Serum levels of S-100β correlate with the clinical status and severity of hypoxic-ischemic encephalopathy in neonates

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ABSTRACT. The clinical significance of serum S-100β levels in neonates with hypoxic-ischemic encephalopathy (HIE), as a reference index to assess HIE severity, was evaluated in this study. On the basis of our strict inclusion and exclusion criteria, relevant high-quality case-control studies reporting the association between HIE and S-100β protein were selected from electronic database searches. The STATA version 12.0 software was used for the statistical analyses. The database search initially retrieved 93 studies (37 in English and 56 in Chinese), and following a multistep screening process, 13 high-quality studies were eventually included in our meta-analysis. The 13 case-control studies included a total of 646 HIE neonates and 381 healthy controls. The results of this meta-analysis revealed that serum S-100β levels in mild, moderate, and severe HIE

neonates were significantly higher than those in healthy controls, and the differences were statistically significant. Importantly, the serum S-100β levels increased incrementally with HIE severity. Our results support the hypothesis that S-100β is an important biological indicator of HIE and serum S-100β levels can be used as a reference index to assess HIE severity.

Key words: S-100β protein; Hypoxic-ischemic encephalopathy; Meta-analysis

INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE) is a clinical syndrome characterized by the appearance of neurological symptoms, including a low level of consciousness, hypotonia, and difficulty in initiating and maintaining respiration. It is the leading cause of mortality and long-term morbidity in neonates worldwide (Liu et al., 2011b). HIE results from a combination of reduced perfusion and/or decreased cerebral oxygenation, resulting in debilitating disorders such as cerebral palsy, epilepsy, mental retardation, and other sensorineural impairments and disabilities (Jacobs et al., 2011). It can occur during conception or parturition, and its severity is classified as mild, moderate, and severe (Perlman and Shah, 2011). It has an incidence rate of 0.5 to 1 per 1000 infants worldwide, and the mortality rate is 20-37% for moderate HIE and 50-89% for severe HIE (Lynch et al., 2012). HIE is multifactorial in neonates and mainly involves perinatal asphyxia, and several other etiologies such as infection, uterine rupture, maternal malnutrition, cord prolapse, and placental abruption also contribute to HIE (Pimentel-Coelho et al., 2012). Studies show that 30% of moderate HIE survivors and 100% of severe HIE survivors show long-term neurological disability because clinical treatment and prevention strategies for HIE are yet to be established, indicating a significant need for focused research in this area (Kim et al., 2013; Taniguchi et al., 2014). Currently, the S-100β protein biomarker level is suggested as a potential reference index for the diagnosis and treatment of HIE (Douglas-Escobar et al., 2010).

The S100 proteins are a family of Ca^{2+}-binding cytosolic proteins that are expressed in multiple cell types and contain 2 subunits, i.e., α and β; these proteins are involved in cell cycle progression, cell development, cytoskeletal-membrane interactions, and cell differentiation (McKiernan et al., 2011; Sun et al., 2012). The S-100β is a small molecule of molecular weight 10.5 kDa and is expressed in glial cells in the nervous system (Modi and Kanungo, 2010). It is secreted into the extracellular space, and is also found in the serum, and is believed to mediate cellular activities in an autocrine, paracrine, or endocrine manner (Marks and Allore, 1990; Patro et al., 2009). The S-100β exerts significant influence on cellular metabolism, Ca^{2+} homeostasis, cytoskeletal modification, cell proliferation, and cell differentiation, mainly by binding to synaptic proteins and inhibiting their phosphorylation (Modi and Kanungo, 2010). This protein is also associated with neurodegenerative diseases, Alzheimer's disease, Down's syndrome, apoptosis, and brain injury (Nogueira et al., 2009). Multiple studies have suggested that the serum S-100β concentration is an important biological indicator of HIE severity (Beharier et al., 2012; Douglas-Escobar and Weiss, 2012b). However, one study could not confirm the relationship between serum S-100β levels and HIE severity (Qian et al., 2009). In light of these conflicting findings, we performed a meta-analysis to investigate the association between HIE and the S-100β protein.

MATERIAL AND METHODS

Literature search

A comprehensive literature search was performed to identify relevant studies published before September 2014. Accordingly, published studies reporting the association between HIE and S-100β protein were retrieved from the following computerized bibliographic databases: PubMed, EBSCO, Ovid, SpringerLink, Wiley, Web of Science, Wanfang, China National Knowledge Infrastructure, and VIP. The highly sensitive search terms included MeSH and Medline medical index terms and free text terms such as "hypoxia-ischemia, brain", "brain hypoxia ischemia", "hypoxic ischemic encephalopathy", "anoxic ischemic encephalopathy", "cerebral hypoxia ischemia", "anoxia ischemia, brain", or "cerebral anoxia ischemia", and each was crossed with the word "S-100β". Additionally, cross-references of key studies were manually searched to identify additional relevant literature.

Inclusion and exclusion criteria

The following inclusion criteria were applied for selecting studies in the current meta-analysis: 1) case-control studies; 2) study subjects including HIE neonates and healthy controls; 3) English or Chinese studies; 4) studies reporting the association between HIE and S-100β; 5) complete data available such as country, ethnicity, publication year, sample size, gestational age, gender, detection method, HIE severity, and S-100β expression levels. The exclusion criteria were as follows: 1) ambiguous diagnosis of study subjects; 2) duplicate publications; 3) incomplete data.

Data extraction

In order to reduce bias and enhance confidence, 2 researchers separately extracted information from the retrieved papers by using a standard data abstraction form and reached a consensus on all the items through discussion and reexamination by multiply researchers. The following relevant data were extracted from the eligible studies: surname and initials of the first author, year of submission, country, language, ethnicity, gestational age, gender, detection method, HIE severity, and S-100β expression levels.

Statistical analysis

Standardized mean difference (SMD) and 95% confidence interval (95%CI) values were calculated for studies investigating S-100β expression, and the Z-test was performed to determine the significance of the overall effect size. This meta-analysis used Cochran's Q-statistic (P < 0.05 was considered to be significant) and the I^2 test to assess heterogeneity among studies (Zintzaras and Ioannidis, 2005a). If significant heterogeneity existed (P < 0.05 or I^2 test exhibited >50%), a random-effect model was used; otherwise, a fixed-effect model was used (Higgins and Thompson, 2002; Zintzaras and Ioannidis, 2005b). Sensitivity analyses were performed to assess the influence of each individual study on the overall estimate, by the sequential removal of individual studies. Furthermore, we used contour-enhanced funnel plots and the Egger linear regression test to detect publication bias (P < 0.05 was considered to

be significant). All tests were two-sided, and all statistical analyses were performed using the STATA software (version 12.0, Stata Corporation, College Station, TX, USA). A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Studies included

A total of 93 studies were initially retrieved through electronic database searches and manual searches. On the basis of the predetermined exclusion criteria, we excluded 4 studies that were duplicates, 4 studies that were letters and reviews, 22 studies that did not include human research, and 25 studies that had no relationship with our research topic. Further, 23 studies were excluded after reading the full text, and 2 studies were excluded because of a lack of highly related data. Finally, 13 case-control studies (Martins et al., 2006; Cheng et al., 2007; Murabayashi et al., 2008; Gazzolo et al., 2004, 2009; Sun et al., 2009; Jin et al., 2011; Liu et al., 2011a; Ding, 2013; Li et al., 2008, 2013; Ma et al., 2013; Nong et al., 2013), published between 2004 and 2013, were incorporated in this meta-analysis. The 13 studies included a total of 646 HIE neonates (150 mild HIE, 106 moderate HIE, and 390 severe HIE neonates) and 381 healthy controls, with sample sizes ranging from 21 to 121. Of the 13 case-control studies, 10 included Asian subjects (specifically, 9 included Chinese subjects and 1 included Japanese subjects) and 3 included Caucasian subjects (specifically, 2 included Italian subjects and 1 included Brazilian subjects). In the studies included, enzyme-linked immunosorbent assay and radioimmunoassay were used for S-100β estimation. The detailed characteristics of the studies included are summarized in Table 1. In the present study, HIE severity was classified according to the clinical staging of HIE.

Table 1. Baseline characteristics of the included studies.

First author	Country	Ethnicity	Gender (M/F)				Age (years)				Method
			Case	Case	Case	Control	Case	Case	Case	Control	
Nong (2013)	China	Asians	8/7	7/4	5/3	9/7	271.83 ± 15.11	272.05 ± 14.00	273.77 ± 16.05	273.69 ± 13.87	ELISA
Ma (2013)	China	Asians	9/6	-	10/11	9/7	271 ± 10	-	281 ± 8	287 ± 7	ELISA
Li (2013)	China	Asians	10/11	11/9	11/12	15/15	-	-	-	-	RIA
Ding (2013)	China	Asians	-	-	25/23	13/12	-	-	264.74 ± 4.56	266.98 ± 5.11	ELISA
Liu (2011a)	China	Asians	-	-	-	-	-	-	-	-	ELISA
Jin (2011)	China	Asians	15/18	11/10	9/12	10/11	273 ± 11.2	270.2 ± 22.61	270.2 ± 22.61	270.9 ± 6.09	ELISA
Sun (2009)	China	Asians	-	-	-	-	-	-	-	-	ELISA
Gazzolo (2009)	Italy	Caucasians	-	-	37/34	25/23	-	-	-	-	ELISA
Li (2008)	China	Asians	72/32			11/6	-	-	-	-	ELISA
Murabayashi (2008)	Japan	Asians	-	-	-	-	-	-	-	-	ELISA
Cheng (2007)	China	Asians	-	-	-	-	275.8 ± 9.1			281.4 ± 6.3	ELISA
Martins (2006)	Brazil	Caucasians	-	-	-	-	-	-	-	-	ELISA
Gazzolo (2004)	Italy	Caucasians	-	-	-	-	-	-	-	-	ELISA

M = male; F = female; ELISA = enzyme-linked immunosorbent assay; RIA = radioimmunoassay.

Pooled outcome of the meta-analysis

All 13 studies reported differences in the S-100β expression levels between severe HIE neonates and healthy controls. The random-effect model was used because of the heterogeneity

of the studies included (I^2 = 95%, P < 0.001). The results of our meta-analysis showed significantly higher S-100β expression levels in severe HIE neonates than in healthy controls (SMD = 1.57, 95%CI = 1.38-1.75, P < 0.001) (Figure 1A). Seven of the 13 studies reported the S-100β expression levels in mild HIE neonates. The random-effect model was used because of the heterogeneity of these studies (I^2 = 84.6%, P < 0.001). The results showed significantly higher S-100β expression levels in mild HIE neonates than in healthy controls (SMD = 1.42, 95%CI = 1.15-1.69, P < 0.001) (Figure 1B). Five studies reported differences in the S-100β expression levels between moderate HIE neonates and healthy controls. Furthermore, the random-effect model was used because of the significant heterogeneity of these studies (I^2 = 80.5%, P < 0.001). The results showed significantly higher S-100β expression levels in moderate HIE neonates than in healthy controls (SMD = 3.08, 95%CI = 2.67-3.48, P < 0.001) (Figure 1C). Seven of the 13 studies reported differences in the S-100β expression levels between mild and severe HIE neonates, and the random-effect model was used because of the heterogeneity of these studies (I^2 = 86.0%, P < 0.001). The results showed significantly higher S-100β expression levels in severe HIE neonates than in mild HIE neonates (SMD = 1.97, 95%CI = 1.67-2.27, P < 0.001) (Figure 1D). Five studies reported differences in the S-100β expression levels between moderate and severe HIE neonates, and the random-effect model was used because of the heterogeneity of these studies (I^2 = 74.2%, P < 0.001). The results showed significantly higher S-100β expression levels in severe HIE neonates than in moderate HIE neonates (SMD = 1.16, 95%CI = 0.86-1.45, P < 0.001) (Figure 1E). Five of the 13 studies reported differences in the S-100β expression levels between mild and moderate HIE neonates, and a fixed-effect model was used because of a lack of heterogeneity of the studies (I^2 < 50%, P = 0.505). The results showed significantly higher S-100β expression levels in moderate HIE neonates than in mild HIE neonates (SMD = 1.86, 95%CI = 1.54-2.17, P < 0.001) (Figure 1F).

Subgroup analysis by ethnicity revealed significantly higher S-100β expression levels in severe HIE neonates than in healthy controls in both Asians and Caucasians (Asians: SMD = 2.34, 95%CI = 2.09-2.60, P < 0.001; Caucasians: SMD = 0.73, 95%CI = 0.47-0.10, P < 0.001) (Figure 2A). In addition, in Asian subjects, the S-100β expression levels were significantly higher in mild HIE neonates than in healthy controls and in severe HIE neonates than in mild HIE neonates (mild HIE *vs* healthy controls: SMD = 1.52, 95%CI = 1.24-1.79, P < 0.001; severe *vs* mild HIE: SMD = 2.09, 95%CI = 1.77-2.40, P < 0.001) (Figure 2B and C). However, in Caucasian subjects, the differences in the S-100β expression levels between mild HIE neonates and healthy controls and between severe and mild HIE neonates were not statistically significant (mild HIE *vs* healthy controls: SMD = 0.0, 95%CI = -1.09-1.09, P = 1.00; severe *vs* mild HIE: SMD = 0.59, 95%CI = -0.49-1.68, P = 0.284) (Table 2, Figure 2B and C).

Sensitivity analysis and publication bias

The results of the sensitivity analyses showed that each individual study exerted no obvious influence on the overall effect size, i.e., the SMD (Figure 3A-F). Symmetrical contour-enhanced funnel plots and the Egger linear regression test for the differences in the S-100β expression levels between HIE neonates and healthy controls indicated no publication bias (P > 0.05 in all) (Figure 4A-F).
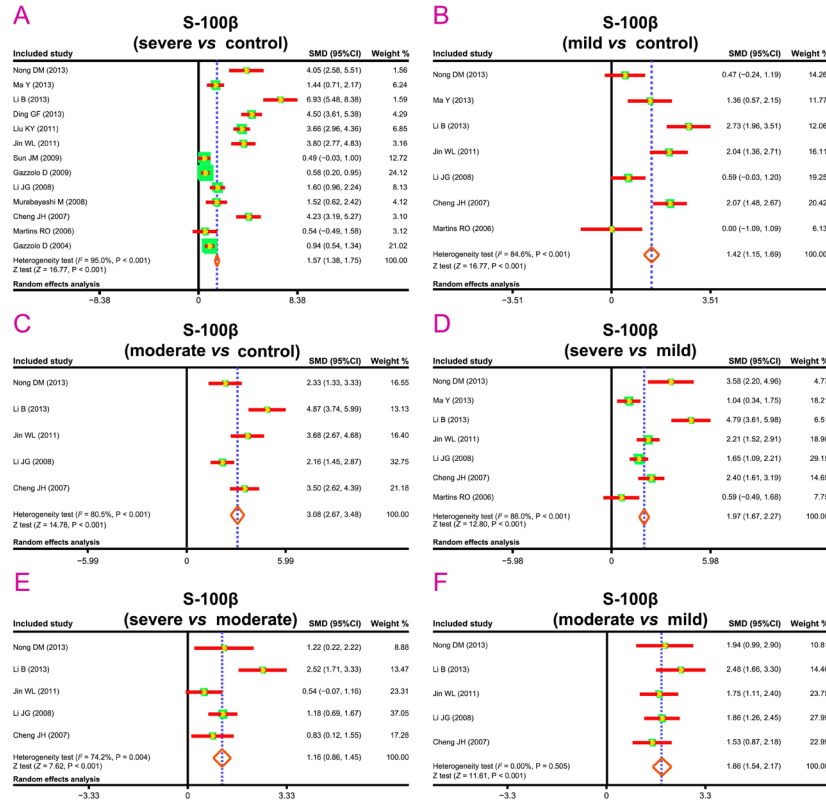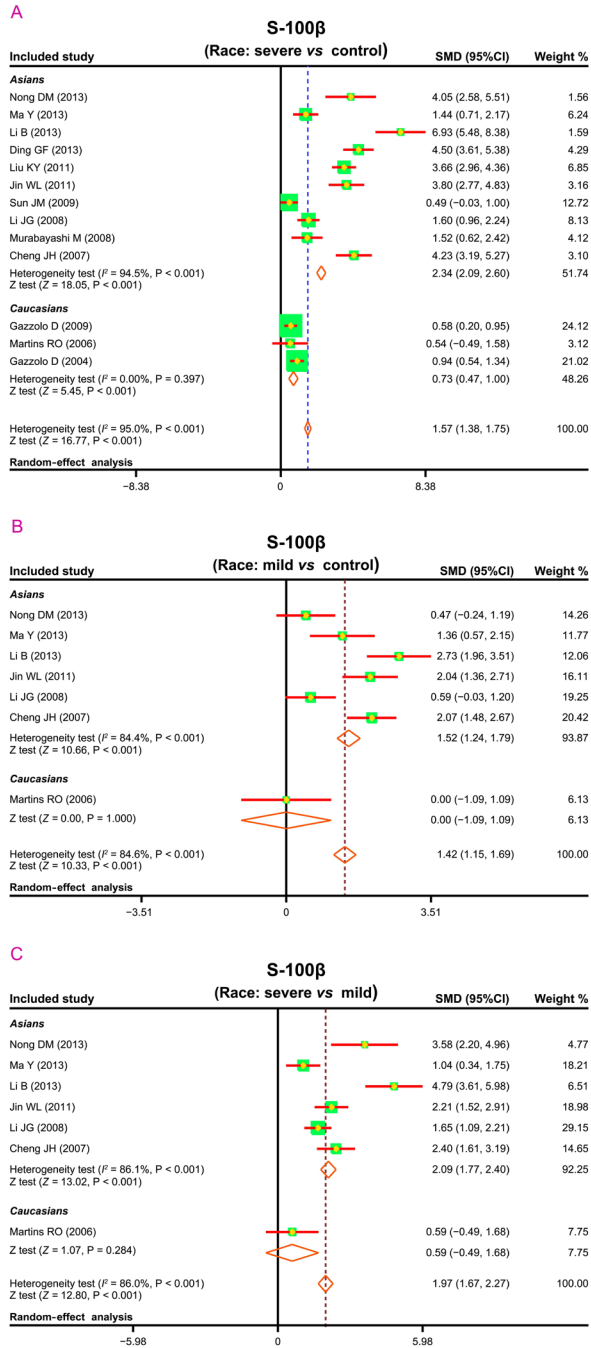
Figure 1. Forest plots of the association between HIE and S-100β protein. Dotted line means the equilibrium position of overall effect. SMD = standard mean difference; 95%CI = 95% confidence interval.

Table 2. Expression levels of the S-100β protein in neonates with different HIE severities.

		Overall	Ethnicity	
			Asians	Caucasians
Severe *vs* control	SMD	1.57	2.34	0.73
	95%CI	1.38-1.75	2.09-2.60	0.47-0.10
	P	0.001	0.001	0.001
Mild *vs* control	SMD	1.42	1.52	0.0
	95%CI	1.15-1.69	1.24-1.79	-1.09-1.09
	P	0.001	0.001	1.00
Moderate *vs* control	SMD	3.08	-	-
	95%CI	2.67-3.48	-	-
	P	0.001	-	-
Severe *vs* mild	SMD	1.97	2.09	0.59
	95%CI	1.67-2.27	1.77-2.40	-0.49-1.68
	P	0.001	0.001	0.284
Severe *vs* moderate	SMD	1.16	-	-
	95%CI	0.86-1.45	-	-
	P	0.001	-	-
Moderate *vs* mild	SMD	1.86	-	-
	95%CI	1.54-2.17	-	-
	P	0.001	-	-

SMD = standardized mean difference; 95%CI = 95% confidence interval.

Figure 2. Forest plots of the association between HIE and S-100β protein according to subgroup analysis by race. Dotted line means the equilibrium position of overall effect. SMD = standard mean difference; 95%CI = 95% confidence interval.
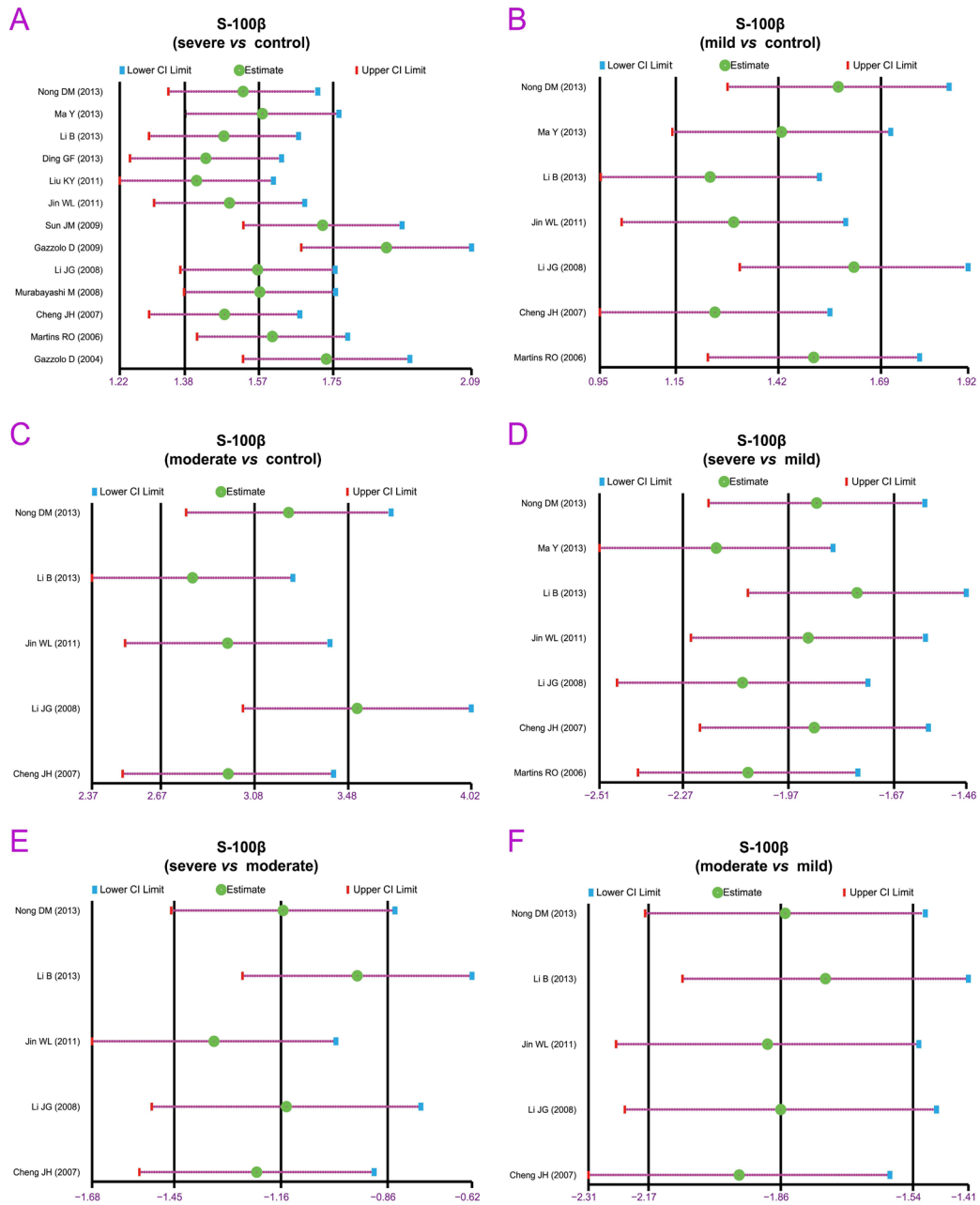
Figure 3. Sensitivity analysis of the association between HIE and S-100β protein.
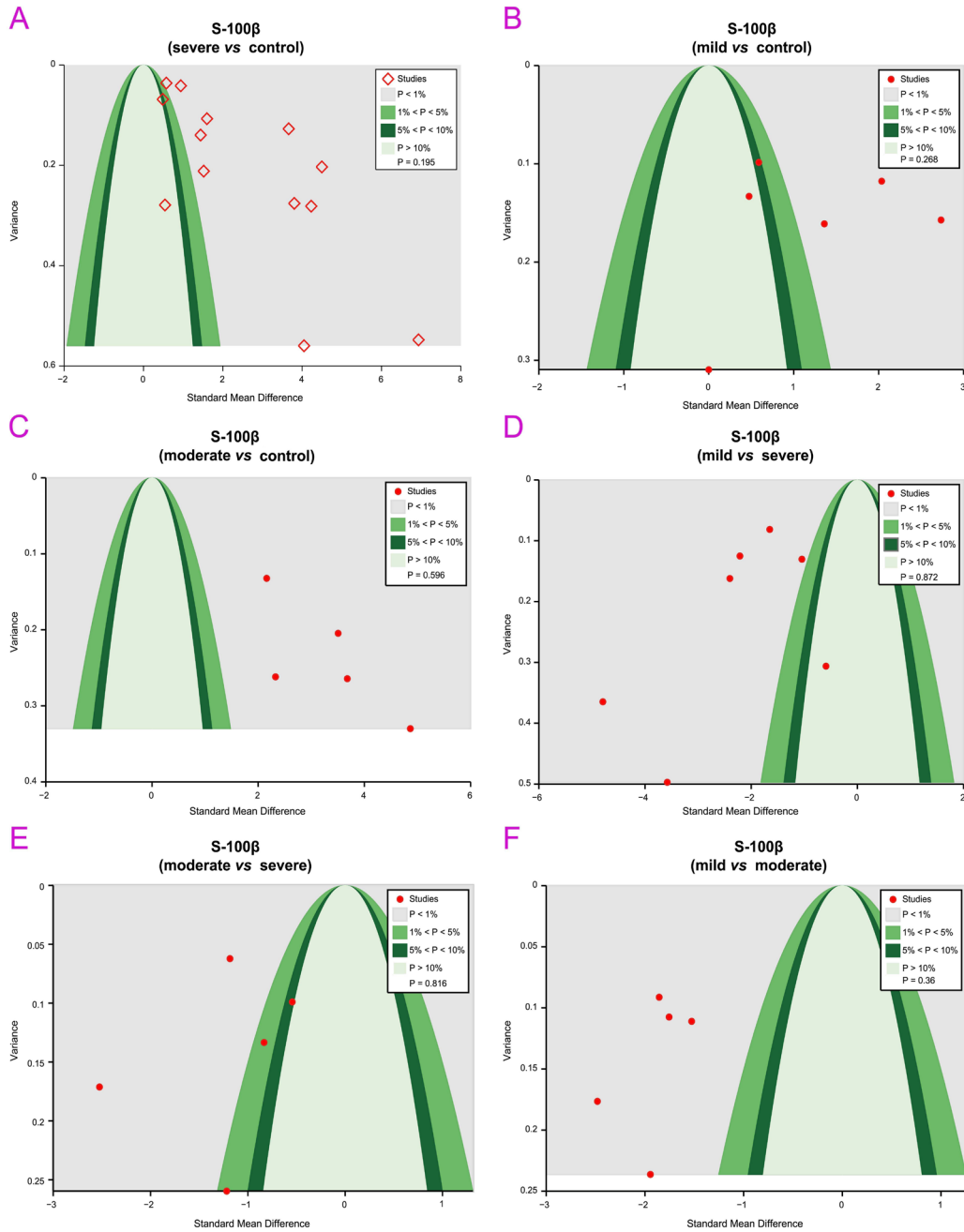
Figure 4. Publication bias assessment of the association between HIE and S-100β protein.

DISCUSSION

HIE causes death in severe cases in newborn infants and frequently results in lifetime neurobehavioral and cognitive dysfunction (Beharier et al., 2012; Zalewska et al., 2015). Because reliable tools for diagnosis are currently unavailable, it is essential to thoroughly evaluate potential biomarkers and their applications in HIE settings. Using data extracted from previous studies, we performed a comprehensive meta-analysis to investigate the association between HIE and the S-100β protein. The main outcome of this meta-analysis is that the serum S-100β level is a reliable diagnostic marker for HIE, and serum S-100β levels can be used as a reference index to assess HIE severity. The S-100β protein is expressed in glial and neuronal cells (Gazzolo et al., 2010; Serpero et al., 2013). It has dual effects depending on its concentration; that is, at nanomolar concentrations, the protein is neurotrophic (Pustylnyak et al., 2011). However, when the S-100β protein is overexpressed at micromolar concentrations, it has neurotoxic effects and increases neuroinflammation and neuronal cell apoptosis (Douglas-Escobar and Weiss, 2012a). This protein normally cannot pass through the blood-brain barrier (BBB) owing to its high molecular weight. However, in HIE, because of enhanced BBB permeability and/or compromised membrane integrity, the S-100β protein is rapidly released into the blood stream from the injured brain, thus elevating serum S-100β levels (Douglas-Escobar and Weiss, 2012a; Hagberg and Ek, 2014). Therefore, higher serum S-100β levels are observed in HIE, and serum S-100β levels can be used as a reference index to determine HIE severity.

We investigated other factors that may influence the diagnostic value of S-100β in HIE. Accordingly, a subgroup analysis was performed by ethnicity. The results of the ethnicity-stratified analysis showed significantly higher S-100β expression levels in severe HIE neonates than in healthy controls. In Asians, significantly higher S-100β expression levels were found in moderate HIE neonates than in healthy controls, and similarly, significantly higher S-100β levels were found in severe HIE neonates than in moderate HIE neonates. However, there were no differences in the S-100β expression levels between moderate HIE neonates and healthy controls and between moderate and severe HIE neonates, which might be owing to the different geographical locations and environmental and genetic factors of different individuals in different countries.

Our study had some limitations. First, there was a lack of data on the S-100β expression levels in mild and moderate HIE neonates, which prospectively reduced the strength of the overall results. Second, the sample sizes of the studies included were relatively small, which may influence the application range of our results. Third, our meta-analysis included only 13 studies, which may have potentially lowered the strength of the results. Finally, the majority of the studies included involved Chinese subjects, and therefore, ethnicity bias may have affected the overall results.

In summary, our meta-analysis demonstrated that the serum S-100β level is an important diagnostic marker of HIE, and serum S-100β levels can be used as a reference index to assess HIE severity. However, because of the abovementioned limitations, further studies with larger sample sizes and better designs are required to confirm our findings on the association between HIE and the S-100β protein.

Conflicts of interest

The authors declare no conflict of interest.

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