese population: a meta-analysis of 20 research studies involving 3332 individuals

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Keywords:	Biochemistry < BASIC SCIENCES, Hypertension < CARDIOLOGY, MOLECULAR BIOLOGY

Associations of pregnancy-associated plasma protein-A level with essential hypertension and hypertensive disorders in pregnancy in Chinese population: a meta-analysis of 20 research studies involving 3332 individuals

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Abstract:

Objective: To explore the associations between serum pregnancy-associated plasma protein-A (PAPP-A) level and essential hypertension (EH) and hypertensive disorders in pregnancy (HDP) in Chinese population.

Methods: Pertinent studies were independently searched in PubMed, Embase, Cochrane Library, Chinese Biomedical Database (CBM), Wanfang databases and China National Knowledge Infrastructure (CNKI). The standardized mean difference (SMD) with 95% confidence intervals (CI) was used to estimate the size of the effect. The subgroup analyses and meta-regression analysis were performed to identify the sources of heterogeneity among studies. Sensitivity analysis was conducted to assess the stability of result. The publication bias between studies was examined by using Begg's funnel plots and Egger's test.

Results: A total of 20 studies involving 1493 patients and 1839 controls were included in the current meta-analysis. The PAPP-A level was significantly higher in EH patients than in controls (SMD=1.960, 95% CI=1.305-2.615, P<0.001) and significant associations were observed in all subgroups. The PAPP-A level was also significantly higher in HDP patients than in healthy pregnant women (SMD=2.249; 95% CI 1.324-3.173, P<0.001). The positive association between PAPP-A level and the risk of HDP was consistently observed in all subgroups except in the subgroup with the low NOS score.

Conclusion: The present meta-analysis suggests that the elevated PAPP-A level may be associated with the susceptibilities to EH and HDP.

Strengths and limitations of this study

1. To our knowledge, the present study is the first meta-analysis focused on the associations of PAPP-A level with EH and HDP in Chinese population.

2. The results suggested that PAPP-A level were higher in patients with EH or HDP than in healthy controls.

3. Multicentre, multi-ethnicity and larger scale studies are required to clarify the actual pathogenic mechanism of PAPP-A underlying EH and HDP.

Keywords: pregnancy-associated plasma protein-A; essential hypertension; hypertensive disorders in pregnancy; Chinese

INTRODUCTION

As a growing public health problem in developing countries, essential hypertension (EH) is considered as a major risk factor for cardiovascular diseases, such as coronary heart disease, ischemic stroke, heart failure, etc. In the last decade, the prevalence of EH in China has increased significantly.[1] In general, the etiology and the development of EH are affected by both genetic and environmental factors.[2, 3] Recently, numerous studies have shown that EH is closely related to inflammation and inflammation plays an important role in the pathogenesis and the maintenance of EH.[4]

Hypertensive disorders in pregnancy (HDP) remain as a major cause of both fetal and maternal mortality.[5] In general, HDP has five subtypes: 1) gestational hypertension, 2) preeclampsia, 3) eclampsia, 4) chronic hypertension in pregnancy, and 5) preeclampsia superimposed on chronic hypertension. The true etiology and the pathophysiology of HDP remain unclear. The genetic susceptibility and other biophysiological factors (such as endothelial dysfunction, immune imbalance, trophoblast cell ischemia, oxidative stress, etc.) may also contribute to the pathogenesis.

Pregnancy-associated plasma protein-A (PAPP-A), first identified by Lin et al.[6] in 1974, belongs to the matrix metalloproteinase family. PAPP-A is synthesized and secreted not only by placental trophoblast cells, but also by fibroblast cells, vascular smooth muscle cells, mesenchymal cells, etc. In the last decade, the role of PAPP-A in the diagnosis and prognosis of coronary heart disease has been investigated in depth.[7, 8]

Several recent studies have indicated that an increased serum PAPP-A level may be a biomarker of EH and HDP based on its important effects on the proliferation of vascular smooth muscle cells and on the reconstruction of extracellular matrix.[9-27] But some results are inconsistent.[28, 29] At the same time, the sample size of individual study was relatively small. Therefore, it is important to perform a meta-analysis of all available data to assess the value of peripheral blood PAPP-A levels for the diagnosis of EH and HDP.

METHODS

Studies selection

The current study was conducted according to the Meta-analysis of Observational Studies in Epidemiology group (MOOSe) [30] and the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines (PRISMA).[31]

Studies, published prior to December 1st, 2014, without language restriction, were searched from the following electronic databases: PubMed, Embase, Cochrane Library, Chinese Biomedical Database (CBM), Wanfang databases and China National Knowledge Infrastructure (CNKI). The following keywords and Medical Subject Headings (MeSH) terms were used: (‘‘PAPP-A’’ or ‘‘pregnancy associated plasma protein-A’’) and (‘‘hypertension’’ or ‘‘essential hypertension’’ or ‘‘high blood pressure’’ or ‘‘hypertension in pregnancy’’ or ‘‘hypertensive disorders in pregnancy’’ or ‘‘pregnancy-induced hypertension syndrome’’ or ‘‘hypertensive disorder complicating pregnancy’’ or ‘‘preeclampsia’’ or ‘‘eclampsia’’ or ‘‘gestational hypertension’’). Literatures in Chinese were searched by using the above terms in Chinese. For example, the full search strategy used in the CNKI database is: Subject term = 'PAPP-A' + 'pregnancy associated plasma protein-A' and (Subject term = 'essential hypertension' + 'hypertensive disorders in pregnancy' or Keyword = 'hypertension in pregnancy' + 'eclampsia' + 'hypertensive disorder complicating pregnancy' + 'gestational hypertension' + 'hypertension'). A manual search was conducted by checking the references of the relevant studies to reduce the omissions.

Inclusion criteria

The included studies must meet all the following criteria: (1) the study design must be a case-control or a cohort study; (2) the study must be used to evaluate the relationships between PAPP-A and EH or HDP; (3) the diagnostic criteria of EH was based on the criteria of World Health Organization/International Society of Hypertension (1999 or 2005); the HDP was defined that high blood pressure was detected only after 20 weeks of pregnancy; (4) the study must be conducted in Chinese population; (5) the specimen must be serum or plasma, rather than placental tissue; (6) the mean and standard deviation must be clear in the case and the control groups. If the results of one population were published in multiple studies, we selected the most comprehensive study. If we had doubts about the data, we contacted the corresponding author by email.

Data extraction

Two investigators (Cai and Zhang) independently evaluated the eligibility of all retrieved studies and extracted the original information, including the first author's name, year of publication, average age, type of disease, geographic distribution, sample size of cases and controls, peripheral blood PAPP-A level, detecting methods, specimen type. Any disagreement was resolved by discussion or in consultation with another author (Shi).

Quality assessment

A quality score was used to assess the quality of the studies according to the Newcastle-Ottawa Scale (NOS) criteria,[32] including subject selection (0-4), comparability (0-2) and exposure (0-3). The quality of a paper was considered as high if the NOS scores were more than 7.[33]

Statistics and analyses

Because the unit weights of PAPP-A level in each study were not consistent, the standardized mean difference (SMD) with 95% confidence intervals (CI) was used to estimate the size of the effect in present study.

Heterogeneity across studies was calculated by using Cochran's Chi-square based Q-test. At the same time, it was also estimated by using the I^2 test. If the heterogeneity was statistically significant (P<0.01, I^2>50%), the random-effects model (a Dersimonian-Laird method) was used. Otherwise, the fixed-effects model (a Mantel-Haenszel method) was applied.[34] In order to identify the sources of heterogeneity, the subgroup analyses were performed. The studies were stratified by sample size (>100 or ≤100), specimen type (serum or plasma), detecting method (ELISA or Chemiluminescence), NOS score (≥7 or <7), type of disease (EH or HDP) and geographical location (North China or South China). The meta-regression analysis was also performed to identify the sources of heterogeneity among studies. To assess the stability of result, sensitivity analysis was used by calculating the pooled effect again when omitting single study one by one.

The publication bias was assessed via Begg's funnel plot and Egger's regression test. If the

funnel plot was asymmetric, the trim-and-fill method was used to identify and correct the publication bias.

The STATA version 12.0 (StataCorp LP, College Station, Texas 77845 USA) was used for the statistical analysis. A *P* value <0.05 was considered statistically significant.

RESULTS

Study selection

A total of 214 potentially pertinent articles were identified, by using the above search strategy. 173 articles were excluded after the title and abstract review. Another 21 studies were excluded, after we reviewed the full article texts. Finally, a total of 20 studies involving 1493 patients and 1839 controls met the inclusion criteria and were included for the current meta-analysis. The detailed process of literature search and study selection is described in figure 1.

Study characteristics

Among the 20 studies, six studies involving 395 cases and 233 controls were focused on the relationship between the level of PAPP-A and EH , and fourteen studies involving 1098 cases and 1606 controls were focused on PAPP-A and HDP. The characteristics of the 20 studies are summarized in table 1. All of the 20 studies were published in Chinese. Publication years of the eligible studies ranged from 2005 to 2014. The sample size of each study varied from 80 to 814. Nineteen studies were conducted in Han population and one study was conducted in Kazakhs population. Seventeen studies used serum specimens to examine the PAPP-A level, and three studies used plasma. Nineteen studies except Zhu's used ELISA as a method to detect PAPP-A level. [27] The NOS scores in seventeen of the 20 studies were above seven.

Association between the PAPP-A level and EH

The pooled SMD was calculated by random-effects model because there was significant heterogeneity between studies (I^2=89.7%, P<0.001). As shown in figure 2, the pooled analysis indicated that the PAPP-A level was significantly higher in EH patients than in controls (SMD=1.960, 95% CI=1.305-2.615, P<0.001).

In the stratified analyses based on sample size, specimen type, detecting methods, NOS score and geographical location, significant associations were observed in all the subgroups (table 2).

The stability of the study was detected by sensitivity analysis, through re-meta-analysis by excluding one individual study each time. As shown in figure 3, no individual study significantly affects the pooled SMD.

The publication bias was assessed by Begg's funnel plot and Egger's test. The funnel plot was symmetric and the Egger's test also suggested no publication bias (P=0.450) (figure 4).

Association between the PAPP-A level and HDP

An obvious heterogeneity was found (I^2= 98.6%, P<0.001), so the pooled SMD was also calculated by random-effects model. As shown in figure 5, the results have demonstrated that the PAPP-A level was significantly higher in HDP patients than in healthy pregnant women (SMD=2.249; 95% CI=1.324-3.173, P<0.001).

Stratified analyses were conducted based on sample size, detecting methods, NOS score and geographical location. A positive association between PAPP-A level and the risk of HDP was consistently observed in all subgroups except the subgroup with low NOS score (table 2).

The sensitivity analysis suggested that only the study of Hu Y [28] appeared to have a large influence, but the result did not change after this study was excluded, suggesting that the results have sufficient statistical power (figure 6).

Due to the significant heterogeneity between studies, besides the subgroup analyses, a meta-regression was also performed to further identify the potential source of heterogeneity. Sample size, detecting methods, NOS score and geographical location, were included in regression analysis. The result indicated that only geographical location might partially contribute to the heterogeneity ($P_{\text{meta-regression}}$= 0.025) and can explain 30.61% of the heterogeneity. Other factors, such as NOS score ($P_{\text{meta-regression}}$= 0.812), sample size ($P_{\text{meta-regression}}$= 0.507) and detecting methods ($P_{\text{meta-regression}}$= 0.605), might not be the sources of heterogeneity.

The obvious asymmetry of the funnel plot was visualized (Egger's test P=0.000) (figure 7). The trim-and-fill method was used to identify and correct the publication bias.[35] The number

of missing studies was estimated by the linear method. After two iterations, the value of diff became zero and the number of estimated deficient study was zero, indicating that result was stable.

DISCUSSION

To our knowledge, the present study is the first meta-analysis focused on the associations of PAPP-A level with EH and HDP. We found that PAPP-A level were higher in patients with EH or HDP than in healthy controls.

In 2011, a multi-variable study of large-scale samples, involving 112,386 pregnant women, was conducted to estimate the prevalence of HDP in China.[36] This epidemiological investigation revealed that the prevalence of HDP in China was about 5.22%, though smaller than 8-10% in other studies.[37] Although numerous studies have been conducted, the precise mechanism underlying the pathogenesis of EH and HDP remains poorly understood.

PAPP-A, about 750~820 kDa in size, plays an important role in the proliferation of vascular smooth muscle cells and in the reconstruction of extracellular matrix. Previous studies indicated that the low serum PAPP-A concentrations in pregnant women were associated with adverse pregnancy outcomes, such as premature delivery and small for gestational age infants,etc.. [38] Recently, a growing number of studies have suggested elevated serum level of PAPP-A might be involved in the development of EH and HDP by the following mechanisms: 1) PAPP-A may increase the IGF-1 level, which can promote the proliferation of vascular smooth muscle cells and the remodeling of extracellular matrix; 2) The elevated IGF-I level also increases the degradation of the NO synthetase, which can lead to a sustained contraction of vessel wall; 3) PAPP-A can promote the release of inflammatory cytokines, which can increase the arterial endothelial injury; 4) In HDP patients, hypoxia causes the trophoblast cells to secret more PAPP-A, which can augment the placenta local oxidative stress and aggravate the placenta hypoxia.

Although most of the studies reached positive conclusions, the results were not consistent and the sample sizes in each study were relatively small. To resolve this problem, we conducted the current meta-analysis to examine the associations of PAPP-A level with risk of

EH and HDP.

In the present meta-analysis, 20 studies met the inclusion criteria. Six of them were focused on EH and 14 of them were focused on HDP. According to the results, we found that the PAPP-A level was significantly higher in EH (SMD=1.960, 95% CI=1.305-2.615, P<0.001) and HDP patients (SMD=2.249; 95% CI 1.324-3.173, P<0.001) than in controls.

A significant heterogeneity was found among studies. A meta-regression was performed to identify the sources of heterogeneity in HDP group. We found that only the geographical location can explain 30.61% of the heterogeneity. In the previous studies, the prevalence of EH and HDP in Chinese population represents a characteristic of geographical location, which is higher in North China than in South China. Although the exact mechanism was not clear, the cold-induced vasoconstriction might trigger the onset of these diseases. Our meta-analysis suggested that geographical location might be a source of the heterogeneity. Subgroup analyses were also performed in EH and HDP groups. The stratified analyses were conducted according to sample size, detecting methods, NOS score and geographical location. The positive associations of PAPP-A level with EH and HDP were consistently observed in all of the subgroups except in the subgroup with the low NOS score. We also preformed the sensitivity analysis to assess the influence of individual study on the pooled SMD. No single study can influence the stability of the whole study.

Although our meta-analysis is a practical way to generate a more powerful result with less random error than each individual study, our study has some limitations. First of all, the total sample size of the studies included in this meta-analysis was relatively small. Secondly, all subjects in studies came from hospital, which may lead to the selection bias. Thirdly, researches have revealed that the PAPP-A levels vary among different subtypes of the disease. The PAPP-A level is even different between in early-onset preeclampsia and late-onset preeclampsia. [28] At the same time, it is increasingly accepted that early-onset preeclampsia and the late-onset preeclampsia have the different pathogenic mechanisms.[39] However, some studies included in our analysis were not divided into subgroups. Fourthly, the blood collection time was not described in studies, which could contribute to the heterogeneity. Fifthly, an obvious publication bias was found in HDP group: it is more difficult to publish

negative results and the study population was also limited in the Chinese population. Although an obvious publication bias exists, sensitivity analysis of trim-and-fill method showed the result was reliable. Finally, a major confounding factor was the heterogeneity among studies. In EH group, due to the number of study was smaller than ten, the meta-regression method was not repeated to further identify the source of heterogeneity. In the HDP group, although the meta-regression was performed, only part of the heterogeneity could be explained by geographical location.

CONCLUSIONS

In conclusion, our meta-analysis suggests that the PAPP-A level is significantly higher in EH and HDP patients than in controls. However, multicentre, multi-ethnicity, and larger-scale studies are required to clarify the actual pathogenic mechanism of PAPP-A underlying EH and HDP.

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The authors declare that they have no competing interests.

Supplementary file: PRISMA-2009-Checklist-MS

Author Contributions

Conceived and designed the experiments: GJC, BFZ. Performed the experiments: GJC, BFZ, WJW, LPY, GWS, SLX, XLF. Analyzed the data: GJC, BFZ, XLF. Contributed reagents/materials/analysis tools: GJC, BFZ. Wrote the paper: GJC, BFZ, XLF.

Data sharing statement: No additional data are available.

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Table 1. Characteristics of 20 studies on PAPP-A and risk of EH and HDP

Author	Year	Type of disease	Geographic distribution	Age (case/control)	Case			Control			Detecting method	Unit	Specimen type	NOS
					N	M	SD	N	M	SD				
Li CF [9]	2005	HDP	Shanxi	26.5±4.3/ -	60	58.85	21.05	60	36.8	10.4	ELISA	IU/L	serum	8
Xia JX [10]	2005	HDP	Guangdong	27.85±5.20/27.50±5.47	95	46.36	14.83	103	23.6	6.1	ELISA	mIU/m	serum	8
Wang LY [11]	2007	EH	Jilin	-/ 60±12	70	3	0.6	56	1.5	0.5	ELISA	mU/L	serum	6
Zhou Z [12]	2007	HDP	Jiangsu	5.8±4.8/ 26.5±4.3	109	57.84	12.25	105	29.4	9.65	ELISA	mg/l	serum	8
Fan JF [13]	2009	EH	Anhui	64.19±13.5/61.78±16.9	84	8.66	3.26	22	5.17	3.15	ELISA	mIU/L	serum	6
Liu JG [22]	2010	HDP	Hunan	29.7±5.7/ 30.2±4.1	40	2.61	0.96	40	1.83	0.75	ELISA	ug/l	serum	8
Pan QR [14]	2010	HDP	Jiangsu	26.8±4.7/ 26.3±3.8	92	58.34	12.19	35	28.4	8.57	ELISA	U/L	serum	8
Wang J [15]	2010	EH	Anhui	64±12/ 61±16	80	11.91	3.42	15	7.1	1.9	ELISA	mU/L	serum	6
Zhang JY [16]	2010	HDP	Hebei	23-36/ 22-35	115	181	32	127	69	5	ELISA	mU/ml	serum	6
Meng ZH [17]	2011	EH	Xinjiang	56.6±11.7/ 56.4±13.2	80	6.04	1.07	30	3.9	0.64	ELISA	mg/l	plasma	9
Xu XL [18]	2011	HDP	Zhejiang	25.7±5.7/ -	70	58.8	12.47	70	30.5	10.5	ELISA	IU/L	serum	8
Zhang CY [19]	2011	EH	Shandong	61±9/ 52±13	39	21.22	4.12	64	7.97	4.36	ELISA	mU/L	plasma	5
Cui FL [20]	2012	HDP	Yunnan	27.3±2.4/ 27.1±2.6	72	51.2	10.4	75	32.7	9.6	ELISA	IU/L	serum	8
Gao L [21]	2012	HDP	Sichuan	32.49±2.1/ 29.15±2.3	120	73.39	5.33	40	38.2	4.56	ELISA	U/L	serum	8
Hu Y [28]	2012	HDP	Henan	-/ -	88	19.98	5.36	726	22.5	1.62	ELISA	U/L	serum	5
Peng HY [23]	2012	HDP	Guangdong	8.6±7.7/ 28.8±7.6	45	56.7	12.6	45	29.1	12.2	ELISA	mU/L	serum	8
Zhong CX [24]	2012	HDP	Guangdong	27±4.2/ 28.2±4.0	50	2.6	0.94	50	1.84	0.73	ELISA	ug/l	serum	7
Liu Z [25]	2014	HDP	Jilin	28.27±3.00/27.13±2.80	50	62.7	11.7	50	28.2	13.1	ELISA	mIU/L	serum	8
Zhang L [26]	2014	EH	Guangdong	37±8/ 35±9	42	65.7	16.5	46	48.8	10.4	ELISA	ug/l	plasma	8
Zhu F [27]	2014	HDP	Jiangsu	27.5±5.3/ 26.2±4.5	92	63.06	21.98	80	40.3	11.5	Chemiluminescence	U/L	serum	7

HDP, hypertensive disorders in pregnancy; EH, essential hypertension; N, number; M, mean, SD, standard deviation; ELISA, enzyme linked immunosorbnent assay; NOS, Newcastle-Ottawa Scale;

Table 2. Effect estimates in subgroup analyses

Subgroup according to	EH				HDP			
	N	SMD (95%CI)	I^2 (%)	$P_{heterogeneity}$	N	SMD (95%CI)	I^2 (%)	$P_{heterogeneity}$
Total	6	1.960(1.305-2.615)	89.7	<0.0001	14	2.249 (1.324-3.173)	98.6	<0.0001
NOS score								
≥7	2	1.711(0.770-2.652)	86.7	0.006	12	2.270(1.694-2.846)	95.3	<0.0001
<7	4	2.086(1.140-3.033)	92.0	<0.0001	2	1.962(-4.005-7.929)	99.8	<0.0001
Specimen type								
Serum	3	1.755(0.752-2.757)	91.0	0.001	14	2.249 (1.324-3.173)	98.6	<0.0001
Plasma	3	2.167(1.117-3.217)	92.0	<0.0001	0	-	-	
Sample size								
>100	3	1.988(1.042-2.935)	90.8	<0.0001	10	2.472(1.254-3.691)	99.0	<0.0001
≤100	3	1.935(0.807-3.061)	92.3	<0.0001	4	1.690(0.783-2.597)	92.9	<0.0001
Geographical location								
North China	3	2.649(2.155-3.142)	62.2	0.071	10	3.969(1.720-6.218)	98.6	<0.0001
South China	3	1.242(0.950-1.533)	0	0.582	4	1.574(0.649-2.500)	98.4	<0.0001
Detecting methods								
ELISA	6	1.960(1.305-2.615)	89.7	<0.0001	13	2.327(1.307-3.346)	98.7	<0.0001
Chemiluminescence	0	-	-	-	1	1.272(0.943-1.601)	0	

HDP, hypertensive disorders in pregnancy; EH, essential hypertension; N, number; SMD, standardized mean difference; CI, confidence intervals; ELISA, enzyme linked immunosorbnent assay; NOS, Newcastle-Ottawa Scale;

Figure legends:

Figure 1. Flow diagram of article selection process

Figure 2. Forest plot of relationship between the level of PAPP-A and EH

Figure 3. Analysis of influence of individual study on the pooled estimate in EH group

(Open circle indicate the pooled SMD. Horizontal lines represent the 95% confidence intervals)

Figure 4. Funnel plot of PAPP-A associated with EH.

(Each point represents a separate study for the indicated association)

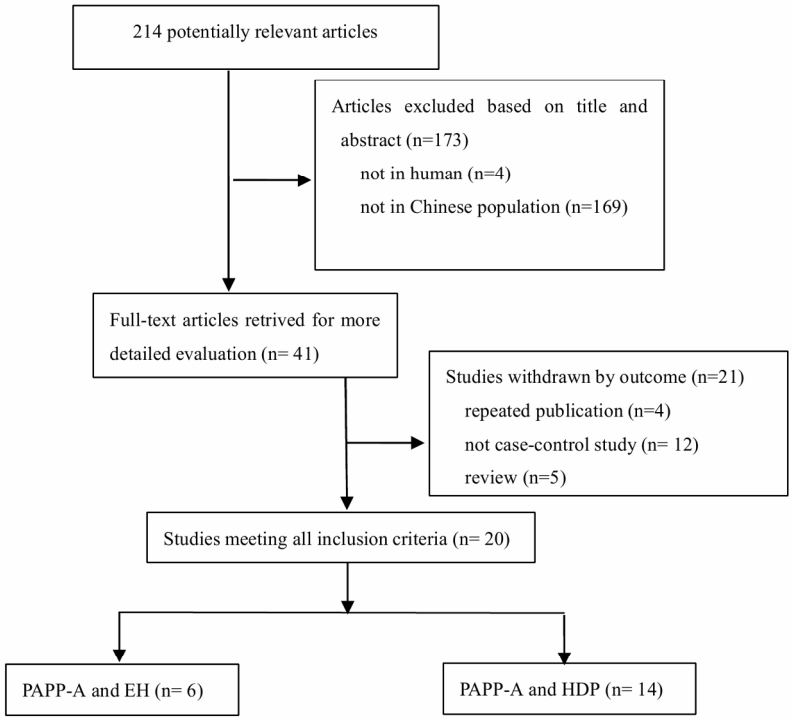
Figure 5. Forest plot of relationship between the level of PAPP-A and HDP

Figure 6. Analysis of influence of individual study on the pooled estimate in HDP group

(Open circle indicate the pooled SMD. Horizontal lines represent the 95% confidence intervals)

Figure 7. Funnel plot of PAPP-A associated with HDP.

(Each point represents a separate study for the indicated association.)



Flow diagram of article selection process
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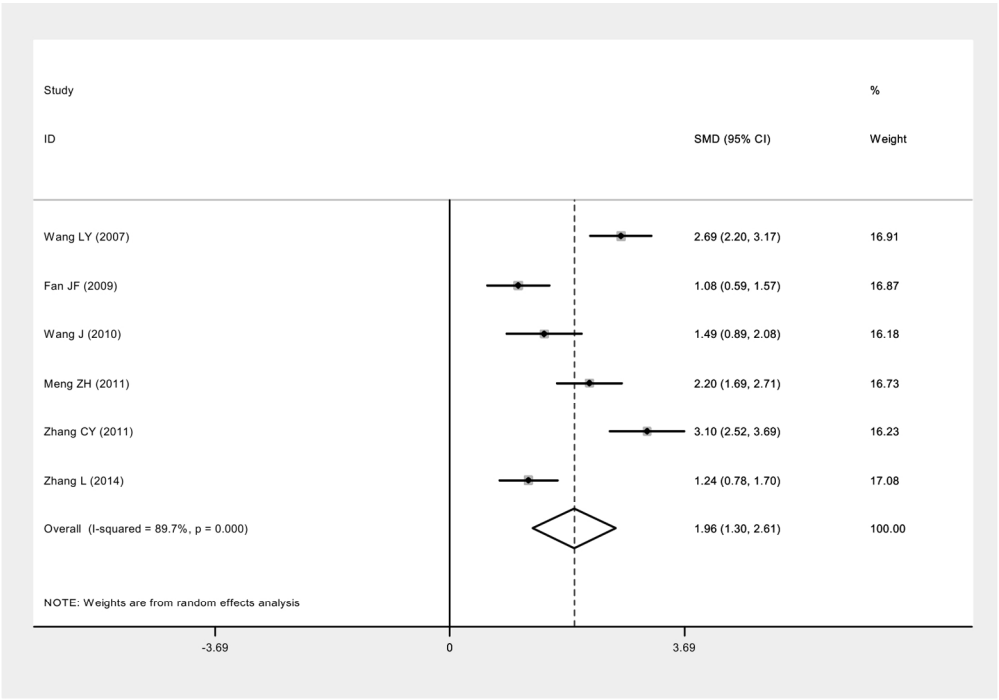

Forest plot of relationship between the level of PAPP-A and EH
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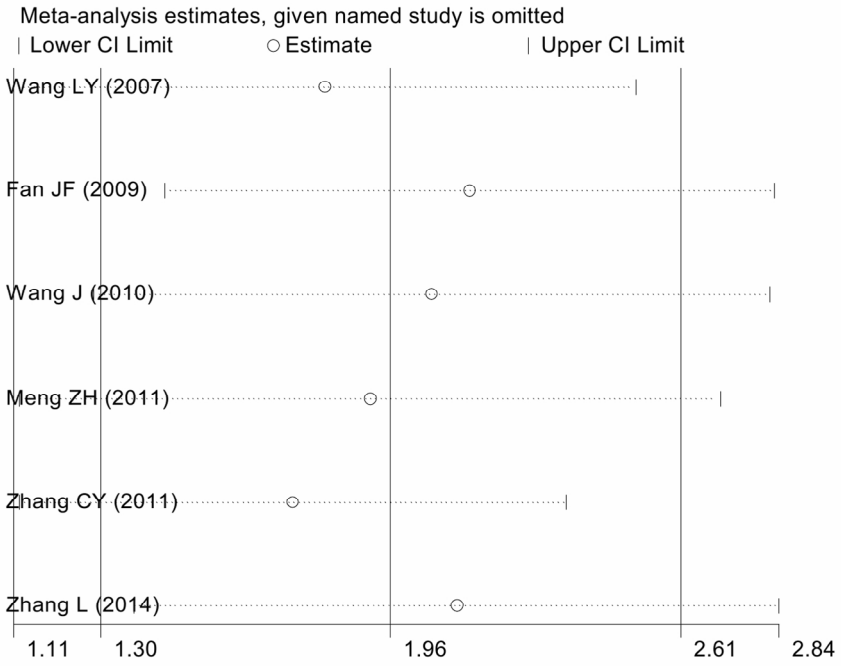

Analysis of influence of individual study on the pooled estimate in EH group
(Open circle indicate the pooled SMD. Horizontal lines represent the 95% confidence intervals)

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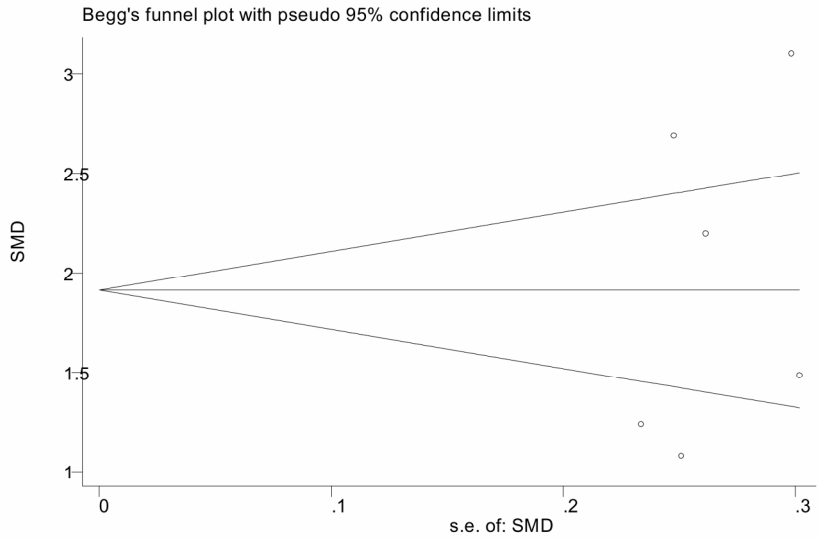

Funnel plot of PAPP-A associated with EH.
(Each point represents a separate study for the indicated association)

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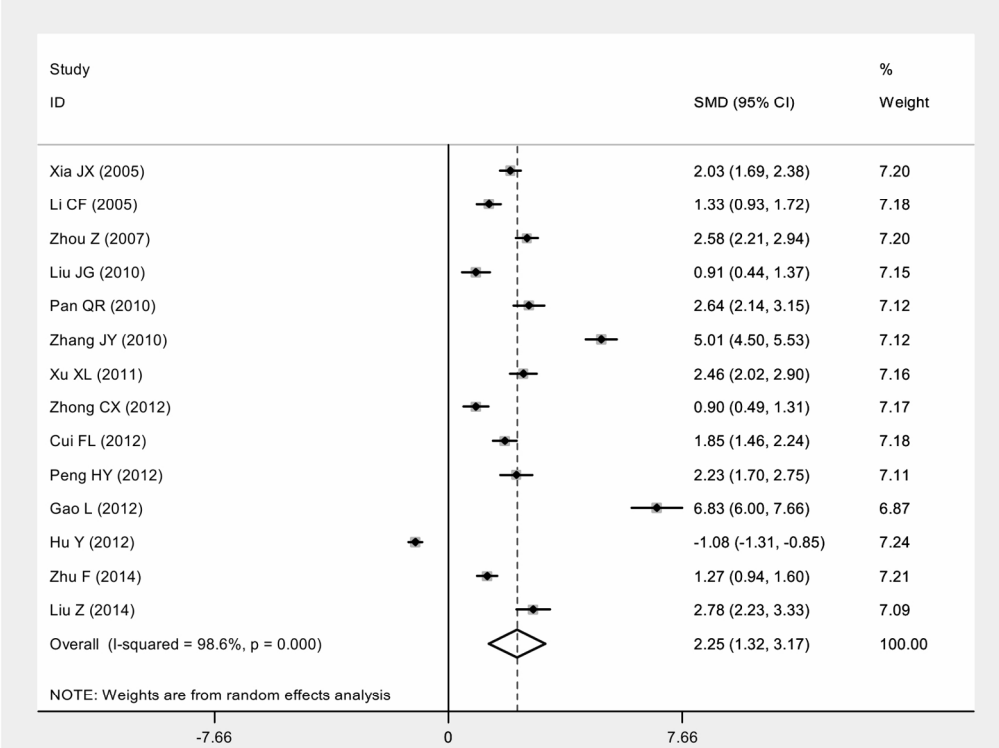
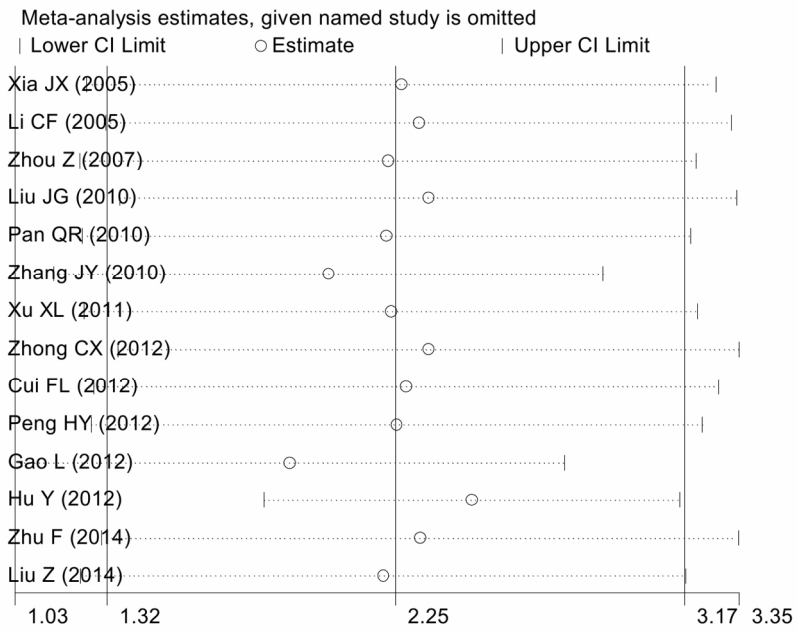
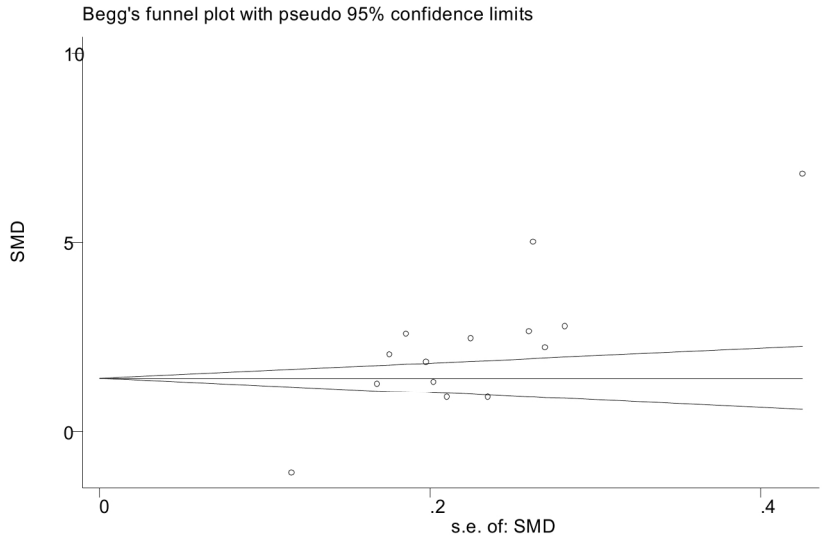


Forest plot of relationship between the level of PAPP-A and HDP
144x114mm (300 x 300 DPI)



Analysis of influence of individual study on the pooled estimate in HDP group
(Open circle indicate the pooled SMD. Horizontal lines represent the 95% confidence intervals)

139x102mm (300 x 300 DPI)



Funnel plot of PAPP-A associated with HDP.
(Each point represents a separate study for the indicated association.)

171x107mm (300 x 300 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	4
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6-7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	6-7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	6-7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	6-7
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	6-7
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8-9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	10

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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