

Original Article

Serum IL-6 and TNF- α as a biomarker in the diagnosis of hypoxic-ischemic encephalopathy-induced brain injury: a meta-analysis of randomized controlled trials

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Abstract: Purpose: Inflammation is reported to play an important part in mediating injury in neonatal hypoxic-ischemic encephalopathy (HIE). Whether inflammatory-related cytokine such as IL-6 and TNF- α is associated with risk of HIE remains unknown. Method: In this meta-analysis of 29 clinical trials published to date in Chinese VIP, Wangfang, CNKI, Pubmed, Google Scholar database using the keywords "IL-6", "TNF- α " and "hypoxic-ischemic encephalopathy/HIE", we selected eligible studies which met the inclusion criterion, extract interested information and analyzed outcomes using the Review Manage 5.3 software. Results: Meta-analysis including 1618 HIE newborns and 790 healthy controls showed that serum IL-6 was significant up-regulated in different states (mild, moderate, severe) of neonatal hypoxic-ischemic encephalopathy (HIE) with the value of IV was 2.62, 4.91, 6.55, 95% CI [2.03, 3.22], [4.01, 5.80], [5.46, 7.64] and $P < 0.01$. Similarly, compared to healthy controls, elevated serum TNF- α was also found to be associated with risk of neonatal HIE, the value of Std. Mean. Difference was 4.00, 4.75, 8.24, 95% CI was [3.60, 4.40], [4.07, 5.43], [7.38, 9.11] and P value < 0.01 . Conclusion: Meta-analysis suggested that elevated serum IL-6 and TNF- α level in newborns is associated with high risk of hypoxic-ischemic encephalopathy (HIE)-induced brain injury. Further studies are needed to validate these observations.

Keywords: Hypoxic-ischemic encephalopathy (HIE), IL-6, TNF- α , meta-analysis

Introduction

Newborns with hypoxic-ischemic encephalopathy (HIE), occurs at an approximate rate of 1 to 2 per 1000 live birth [1, 2], is one of leading causes of neonatal death and long-term disability, such as cerebral palsy, seizures, visual and learning impairment and abnormal mental development [3, 4]. Ischemia in brain induces both central and peripheral inflammation leading to a secondary neuron damage [5]. Microglia, which is a major glial components of the CNS and resides inside brain, activates within minutes in ischemia brain and acts as first step of immune system activation followed by infiltration of monocytes, T cells, amplifying immune responses in a stimulated brain [6, 7].

Cerebral ischemic injury not only triggers the activation and expansion of immune cells, but also acts as a cascade of cytokine induction. Cytokine, as important inflammatory mediators, orchestrate and amplify in-situ inflamma-

tion in brain and exaggerate cerebral injury [8]. Of these, tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6) are well-known and most-studied cytokines that take active part in the inflammatory responses.

In a variety of clinical studies, newborn infants with hypoxic-ischemic encephalopathy were found to be associated with abnormal expression of TNF- α and IL-6 in the serum, plasma and cerebral flow. However, due to the limited clinical samples, consensus conclusion of whether serum IL-6 and TNF- α could be an effective biomarker for hypoxic-ischemic-induced brain injury diagnosis in was not drawn yet. Therefore, we performed a meta-analysis study by combining a number of researches related to the association of enhanced expression of serum IL-6 and TNF- α with risk of different state of HIE to find out whether serum IL-6 and TNF- α could be a novel biomarker for testing the early occurrence of HIE.

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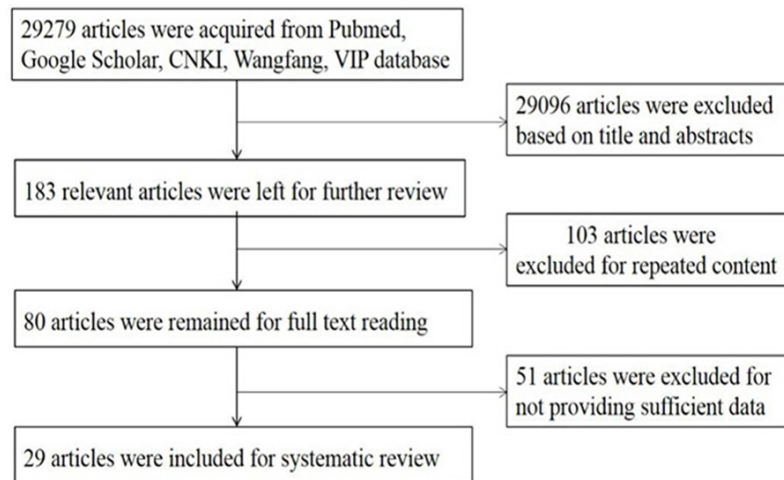


Figure 1. Search flow diagram of included studies.

Methods

Search strategy

The following database: Chinese VIP, Wangfang, CNKI, Pubmed, Google Scholar were searched for the randomized controlled trials using the keywords “IL-6”, “TNF- α ” and “Hypoxic-ischemic encephalopathy/HIE” ever since from database established. There were no limitations for the language.

Inclusion and exclusion criteria

Inclusion criteria: Eligible studies met the following criteria: 1) Enrolled newborns diagnosed of hypoxia-ischemic encephalopathy with different state of illness (mild, moderate and severe); 2) Healthy control group should be included in the study; 3) Serum IL-6 or TNF- α levels should be measured with consensus unit (ng/L).

Exclusion criteria: 1) Reviews, editorials and dissertations; 2) Animal models (mice, rats, rabbits and so on); 3) Cell line and in-vitro study; 4) Repeated publications (in different language); 5) Plasma or cell flow IL-6 or TNF- α levels; 6) Data was not displayed with mean \pm SEM.

Data extraction

Identified studies were reviewed and data was extracted from two reviewers independently using the extracting data form. The following information should be extracted: 1) First

author’s family name and publication year; 2) Sample size including different gender of each group; 4) The number of different degree of HIE (mild, moderate, severe); 5) The value of IL-6 and TNF- α (mean \pm SEM) of each group; 8) The included measurement (IL-6 or TNF- α). Disagreements were resolved by the decision of a third reviewer.

Statistical analysis

The main outcome was the comparison of serum IL-6 and TNF- α value in different

state of illness in neonatal HIE compared with healthy controls in clinic. Random model was performed using Review Manage 5.3 software for meta-analysis and weighed mean differences (WMD), standardized mean difference (SMD) and 95% CI for the outcome of continuous variables. If the value of I^2 was higher than 50%, the results of included studies with homogeneity ($I^2 < 50\%$) should be represent to verify the stability of results.

Results

Study selection and characteristics

As the search flow diagram of included studies showing in **Figure 1**, a total of 29279 articles were required for initial search in database by the key words. After reading the title and abstracts, only 183 randomized controlled trials which were relevant to the level of IL-6 and TNF- α in neonatal hypoxic-ischemic encephalopathy were remained for further text review. After depleting the repeated articles including same articles published in different languages, 80 articles were left for full text reading. Of these, 29 articles met the inclusion criterion, all were conducted in China.

The interested information was extracted in **Table 1**; of the included 1618 HIE newborns and 790 healthy controls. In short, serum IL-6 and TNF- α were measured by ELISA. Neonatal HIE were all classified into three states: mild, moderate and severe. Most of included studies performed their measurement within 24 hours.

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Table 1. Study characteristics of included studies

Study	Year	Control N	M/F	HIE N	M/F	State of illness (mild/moderate/severe)	TNF α	IL-6
Yang [9]	2011	20	11/9	48	25/23	20/17/11	√	√
Chen [10]	2011	20	12/8	60	34/26	28/ND	√	√
Zou [11]	2014	80	40/40	80	45/35	28/30/22	√	
Wang et al. [12]	2005	10	ND	46	35/11	14/26/6		√
Dai et al. [13]	2015	20	ND	60	32/28	21/21/18	√	√
Zhang et al. [14]	2010	20	12/8	42	23/19	24/10/8		√
Chai et al. [15]	2010	20	11/9	58	32/26	30/ND	√	
Mi et al. [16]	2009	21	10/11	34	19/15	15/10/9	√	√
Guo et al. [17]	2014	42	26/16	98	56/42	40/32/26		√
Gong [18]	2012	20	12/8	60	34/26	28/ND	√	√
Liu [19]	2011	32	18/14	94	51/43	38/31/25	√	
Feng et al. [20]	2008	26	18/8	58	37/21	19/26/13		√
Lin et al. [21]	2002	30	20/10	50	35/15	18/17/15		√
Lu et al. [22]	2006	30	19/11	32	22/10	15/9/8		√
Mao et al. [23]	2005	30	20/10	30	18/12	14/10/6		√
Li [24]	2012	30	19/11	65	39/26	33/19/13	√	
Li et al. [25]	2003	40	21/19	60	33/27	30/22/8		√
Kong [26]	2013	29	16/13	32	15/17	8/13/11		√
Wang et al. [27]	2008	14	7/4	41	29/12	13/17/11		√
Jiang et al. [28]	2011	20	12/8	40	23/17	15/13/12		√
Tian [29]	2014	15	8/7	37	21/16	11/14/12		√
Zhang et al. [30]	2003	12	ND	37	ND	10/15/12	√	√
Li et al. [31]	2004	40	21/19	60	33/27	30/22/8		√
Mao et al. [32]	2011	11	6/5	41	22/19	19/14/8	√	√
Tan [33]	2005	63	33/30	98	50/48	20/38/40		√
Liao et al. [34]	2009	13	ND	46	29/17	12/26/8		√
Huang et al. [35]	2011	22	11/11	103	56/47	53/35/15		√
Xu et al. [36]	2013	30	18/12	46	27/19	15/19/12	√	
Huang et al. [37]	2010	30	16/14	62	36/26	31/20/11	√	

M: Male, F: Female, ND: not described.

Association of serum IL-6 level of newborns with hypoxia-ischemic encephalopathy in different state of illness

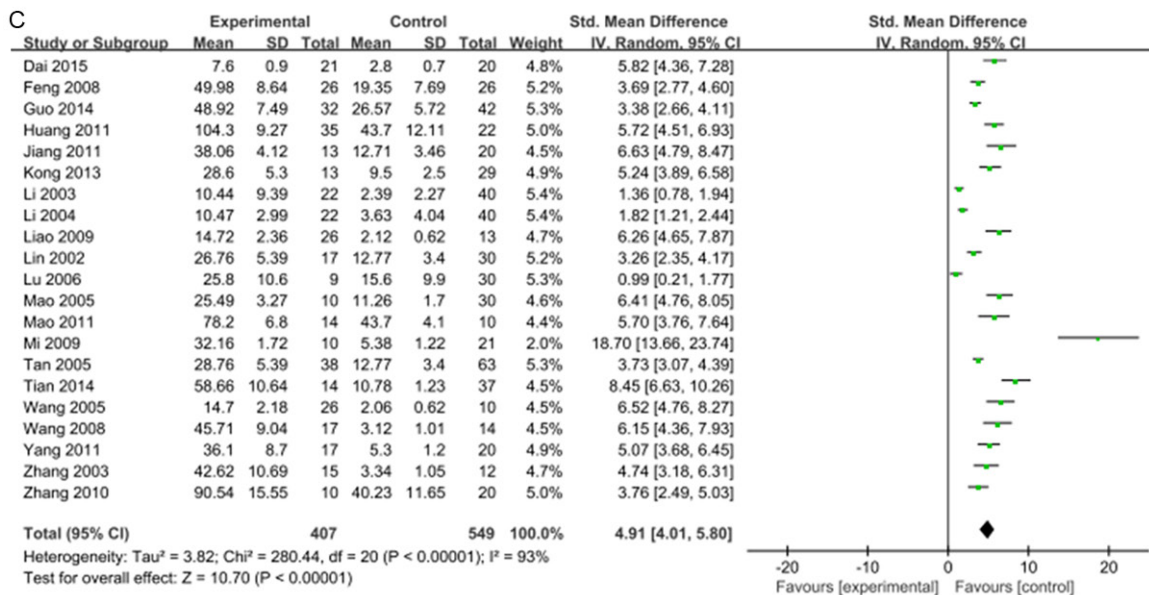
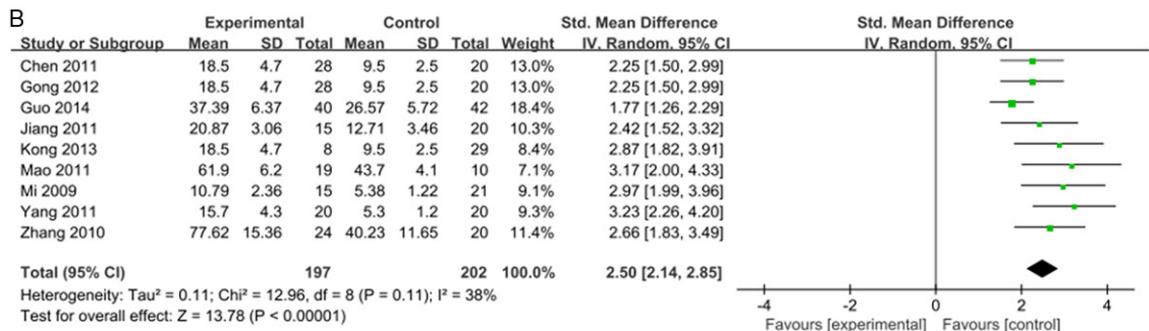
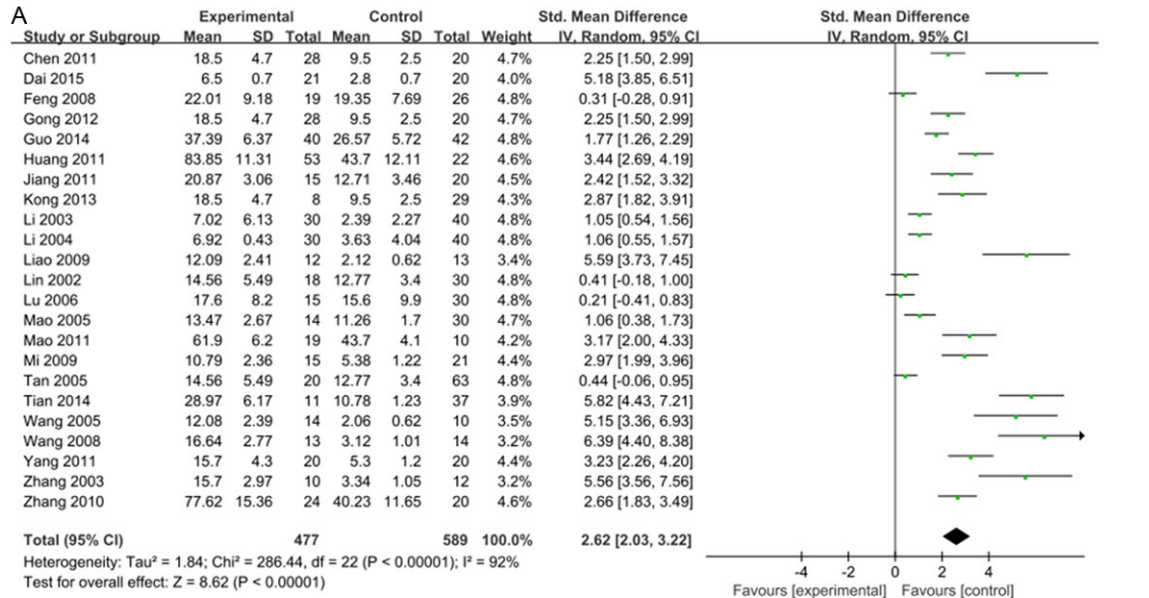
As the summary of results displayed in **Figure 2A, 2C, 2E** (mild, moderate, severe), the total value of Std. Mean. Difference was 2.62, 4.91, 6.55, 95% CI [2.03, 3.22], [4.01, 5.80], [5.46, 7.64] and $P < 0.01$ suggests a significant increase of serum IL-6 in newborns with different state of newborns with HIE. Included studies with homogeneity displayed in **Figure 2B, 2D, 2F** (mild, moderate, severe), ($I^2 = 38\%$, 36% , $43\% < 50\%$), with the value of Std. Mean. Difference 2.50, 3.77, 5.52, 95% CI [2.14, 2.85], [3.43, 4.12], [4.93, 6.12] and $P < 0.01$,

we draw similar conclusion that serum IL-6 might be correlate with occurrence of HIE.

Association of serum TNF- α level of newborns with hypoxia-ischemic encephalopathy in different state of illness

As the summary of results displayed in **Figure 3A, 3C, 3E** (mild, moderate, severe), the total value of Std. Mean. Difference was 3.53, 6.29, 8.35, 95% CI [2.39, 4.67], [4.55, 8.02], [5.92, 10.98] and $P < 0.01$ suggests a significant increase of serum TNF- α in newborns with different state of newborns with HIE. Included studies with homogeneity displayed in **Figure 3B, 3D, 3F** (mild, moderate, severe), ($I^2 = 37\%$,

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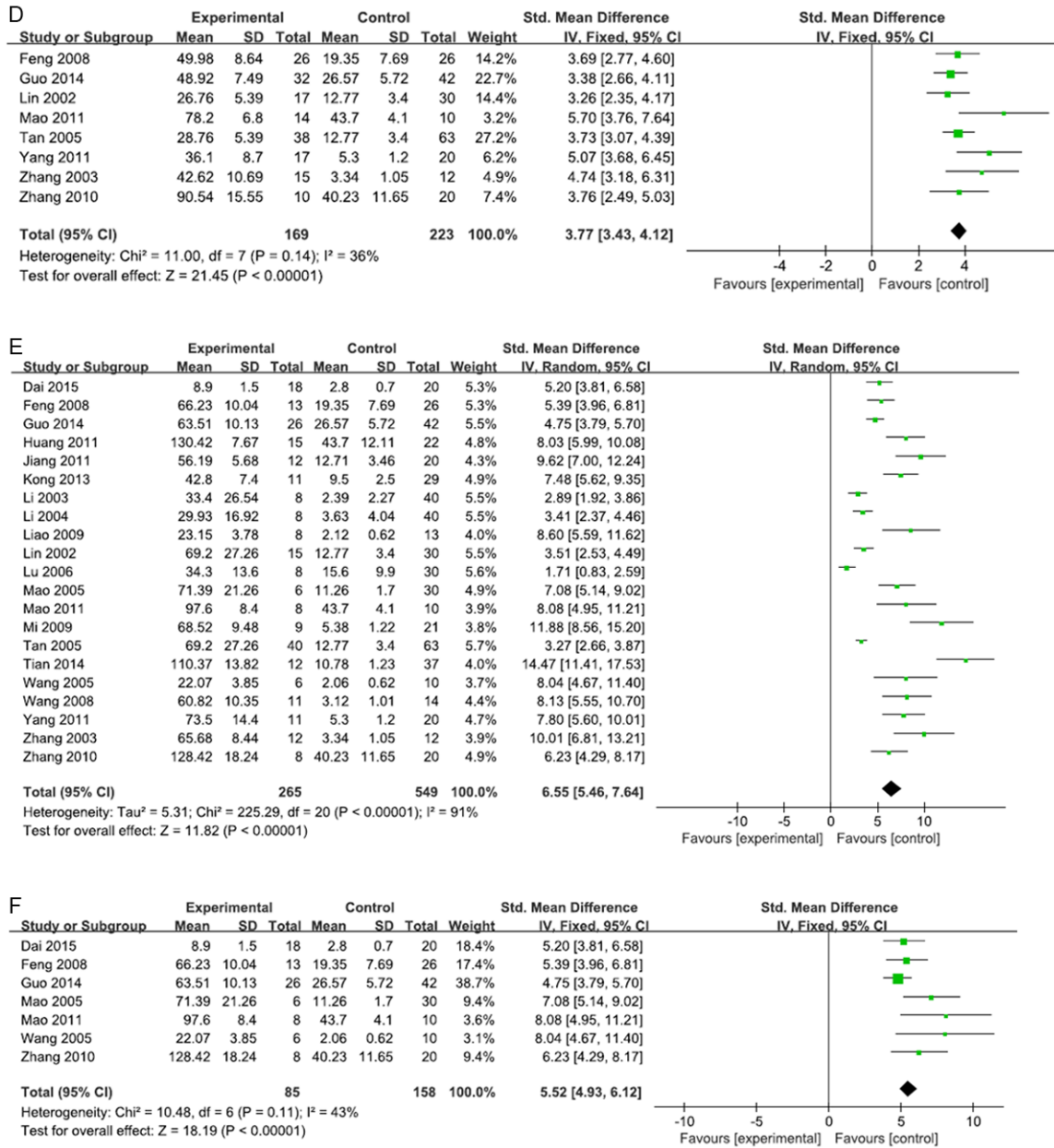


Figure 2. Association of serum IL-6 with different state of neonatal HIE.

0%, 0%<50%), with the value of Std. Mean Difference 4.00, 4.75, 8.24, 95% CI [3.60, 4.40], [4.07, 5.43], [7.38, 9.11] and $P < 0.01$, we draw similar conclusion that elevated serum TNF- α might be correlate with occurrence of HIE.

Sensitivity analysis

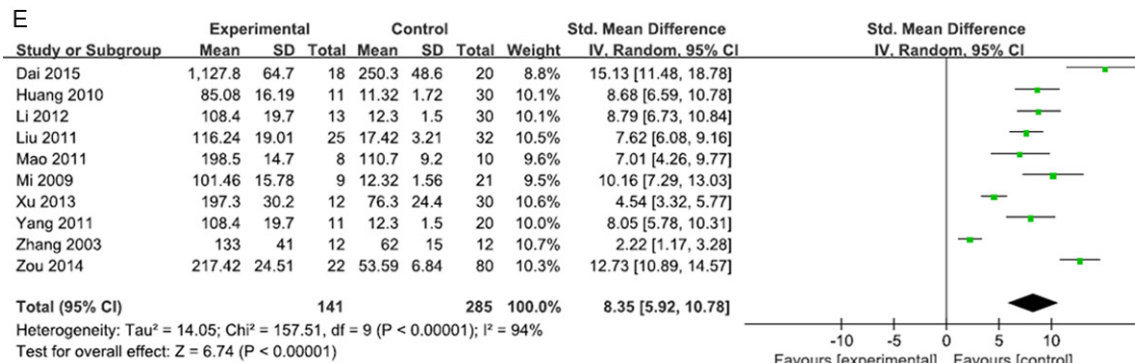
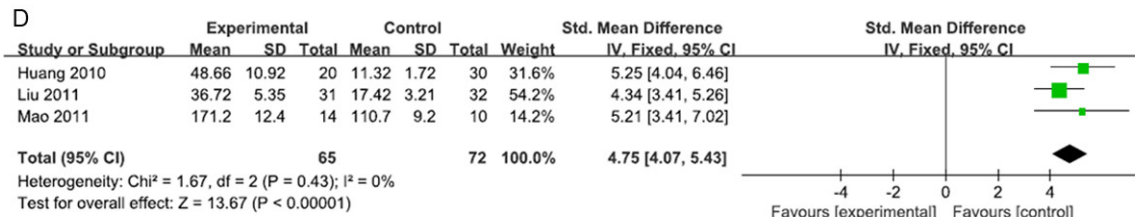
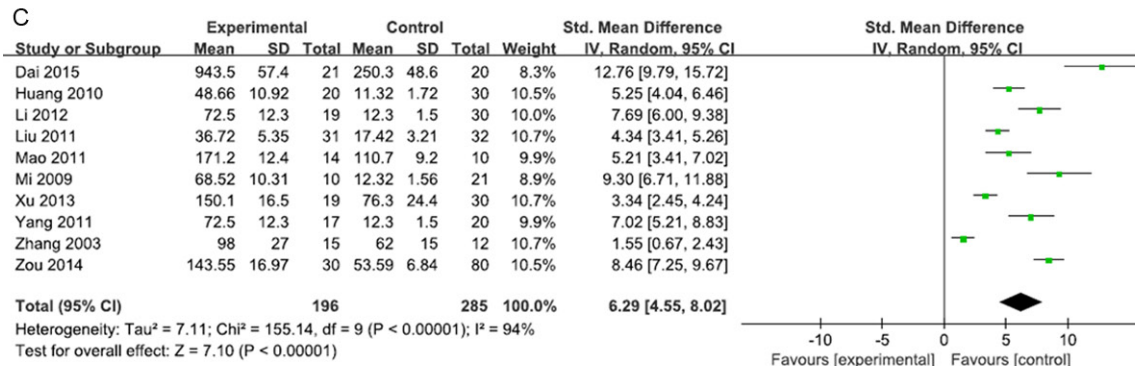
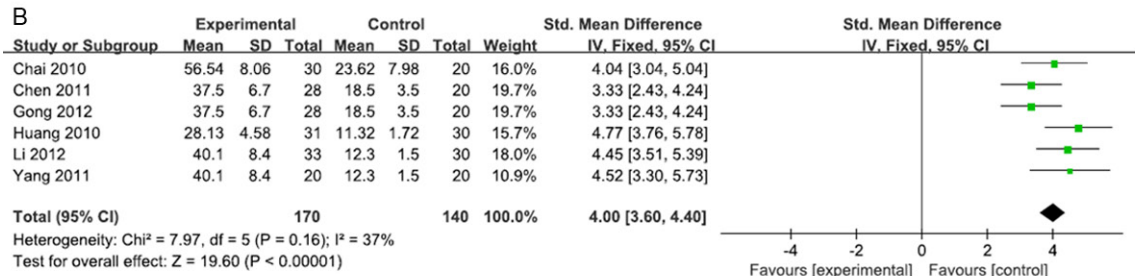
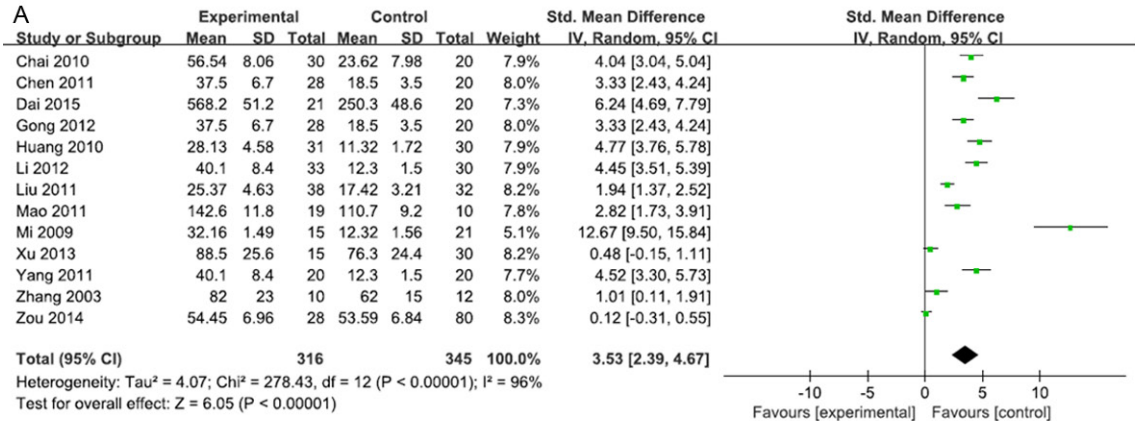
To assess the stability of the results from meta-analysis, we performed a one-study-removed sensitivity analysis for the dominant model.

Statistically insignificant results ($P > 0.05$) were observed after sequentially excluding each of the other studies indicated the stability of established model.

Evaluation of publication bias

Publication bias was tested by funnel plot. The funnel plot is shown in **Figure 4** and publication bias was observed for the overall population. We speculated that random controlled trials with positive results are more likely to be pub-

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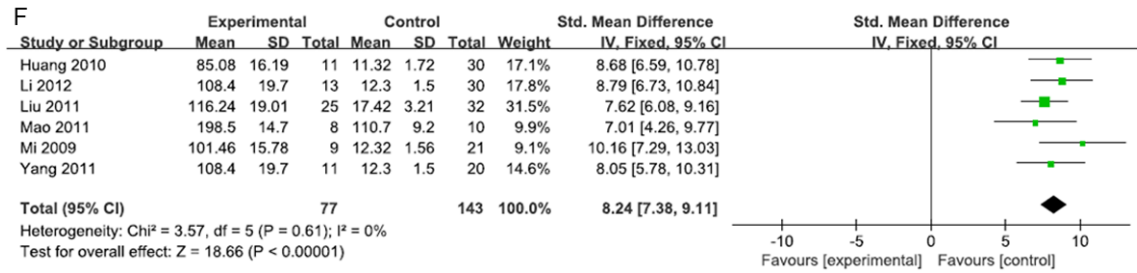


Figure 3. Association of serum TNF- α with different state of neonatal HIE.

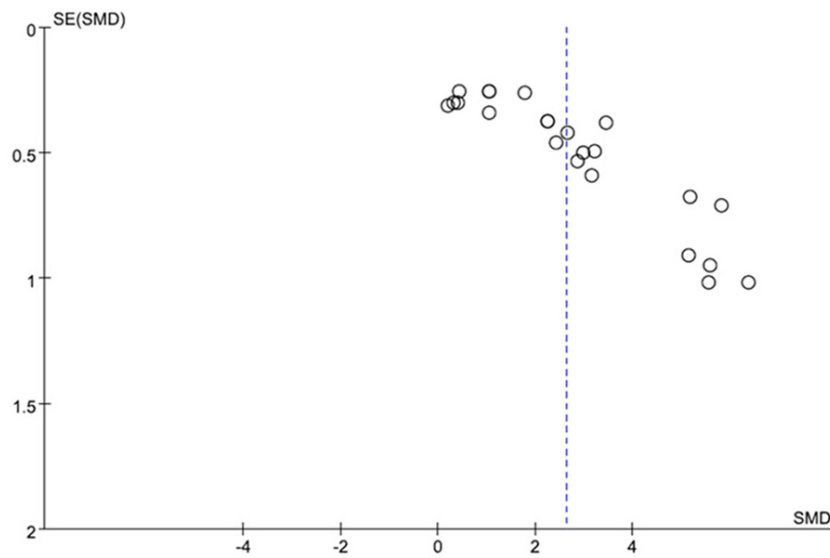


Figure 4. Funnel plot for publication bias in included studies.

By using meta-analysis, a powerful tool which can make combination of results in different randomized controlled studies, 29 clinical trials with 1618 HIE newborns and 790 healthy controls are included to investigate the potential role of IL-6 and TNF- α in different states of hypoxic-ischemic encephalopathy in the present study. Our results showed a significant increase of serum IL-6 and TNF- α level in neonatal HIE ($P < 0.01$). The sample cases are large (>1000), so we considered the

conclusion drawn from our meta-analysis credible.

conclusion drawn from our meta-analysis credible.

Discussion

Hypoxic-ischemic induced brain injury in the infants and neonates is a major cause of mortality and chronic long-term disability in survivors. Animal models have demonstrated that inflammation cytokines take an active part in neonatal hypoxic-ischemic encephalopathy (HIE) [38, 39], IL-6 and TNF- α contribute greatly to brain injury in perinatal asphyxia [40, 41]. It was reported that in newborn rats with hypoxia-ischemia, plasma IL-6 level reached to peak level 6 hours after the insult and the level of TNF- α , on the other hand, increased immediately after hypoxia insults [42, 43]. However, due to limited clinical samples, whether up-regulated serum IL-6 and TNF- α is associated with newborn infants with HIE remains inconclusive.

Our study first revealed the abnormal expression of IL-6 and TNF- α in clinical sample in different states of neonatal hypoxic-ischemic encephalopathy (HIE), our results showed that both serum IL-6 and TNF- α up-regulated in mild, moderate and severe neonatal HIE. With the aggravation of illness, the value of serum IL-6 and TNF- α increased accordingly. Therefore, the results suggest that inflammatory cytokines might not only orchestrate cerebral in-situ inflammatory response but also contribute to exaggerating brain injury. Thus, the value of inflammatory cytokine could be a valuable reference for the state of neonatal HIE.

Not only IL-6 and TNF- α in serum but also in plasma and cerebral flow increases in neonatal HIE, However our efforts are focus on searching related articles only with serum cytokine levels measurement rather than anywhere else. Since

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serum is relatively easier to acquire in clinical patients and the value is accurate by ELISA. Our results provide an effective way of neonatal HIE diagnosis.

Currently, there has not yet any other meta-analysis reported about the relationship of serum IL-6, TNF- α level with occurrence of newborn infants with hypoxic ischemia encephalopathy. Our observation was the first meta-analysis focused on diagnoses of occurrence and states of neonatal HIE using inflammation-related biomarker. Our results highlighted the role of inflammation and immune system in the development of hypoxic ischemia-induced chronic long-term disability and provide a new insight for the therapy of sequelae of neonatal HIE.

Our results also have limitations. The quality of included studies was not as good as a lot of interested information (the gender, state of illness) was missing in some included studies. Moreover, publication bias is obviously seen in funnel plot, thus other relevant published or unpublished studies with null results might have been omitted. Also, the included studies met the inclusion and exclusion criterion was all in Chinese, Therefore, efforts are still needed for further recruiting not only eligible but also high quality studies in other languages.

In conclusion, our study is the first meta-analysis to have assessed the association between serum IL-6, TNF- α level and neonatal HIE risk. The results suggest that up-regulation of serum T IL-6, TNF- α might be associated with an increased hypoxic-ischemic-induced brain injury risk. Larger-scale case controlled studies are required to confirm these findings.

Disclosure of conflict of interest

None.

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