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 encephalopa-thy/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 inflammation 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.



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 ± 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 ± 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 I² was higher than 50%, the results of included studies with homogeneity (I²<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.

Study	Year	Control N	M/F	HIE N	M/F	State of illness (mild/ moderate/severe)	TNFα	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	\checkmark	\checkmark
Zou [11]	2014	80	40/40	80	45/35	28/30/22	\checkmark	
Wang et al. [12]	2005	10	ND	46	35/11	14/26/6		\checkmark
Dai et al. [13]	2015	20	ND	60	32/28	21/21/18	\checkmark	\checkmark
Zhang et al. [14]	2010	20	12/8	42	23/19	24/10/8		\checkmark
Chai et al. [15]	2010	20	11/9	58	32/26	30/ND	\checkmark	
Mi et al. [16]	2009	21	10/11	34	19/15	15/10/9	\checkmark	
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	\checkmark	
Liu [19]	2011	32	18/14	94	51/43	38/31/25	\checkmark	
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	\checkmark	
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	\checkmark	
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	\checkmark	
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		\checkmark
Xu et al. [36]	2013	30	18/12	46	27/19	15/19/12	\checkmark	
Huang et al. [37]	2010	30	16/14	62	36/26	31/20/11		

 Table 1. Study characteristics of included studies

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), (I²=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), (I²=37%,

Meta-analysis of serum IL-6 and TNF- $\!\alpha$ in the diagnosis of neonatal HIE

A	Exp	eriment	tal	c	ontrol			Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Chen 2011	18.5	4.7	28	9.5	2.5	20	4.7%	2.25 [1.50, 2.99]	
Dai 2015	6.5	0.7	21	2.8	0.7	20	4.0%	5.18 [3.85, 6.51]	
Feng 2008	22.01	9.18	19	19.35	7.69	26	4.8%	0.31 [-0.28, 0.91]	
Gong 2012	18.5	4.7	28	9.5	2.5	20	4.7%	2.25 [1.50, 2.99]	
Guo 2014	37.39	6.37	40	26.57	5.72	42	4.8%	1.77 [1.26, 2.29]	-
Huang 2011	83.85	11.31	53	43.7	12.11	22	4.6%	3.44 [2.69, 4.19]	
Jiang 2011	20.87	3.06	15	12.71	3.46	20	4.5%	2.42 [1.52, 3.32]	
Kong 2013	18.5	4.7	8	9.5	2.5	29	4.3%	2.87 [1.82, 3.91]	
Li 2003	7.02	6.13	30	2.39	2.27	40	4.8%	1.05 [0.54, 1.56]	
Li 2004	6.92	0.43	30	3.63	4.04	40	4.8%	1.06 [0.55, 1.57]	
Liao 2009	12.09	2.41	12	2.12	0.62	13	3.4%	5.59 [3.73, 7.45]	
Lin 2002	14.56	5.49	18	12.77	3.4	30	4.8%	0.41 [-0.18, 1.00]	<u>+-</u>
Lu 2006	17.6	8.2	15	15.6	9.9	30	4.8%	0.21 [-0.41, 0.83]	+-
Mao 2005	13.47	2.67	14	11.26	1.7	30	4.7%	1.06 [0.38, 1.73]	
Mao 2011	61.9	6.2	19	43.7	4.1	10	4.2%	3.17 [2.00, 4.33]	
Mi 2009	10.79	2.36	15	5.38	1.22	21	4.4%	2.97 [1.99, 3.96]	
Tan 2005	14.56	5.49	20	12.77	3.4	63	4.8%	0.44 [-0.06, 0.95]	
Tian 2014	28.97	6.17	11	10.78	1.23	37	3.9%	5.82 [4.43, 7.21]	
Wang 2005	12.08	2.39	14	2.06	0.62	10	3.5%	5.15 [3.36, 6.93]	
Wang 2008	16.64	2.77	13	3.12	1.01	14	3.2%	6.39 [4.40, 8.38]	
Yang 2011	15.7	4.3	20	5.3	1.2	20	4.4%	3.23 [2.26, 4.20]	
Zhang 2003	15.7	2.97	10	3.34	1.05	12	3.2%	5.56 [3.56, 7.56]	
Zhang 2010	77.62	15.36	24	40.23	11.65	20	4.6%	2.66 [1.83, 3.49]	
Total (95% CI)			477			589	100.0%	2.62 [2.03, 3.22]	•
Heterogeneity: Tau ² =	1.84; Ch	ni² = 286	6.44, df	= 22 (P	< 0.00	001); l ²	= 92%	-	
			000041	•					-4 -2 0 2 4

Test for overall effect: Z = 8.62 (P < 0.00001)



В	Exp	eriment	al	c	ontrol			Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV. Random, 95% CI	IV. Random, 95% CI
Chen 2011	18.5	4.7	28	9.5	2.5	20	13.0%	2.25 [1.50, 2.99]	
Gong 2012	18.5	4.7	28	9.5	2.5	20	13.0%	2.25 [1.50, 2.99]	
Guo 2014	37.39	6.37	40	26.57	5.72	42	18.4%	1.77 [1.26, 2.29]	
Jiang 2011	20.87	3.06	15	12.71	3.46	20	10.3%	2.42 [1.52, 3.32]	
Kong 2013	18.5	4.7	8	9.5	2.5	29	8.4%	2.87 [1.82, 3.91]	
Mao 2011	61.9	6.2	19	43.7	4.1	10	7.1%	3.17 [2.00, 4.33]	
Mi 2009	10.79	2.36	15	5.38	1.22	21	9.1%	2.97 [1.99, 3.96]	
Yang 2011	15.7	4.3	20	5.3	1.2	20	9.3%	3.23 [2.26, 4.20]	
Zhang 2010	77.62	15.36	24	40.23	11.65	20	11.4%	2.66 [1.83, 3.49]	
Total (95% CI)			197			202	100.0%	2.50 [2.14, 2.85]	•
Heterogeneity: Tau ² =	0.11; Cł	ni² = 12.	96, df =	= 8 (P =	0.11); l ²	2 = 38%			
Test for overall effect:	Z = 13.7	8 (P < 0	0.00001	1)					Favours [experimental] Favours [control]

С	Exp	erimen	tal	Control		:	Std. Mean Difference	Std. Mean Difference		
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV. Random, 95% CI	
Dai 2015	7.6	0.9	21	2.8	0.7	20	4.8%	5.82 [4.36, 7.28]		
Feng 2008	49.98	8.64	26	19.35	7.69	26	5.2%	3.69 [2.77, 4.60]	-	
Guo 2014	48.92	7.49	32	26.57	5.72	42	5.3%	3.38 [2.66, 4.11]	· ·	
Huang 2011	104.3	9.27	35	43.7	12.11	22	5.0%	5.72 [4.51, 6.93]	-	
Jiang 2011	38.06	4.12	13	12.71	3.46	20	4.5%	6.63 [4.79, 8.47]	-	
Kong 2013	28.6	5.3	13	9.5	2.5	29	4.9%	5.24 [3.89, 6.58]		
Li 2003	10.44	9.39	22	2.39	2.27	40	5.4%	1.36 [0.78, 1.94]	-	
Li 2004	10.47	2.99	22	3.63	4.04	40	5.4%	1.82 [1.21, 2.44]	•	
Liao 2009	14.72	2.36	26	2.12	0.62	13	4.7%	6.26 [4.65, 7.87]		
Lin 2002	26.76	5.39	17	12.77	3.4	30	5.2%	3.26 [2.35, 4.17]	-	
Lu 2006	25.8	10.6	9	15.6	9.9	30	5.3%	0.99 [0.21, 1.77]	-	
Mao 2005	25.49	3.27	10	11.26	1.7	30	4.6%	6.41 [4.76, 8.05]		
Mao 2011	78.2	6.8	14	43.7	4.1	10	4.4%	5.70 [3.76, 7.64]		
Mi 2009	32.16	1.72	10	5.38	1.22	21	2.0%	18.70 [13.66, 23.74]		
Tan 2005	28.76	5.39	38	12.77	3.4	63	5.3%	3.73 [3.07, 4.39]	-	
Tian 2014	58.66	10.64	14	10.78	1.23	37	4.5%	8.45 [6.63, 10.26]		
Wang 2005	14.7	2.18	26	2.06	0.62	10	4.5%	6.52 [4.76, 8.27]		
Wang 2008	45.71	9.04	17	3.12	1.01	14	4.5%	6.15 [4.36, 7.93]	-	
Yang 2011	36.1	8.7	17	5.3	1.2	20	4.9%	5.07 [3.68, 6.45]	-	
Zhang 2003	42.62	10.69	15	3.34	1.05	12	4.7%	4.74 [3.18, 6.31]		
Zhang 2010	90.54	15.55	10	40.23	11.65	20	5.0%	3.76 [2.49, 5.03]	-	
Total (95% CI)			407			549	100.0%	4.91 [4.01, 5.80]	•	
Heterogeneity: Tau ² =	3.82; Ch	ni² = 280	0.44, df	= 20 (P	< 0.00	001); l²	= 93%	-		
Test for overall effect:	Z = 10.7	0 (P < 0	0.00001)					Favours [experimental] Favours [control]	

D	Experimental		c	ontrol			Std. Mean Difference	Std. Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% Cl	IV, Fixed, 95% CI
Feng 2008	49.98	8.64	26	19.35	7.69	26	14.2%	3.69 [2.77, 4.60]	
Guo 2014	48.92	7.49	32	26.57	5.72	42	22.7%	3.38 [2.66, 4.11]	
Lin 2002	26.76	5.39	17	12.77	3.4	30	14.4%	3.26 [2.35, 4.17]	
Mao 2011	78.2	6.8	14	43.7	4.1	10	3.2%	5.70 [3.76, 7.64]	
Tan 2005	28.76	5.39	38	12.77	3.4	63	27.2%	3.73 [3.07, 4.39]	
Yang 2011	36.1	8.7	17	5.3	1.2	20	6.2%	5.07 [3.68, 6.45]	
Zhang 2003	42.62	10.69	15	3.34	1.05	12	4.9%	4.74 [3.18, 6.31]	
Zhang 2010	90.54	15.55	10	40.23	11.65	20	7.4%	3.76 [2.49, 5.03]	
Total (95% CI)			169			223	100.0%	3.77 [3.43, 4.12]	•
Heterogeneity: Chi ² =	11.00, di	f = 7 (P	= 0.14)	; I ² = 36	%			_	
Test for overall effect:	Z = 21.4	5 (P < 0	0.00001	1)					-4 -2 0 2 4 Favours [experimental] Favours [control]

E	Experimental		Control				Std. Mean Difference	Std. Mean Difference		
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% Cl	
Dai 2015	8.9	1.5	18	2.8	0.7	20	5.3%	5.20 [3.81, 6.58]		
Feng 2008	66.23	10.04	13	19.35	7.69	26	5.3%	5.39 [3.96, 6.81]		
Guo 2014	63.51	10.13	26	26.57	5.72	42	5.5%	4.75 [3.79, 5.70]	-	
Huang 2011	130.42	7.67	15	43.7	12.11	22	4.8%	8.03 [5.99, 10.08]		
Jiang 2011	56.19	5.68	12	12.71	3.46	20	4.3%	9.62 [7.00, 12.24]		
Kong 2013	42.8	7.4	11	9.5	2.5	29	4.9%	7.48 [5.62, 9.35]		
Li 2003	33.4	26.54	8	2.39	2.27	40	5.5%	2.89 [1.92, 3.86]	-	
Li 2004	29.93	16.92	8	3.63	4.04	40	5.5%	3.41 [2.37, 4.46]		
Liao 2009	23.15	3.78	8	2.12	0.62	13	4.0%	8.60 [5.59, 11.62]		
Lin 2002	69.2	27.26	15	12.77	3.4	30	5.5%	3.51 [2.53, 4.49]		
Lu 2006	34.3	13.6	8	15.6	9.9	30	5.6%	1.71 [0.83, 2.59]	-	
Mao 2005	71.39	21.26	6	11.26	1.7	30	4.9%	7.08 [5.14, 9.02]		
Mao 2011	97.6	8.4	8	43.7	4.1	10	3.9%	8.08 [4.95, 11.21]		
Mi 2009	68.52	9.48	9	5.38	1.22	21	3.8%	11.88 [8.56, 15.20]		
Tan 2005	69.2	27.26	40	12.77	3.4	63	5.7%	3.27 [2.66, 3.87]	-	
Tian 2014	110.37	13.82	12	10.78	1.23	37	4.0%	14.47 [11.41, 17.53]		
Wang 2005	22.07	3.85	6	2.06	0.62	10	3.7%	8.04 [4.67, 11.40]		
Wang 2008	60.82	10.35	11	3.12	1.01	14	4.4%	8.13 [5.55, 10.70]		
Yang 2011	73.5	14.4	11	5.3	1.2	20	4.7%	7.80 [5.60, 10.01]		
Zhang 2003	65.68	8.44	12	3.34	1.05	12	3.9%	10.01 [6.81, 13.21]		
Zhang 2010	128.42	18.24	8	40.23	11.65	20	4.9%	6.23 [4.29, 8.17]		
Total (95% CI)			265			549	100.0%	6.55 [5.46, 7.64]	◆	
Heterogeneity: Tau ² =	5.31; Chi	² = 225.	29, df =	= 20 (P ·	< 0.000	01); ² =	91%	-	10 5 0 5 10	
Test for overall effect:	Z = 11.82	2 (P < 0.	.00001)						Favours [experimental] Favours [control]	

F	Expe	eriment	al	c	ontrol			Std. Mean Difference	Std.	Mean Differe	nce	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI		, Fixed, 95%	CI	
Dai 2015	8.9	1.5	18	2.8	0.7	20	18.4%	5.20 [3.81, 6.58]				
Feng 2008	66.23	10.04	13	19.35	7.69	26	17.4%	5.39 [3.96, 6.81]				
Guo 2014	63.51	10.13	26	26.57	5.72	42	38.7%	4.75 [3.79, 5.70]				
Mao 2005	71.39	21.26	6	11.26	1.7	30	9.4%	7.08 [5.14, 9.02]				
Mao 2011	97.6	8.4	8	43.7	4.1	10	3.6%	8.08 [4.95, 11.21]				-
Wang 2005	22.07	3.85	6	2.06	0.62	10	3.1%	8.04 [4.67, 11.40]				-
Zhang 2010	128.42	18.24	8	40.23	11.65	20	9.4%	6.23 [4.29, 8.17]			-	-
Total (95% CI)			85			158	100.0%	5.52 [4.93, 6.12]			•	
Heterogeneity: Chi2 =	10.48, df	= 6 (P =	= 0.11);	l ² = 43%	6			-			-	
Test for overall effect:	Z = 18.19) (P < 0.	.00001))					-10 -5 Favours (experim	ental] Favou	5 rs [control]	10

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 metaanalysis, 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-

Meta-analysis of serum IL-6 and TNF- α in the diagnosis of neonatal HIE

A	Exp	erimer	ntal	С	ontrol			Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% Cl
Chai 2010	56.54	8.06	30	23.62	7.98	20	7.9%	4.04 [3.04, 5.04]	-
Chen 2011	37.5	6.7	28	18.5	3.5	20	8.0%	3.33 [2.43, 4.24]	-
Dai 2015	568.2	51.2	21	250.3	48.6	20	7.3%	6.24 [4.69, 7.79]	
Gong 2012	37.5	6.7	28	18.5	3.5	20	8.0%	3.33 [2.43, 4.24]	-
Huang 2010	28.13	4.58	31	11.32	1.72	30	7.9%	4.77 [3.76, 5.78]	-
Li 2012	40.1	8.4	33	12.3	1.5	30	7.9%	4.45 [3.51, 5.39]	
Liu 2011	25.37	4.63	38	17.42	3.21	32	8.2%	1.94 [1.37, 2.52]	-
Mao 2011	142.6	11.8	19	110.7	9.2	10	7.8%	2.82 [1.73, 3.91]	
Mi 2009	32.16	1.49	15	12.32	1.56	21	5.1%	12.67 [9.50, 15.84]	
Xu 2013	88.5	25.6	15	76.3	24.4	30	8.2%	0.48 [-0.15, 1.11]	-
Yang 2011	40.1	8.4	20	12.3	1.5	20	7.7%	4.52 [3.30, 5.73]	
Zhang 2003	82	23	10	62	15	12	8.0%	1.01 [0.11, 1.91]	-
Zou 2014	54.45	6.96	28	53.59	6.84	80	8.3%	0.12 [-0.31, 0.55]	Ť
Total (95% CI)			316			345	100.0%	3.53 [2.39, 4.67]	•
Heterogeneity: Tau ² =	4.07; CI	hi² = 27	78.43, 0	if = 12 (P < 0.0	00001);	l² = 96%		10 5 0 5 10
Test for overall effect:	Z = 6.05	5 (P < 0	0.00001)					Favours [experimental] Favours [control]
В	Expe	erimen	tal	C	ontrol			Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI	IV, Fixed, 95% CI
Chai 2010	56.54	8.06	30	23.62	7.98	20	16.0%	4.04 [3.04, 5.04]	_
Chen 2011	37.5	6.7	28	18.5	3.5	20	19.7%	3.33 [2.43, 4.24]	
Gong 2012	37.5	6.7	28	18.5	3.5	20	19.7%	3.33 [2.43, 4.24]	

4.77 [3.76, 5.78]

4.45 [3.51, 5.39]

4.52 [3.30, 5.73] 4.00 [3.60, 4.40]

Yang 2011	40.1	8.4	20	12.3	1.5	20	10.9%				
Total (95% CI)			170			140	100.0%				
Heterogeneity: Chi ² = 7.97, df = 5 (P = 0.16); l ² = 37%											
Test for overall effect: Z = 19.60 (P < 0.00001)											

33 12.3 1.5

31 11.32 1.72

30 15.7%

30 18.0%

28.13 4.58

40.1 8.4

Test for overall effect: Z = 7.10 (P < 0.00001)

Huang 2010

Li 2012



С							12	Difference	Chil Mana Difference
	Expe	eriment	ai	C	ontrol			Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Dai 2015	943.5	57.4	21	250.3	48.6	20	8.3%	12.76 [9.79, 15.72]	
Huang 2010	48.66	10.92	20	11.32	1.72	30	10.5%	5.25 [4.04, 6.46]	
Li 2012	72.5	12.3	19	12.3	1.5	30	10.0%	7.69 [6.00, 9.38]	
Liu 2011	36.72	5.35	31	17.42	3.21	32	10.7%	4.34 [3.41, 5.26]	
Mao 2011	171.2	12.4	14	110.7	9.2	10	9.9%	5.21 [3.41, 7.02]	
Mi 2009	68.52	10.31	10	12.32	1.56	21	8.9%	9.30 [6.71, 11.88]	
Xu 2013	150.1	16.5	19	76.3	24.4	30	10.7%	3.34 [2.45, 4.24]	
Yang 2011	72.5	12.3	17	12.3	1.5	20	9.9%	7.02 [5.21, 8.83]	
Zhang 2003	98	27	15	62	15	12	10.7%	1.55 [0.67, 2.43]	-
Zou 2014	143.55	16.97	30	53.59	6.84	80	10.5%	8.46 [7.25, 9.67]	
Total (95% CI)			196			285	100.0%	6.29 [4.55, 8.02]	•
Heterogeneity: Tau ² =	7.11; Chi	² = 155.	14, df =	= 9 (P <	0.000	01); l ² =	94%	-	
			-10 -5 0 5 10						

-10 -5 0 5 10 Favours [experimental] Favours [control]

D	Experimental		Experimental Control					Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI	IV, Fixed, 95% CI
Huang 2010	48.66	10.92	20	11.32	1.72	30	31.6%	5.25 [4.04, 6.46]	
Liu 2011	36.72	5.35	31	17.42	3.21	32	54.2%	4.34 [3.41, 5.26]	
Mao 2011	171.2	12.4	14	110.7	9.2	10	14.2%	5.21 [3.41, 7.02]	
Total (95% CI)			65			72	100.0%	4.75 [4.07, 5.43]	· · · · · · · · · · · · · · · · · · ·
Heterogeneity: Chi ² =	1.67, df :	= 2 (P =	0.43);	$ ^2 = 0\%$				-	-4 -2 0 2 4
Test for overall effect:	Z = 13.6	7 (P < 0	0.00001	1)					Favours [experimental] Favours [control]

Е Experimental Control Std. Mean Difference Std. Mean Difference Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% Cl IV, Random, 95% CI 20 15.13 [11.48, 18.78] Dai 2015 1,127.8 64.7 18 250.3 48.6 8.8% Huang 2010 85.08 16.19 11 11.32 1.72 30 10.1% 8.68 [6.59, 10.78] Li 2012 108.4 19.7 13 12.3 1.5 30 10.1% 8.79 [6.73, 10.84] Liu 2011 116.24 19.01 25 17.42 3.21 32 10.5% 7.62 [6.08, 9.16] Mao 2011 198.5 14.7 8 110.7 9.2 10 9.6% 7.01 [4.26, 9.77] Mi 2009 101.46 15.78 9 12.32 1.56 21 9.5% 10.16 [7.29, 13.03] Xu 2013 197.3 30.2 12 76.3 24.4 30 10.6% 4.54 [3.32, 5.77] Yang 2011 108.4 19.7 11 12.3 1.5 20 10.0% 8.05 [5.78, 10.31] Zhang 2003 133 41 12 62 15 12 10.7% 2.22 [1.17, 3.28] Zou 2014 217.42 24.51 22 53.59 6.84 80 10.3% 12.73 [10.89, 14.57] Total (95% CI) 141 285 100.0% 8.35 [5.92, 10.78] Heterogeneity: Tau² = 14.05; Chi² = 157.51, df = 9 (P < 0.00001); |² = 94% -10 -5 10 0 5

Test for overall effect: Z = 6.74 (P < 0.00001)





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



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

lished and all of the included studies in this meta-analysis are in Chinese rather than any other countries.

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 hypoxiaischemia, 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.

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.

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

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 metaanalysis 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 metaanalysis focused on diagnoses of occurrence and states of neonatal HIE using inflammationrelated 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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