

Nutrition

The association between mercury levels and autism spectrum disorders: A systematic review and meta-analysis

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ABSTRACT

Background & aims: The relationship between mercury and autism spectrum disorders (ASD) has always been a topic of controversy among researchers. This study aimed to assess the relationship between ASD and mercury levels in hair, urine, blood, red blood cells (RBC), and brain through a meta-analysis.

Methods: A systematic search was performed in several databases including PubMed, ISI Web of Science, Cochrane register of controlled trials, Google Scholar, Scopus, and MagIran until June 2017. Case-control studies evaluating concentration of total mercury in different tissues of ASD patients and comparing them to the healthy subjects (control group) were identified. Necessary data were extracted and random effects model was used to calculate overall effect and its 95% corresponding confidence interval (CI) from the effect sizes.

Results: A total of 44 studies were identified that met the necessary criteria for meta-analysis. The mercury level in whole blood (Hedges = 0.43, 95% CI: 0.12, 0.74, $P = 0.007$), RBC (Hedges = 1.61, 95% CI: 0.83, 2.38, $P < 0.001$), and brain (0.61 ng/g, 95% CI: 0.02, 1.19, $P = 0.043$) was significantly higher in ASD patients than healthy subjects, whereas mercury level in hair (-0.14 mg/g, 95% CI: -0.28 , -0.01 , $P = 0.039$) was significantly lower in ASD patients than healthy subjects. The mercury level in urine was not significantly different between ASD patients and healthy subjects (0.51 mg/g creatinine, 95% CI: -0.14 , 1.16, $P = 0.121$).

Conclusions: Results of the current meta-analysis revealed that mercury is an important causal factor in the etiology of ASD. It seems that the detoxification and excretory mechanisms are impaired in ASD patients which lead to accumulation of mercury in the body. Future additional studies on mercury levels in different tissues of ASD patients should be undertaken.

1. Introduction

Autism spectrum disorders (ASD), as a general term refers to a group of complex neurodevelopmental disorders present from early childhood. These disorders are characterized, in varying degrees, by difficulties in social interaction, verbal and non-verbal communication, and repetitive behaviors [1]. Based on studies performed on identical twins, factors such as genetics, environment, and their interactions are important in the etiology of ASD. It was estimated from several previous studies on advanced genetic testing of chromosomal abnormalities in individuals diagnosed with an ASD that about 80% have negative genetic test results, and among those with positive genetic test results only about 7% had *de novo* mutations that could possible play an etiological role in ASD [2,3].

Several neurotoxic environmental pollutants such as lead, mercury

(organic and inorganic), polychlorinated biphenyls, arsenic, and toluene are studied in the disease pathogenesis [4]. Exposure to mercury occurs through dietary and non-dietary sources such as fish and other seafood products, vaccines containing thimerosal, and dental amalgams [5–7]. Mercury can directly pass the blood brain barrier and accumulate in high quantities specially in the visual cortex, cerebellum, and spinal cord. Mercury also binds to the membranous organelles like endoplasmic reticulum, mitochondria, and Golgi complex and disrupt their function [8]. Children's central nervous system (CNS) is developing and therefore, more susceptible to neurotoxic effects of heavy metals than adults [9].

Referring to scientific literature, several studies have explored the relationship between tissue mercury levels and ASD. A group of studies found mercury as a risk factor in developing ASD [10–12]. However, some studies did not find any association between mercury and ASD

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[13–15]. Therefore, this study aimed to assess the relationship between ASD and mercury levels in different tissues (hair, urine, blood, RBC, and brain) through a meta-analysis.

2. Materials and methods

2.1. Review design and search strategy

The study protocol was registered in PROSPERO, the international database of registered systematic reviews with registration number: CRD42016043439. We followed the PRISMA guidelines for the processing and reporting the results of the meta-analysis [16]. A systematic search was performed in several databases including PubMed, ISI Web of Science, Cochrane register of controlled trials, Google Scholar, Scopus, and MagIran until 25 June 2017.

The search strategy was as follow: PubMed was searched by keywords: “Mercury”[Mesh] OR “Mercury Compounds”[Mesh] OR “Mercury Isotopes”[Mesh]) AND (“Autism” OR “Autistic Disorder”[Mesh] OR “Autism Spectrum Disorder”[Mesh]); ISI Web of Science and Cochrane register of control trials were searched by keywords: “Mercury” OR “Mercury Compounds” OR “Mercury Isotopes”) AND (“Autism” OR “Autistic Disorder” OR “Autism Spectrum Disorder”; Scopus and Google Scholar was searched by keywords: “mercury” AND (“autism” OR “autism spectrum disorder” OR “autistic disorder”). MagIran; was searched by keywords: Mercury AND (Autism OR “Autistic Disorder”). Search on the databases was separately performed by all of the authors. Related studies were recognized and their title; abstract; and if necessary full text were carefully read and evaluated. References of the related articles were also searched in order to avoid missing of the information.

2.2. Study selection

Case-control studies evaluating concentration of total mercury in blood, red blood cells (RBC), hair, urine, nail, teeth, and brain of ASD patients and comparing them to the healthy subjects (control group) were identified. For each tissue, if possible, the mercury measurement units were matched and then the studies were included in the meta-analysis. Studies which did not measure the mercury levels in control subjects and only report the tissue mercury concentration of ASD patients were excluded.

2.3. Data extraction and quality assessment

Data of the selected studies were extracted: the first author's last name, year of publication, origin, subjects age and gender, number of cases and controls, tissues in which mercury was measured, method of measurement, and mean \pm standard deviation (SD) of mercury concentration. Two authors (AH and NR) separately extracted the data and discussed to reach a consensus.

The quality of studies was assessed using the 9-star Newcastle-Ottawa scale; in which a study was judged based on the following perspectives: selection of study groups (case and control), comparability of the groups, and ascertainment of exposure [17]. The full score was 9 stars; scores 7–9 stars were defined as high-quality and scores <7 as low-quality studies. Two authors (TJ and AAF) separately assessed the quality of studies and discrepancies was resolved by consensus.

2.4. Statistical analyses

To evaluate the effect size for each study, mean \pm SD of total mercury concentration in ASD and control groups was used. Random effects model was used to calculate overall effect and its 95% corresponding confidence interval (CI) from the effect sizes. Therefore, we could consider the between-study variation [18,19]. For concentration

of mercury in hair, urine, and CNS, un-standardized method was employed to calculate the mean difference and 95% CI; while the Hedges'g method (bias corrected standardized mean difference and its 95% CI) was used for mercury levels in the whole blood and RBC, because matching the mercury measurement units was not possible [20]. The overall effect was separately calculated for each tissue. Subgroup analysis based on the origin of the studies was performed to consider the possible source of heterogeneity. The statistical heterogeneity between the studies was assessed by I-squared (I^2 , ranged from 0 to 100%) and Cochran's Q test [21,22]. Influence analysis was also performed to explore if the overall effects depend on a particular study or group of studies. Publication bias was evaluated by visual inspection of Begg's funnel plot asymmetry and Egger's test [23,24]. Statistical analyses were performed using Stata, version 11.2 (Stata Corp, College Station, TX, USA). *P* values less than 0.050 were considered statistically significant.

3. Results

3.1. Study selection and identification

A total number of 2185 studies were found by our systematic search on the mentioned databases. We excluded 998 duplicate studies and 1057 irrelevant topics. In the remained 130 articles, 85 reviews and clinical trials were also excluded. Finally, we found 44 case-control studies [10–15,25–62] in which the concentration of mercury was compared between ASD and normal subjects. Fig. 1 shows the search process. In 2 studies [9,63], mercury level was measured in children tooth enamels and in 1 study [43], it was measured in nails. Due to the lack of data, we did not perform meta-analysis on these tissues.

According to the 9-star Newcastle-Ottawa scale, 36 studies [10–15,25–28,30–44,46,47,49,51,52,54,56–62] were classified as high-quality studies (score \geq 7) and 6 studies [29,45,48,50,53,55] as low-quality studies (score <7; Table 1).

3.2. Association between mercury levels of different tissues and autism

3.2.1. Hair

Mercury status in hair had been compared in 23 studies with 1038 ASD patients and 933 healthy subjects (Table 1). Results of meta-analysis on these studies demonstrated that mercury concentration was not significantly different in the hair of ASD patients comparing to healthy subjects (Fig. S1: 0.63 mg/g, 95% CI: $-0.21, 1.46, P = 0.141$). The Egger's test and Begg's funnel plot showed that there was no publication bias among the studies (Egger's test $P = 0.773$). According to I^2 (97.4%) and Cochran Q test ($P < 0.001$), there was heterogeneity between the studies. Influence analysis showed that the heterogeneity belonged to the studies of Fido and Al-Saad [29], Yassa [50], and Hodgson et al. [48]. The analysis was repeated again without the mentioned 3 studies; and the results showed that concentration of mercury in the hair of ASD patients was significantly lower than healthy subjects (Fig. 2: -0.14 mg/g, 95% CI: $-0.28, -0.01, P = 0.039$). The between studies heterogeneity was also removed ($I^2 = 0.0\%$ and Cochran Q test $P = 0.505$).

3.2.2. Urine

The concentration of mercury in urine had been evaluated in 8 studies with 491 ASD patients and 417 healthy subjects (Table 1). The results showed that the concentration of mercury in the urine was not significantly different between ASD patients and healthy subjects (Fig. S2: 1.80 mg/g creatinine, 95% CI: $-0.10, 3.70, P = 0.063$). There was no publication bias among the studies according to the Begg's funnel plot and Egger's test (Egger's test $P = 0.274$). The between studies heterogeneity was high due to I^2 (92%) and Cochran Q test ($P < 0.001$). Influence analysis showed that the heterogeneity belonged to the study of Metwally et al. [53]. Data was reanalyzed

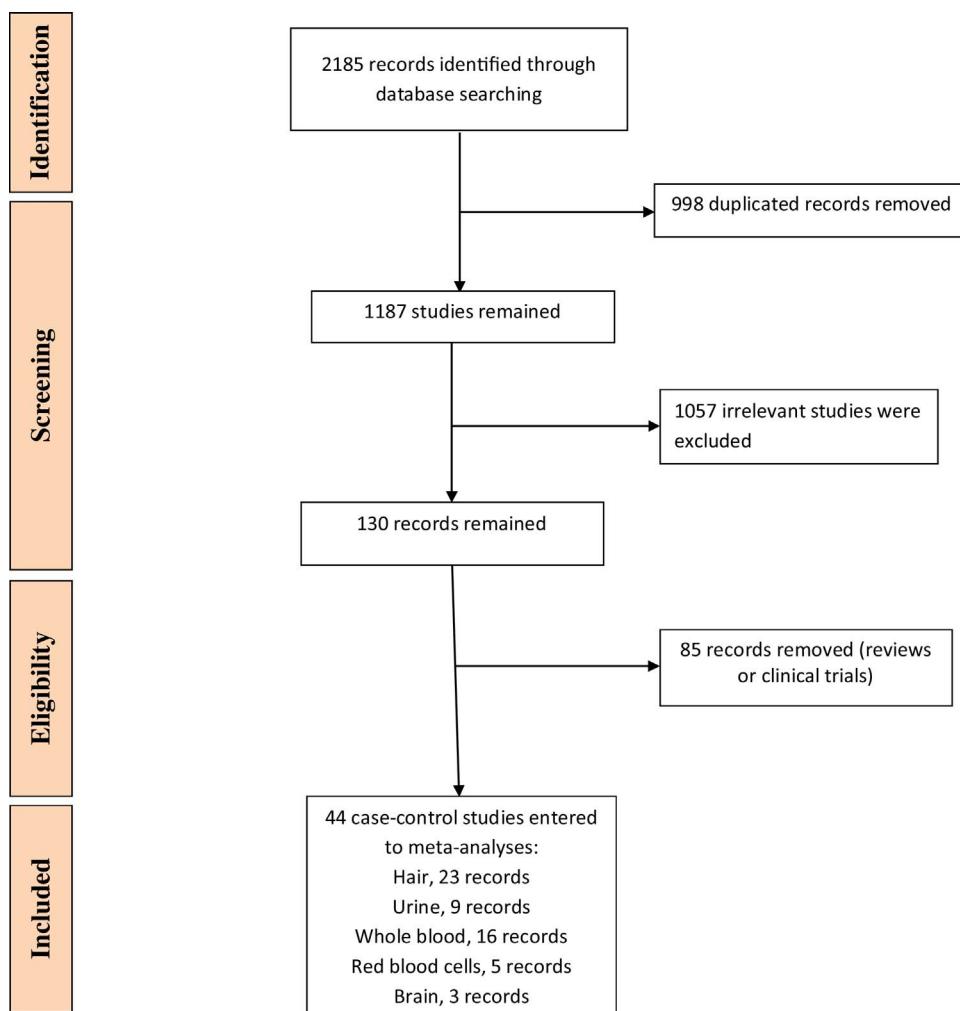


Fig. 1. PRISMA flow diagram of study identification, inclusion and exclusion.

without this study and the heterogeneity was reduced ($I^2 = 24.9\%$ and Cochrane Q test $P = 0.239$). The new results also demonstrated that the concentration of mercury in the urine was not significantly different between ASD subjects and healthy ones (Fig. 3: 0.51 mg/g creatinine, 95% CI: $-0.14, 1.16, P = 0.121$).

3.2.3. Whole blood

Mercury concentration in the whole blood was measured in 16 studies with 1239 ASD patients and 1039 healthy subjects (Table 1). Results demonstrated that blood level of mercury was significantly higher in ASD patients than healthy subjects (Fig. S3: Hedges = 1.13, 95% CI: 0.22, 2.04, $P = 0.014$). There was no publication bias among the studies according to the Begg's funnel plot and Egger's test (Egger's test $P = 0.201$). The between studies heterogeneity was high due to I^2 (91.8%) and Cochrane Q test ($P < 0.001$). Considering the influence analysis, the study of Yassa [45] and the study of Mostafa and Al-Ayadhi [55] were the major source of heterogeneity. After excluding the mentioned studies, the heterogeneity was removed ($I^2 = 22.4\%$ and Cochrane Q test $P = 0.211$). The new results showed that the concentration of mercury was significantly higher in ASD patients compared to the healthy subjects (Fig. 4: Hedges = 0.43, 95% CI: 0.12, 0.74, $P = 0.007$).

3.2.4. RBC

The concentration of mercury was considered in 5 studies with 251 ASD patients and 215 healthy subject (Table 1). Result of meta-analysis on these studies showed that the concentration of mercury in the RBC of ASD patients was significantly higher than healthy subjects (Fig. S4:

Hedges = 1.29, 95% CI: 0.35, 2.24, $P = 0.007$). There was no publication bias according to the Begg's funnel plot and Egger's test (Egger's test $P = 0.062$). The between studies heterogeneity was high due to I^2 (65.6%) and Cochrane Q test ($P = 0.020$) and according to influence analysis, the heterogeneity belonged to the study of Adams et al. [45]. The new analysis re-performed without the mentioned study represented that the concentration of mercury in the RBC of ASD patients was significantly higher than healthy subjects (Fig. 5: Hedges = 1.61, 95% CI: 0.83, 2.38, $P < 0.001$). The between studies heterogeneity was reduced according to I^2 (29.5%) and Cochrane Q test ($P = 0.235$).

3.2.5. Brain

Three studies with 16 ASD patients and 20 healthy subjects measured the concentration of mercury in brain tissue (Table 1). Results of meta-analysis on these studies demonstrated that the concentration of mercury was significantly higher in ASD patients than healthy subjects (Fig. 6: 0.61 ng/g, 95% CI: 0.02, 1.19, $P = 0.043$). According to the Begg's funnel plot and Egger's test (Egger's test $P = 0.336$), there was no publication bias. Results of I^2 (0.0%) and Cochrane Q test ($P = 0.860$) showed that there was no significant heterogeneity between the studies.

3.3. Subgroup analyses

Results of subgroup analyses based on continents are shown in Table 2. Concentration of mercury in the hair of ASD patients in America was significantly lower than healthy subjects while in Asia, Africa, and Europe the results were not statistically significant. Subgroup analysis also revealed that the concentration of mercury in the

Table 1
Characteristics of studies enrolled in meta-analyses.

Study/Year	Country	Study quality ^a	No. of subjects		Age (mean ± SD), year		Gender	Tissue	Method of Hg measurement
			Case	Control	Case	Control			
Wecker et al. [25]	USA	7	12	22	5.67 ± 2.39	4.18 ± 1.83	Male & female	Hair	AAS
Bradstreet et al. [26]	USA	8	221	18	6.25 ± 3.06	8.85 ± 3.25	Male & female	Urine	ICP-MS
Holmes et al. [27]	USA	8	94	45	7 ± 3.25	7 ± 1.02	Male & female	Hair	ICP-MS
Ip et al. [10]	Hong Kong	8	82	55	7.2 ± 0.2	7.8 ± 0.4	Male & female	Hair, blood	AAS
Al-Ayadhi [28]	Saudi Arabia	7	65	80	9 ± 2.42	7.2 ± 6.21	Male & female	Hair	AAS
Fido and Al-Saad [29]	Kuwait	5	40	40	4.2 ± 2.2	4.3 ± 2.6	Male	Hair	ICP-MS
Adams et al. [30]	USA	9	51	40	7.1 ± 3	7.5 ± 3	Male & female	Hair	ICP-MS
Kern et al. [31]	USA	9	45	45	3 ± 1.4	3 ± 1.4	Male & female	Hair	ICP-MS
Mostafa and Refaei [11]	Egypt	7	40	40	5.38 ± 1.85	5.25 ± 1.80	Male & female	Blood	HG-AAS
Adams et al. [32]	USA	8	78	31	1.38 ± 0.37	1.37 ± 0.42	Male & female	Hair	CV-AFS
Sajdel-Sulkowska et al. [33]	USA	7	6	9	12.1 ± 8.09	13.4 ± 7.14	Male & female	Cerebella	CV-AFS
Williams et al. [34]	Egypt	7	15	16	2–6 ^b	2–6 ^b	Male & female	Hair	ICP-MS
El-Baz et al. [35]	Egypt	8	32	15	6.75 ± 3.26	5.53 ± 2.75	Male & female	Hair	HG-AAS
Geier et al. [36]	USA	8	83	89	7.3 ± 3.7	11.4 ± 2.2	Male & female	RBC	CV-AAS
Hertz-Picciotto et al. [37]	USA	8	249	143	2–5 ^b	2–5 ^b	Male & female	Blood	ICP-MS
Majewska et al. [38]	Poland	8	30	19	3.6 ± 0.55	3.5 ± 0.44	Male & female	Hair	CV-AAS
Woods et al. [39]	USA	7	59	59	6.01 ± 2.14	6.39 ± 3.06	Male	Urine	CV-AFS
Woods et al. [39]	USA	7	5	55	4.60 ± 1.82	6.93 ± 2.85	Female	Urine	CV-AFS
Blaurock-Busch et al. [40]	Saudi Arabia	7	25	25	5.29 ± 1.90	6.25 ± 2.31	Male & female	Hair, urine	ICP-MS
Elshestawy et al. [41]	Egypt	8	32	32	4.1 ± 0.8	4 ± 0.8	Male & female	Hair	HG-AAS
Obrenovich et al. [42]	USA	8	26	39	<6	<6	Male & female	Hair	ICP-MS
Priya and Geetha [43]	India	7	50	50	4–12 ^b	4–12 ^b	Male & female	Hair	CV-AAS
Albizzati et al. [44]	Italy	8	17	20	11.5 ± 3.20	10.4 ± 3.05	Male & female	Hair, urine, blood	ICP-MS
De Palma et al. [13]	Italy	8	44	61	9 ± 4	8.4 ± 3.1	Male & female	Hair	ICP-MS
Wright et al. [14]	United Kingdom	8	54	121	9.6 ± 3.6	8.8 ± 2.4	Male & female	Urine	ICP-MS
Adams et al. [45]	USA	6	55	44	10.0 ± 3.1	11.0 ± 3.1	Male & female	Urine, blood, RBC	ICP-MS
Rahbar et al. [46]	Jamaica	8	65	65	2–8 ^b	2–8 ^b	Male & female	Blood	ICP-MS
Alabdali et al. [47]	Saudi Arabia	7	58	32	7 ± 2.34	7.2 ± 2.14	Male	RBC	AAS
Hodgson et al. [48]	Oman	6	27	27	5.3 ± 1.5	5.5 ± 1.4	Male & female	Hair	ICP-MS
Khan et al. [49]	USA	9	10	11	4–15 ^b	5–16 ^b	Male & female	Cerebella, brainstem	ICP-MS
Yassa [50]	Egypt	5	45	45	2–10 ^b	2–10 ^b	Male & female	Hair, blood	ICP-MS
Yau et al. [15]	USA, Mexico	9	76	147	3–4 ^b	3–4 ^b	Male & female	Blood	ICP-MS
Macedoni Lukšič et al. [51]	Slovenia	8	42	14	6.2 ± 3	6.6 ± 3.7	Male & female	Blood	CV-AAS
McKean et al. [52]	USA	8	164	58	2–5 ^b	2–5 ^b	Male & female	Blood	ICP-MS
Metwally et al. [53]	Egypt	6	55	75	4.01 ± 1.91	4.02 ± 1.33	Male & female	Urine	ICP-MS
Mohamed et al. [54]	Egypt	7	100	100	6.24 ± 2.43	6.80 ± 3.04	Male & female	Hair	HG-AAS
Mostafa and Al-Ayadhi [55]	Egypt	6	100	100	8.1 ± 1.7	8.3 ± 1.6	Male & female	Blood	CV-AAS
Domingues et al. [56]	Italy	8	21	19	6.9 ± 2.33	7.4 ± 2.11	Male & female	Hair	ICP-OES
El-Ansary [57]	Saudi Arabia	7	20	20	7 ± 4	7 ± 4	Male & female	RBC	AAS
Khaled et al. [12]	Egypt	8	40	40	4.12 ± 0.94	5.23 ± 1.25	Male & female	Blood	CV-AAS
Mostafa et al. [58]	Egypt	7	84	84	6.8 ± 1.5	7 ± 1.8	Male & female	Blood	CV-AAS
El-Ansary et al. [59]	Saudi Arabia	7	35	30	7.0 ± 2.34	7.2 ± 2.14	Male	RBC	AAS
Li et al. [60]	China	7	180	184	5.06 ± 1.37	6.12 ± 1.69	Male & female	Blood	AFS
Skalny et al. [61]	Russia	8	74	74	5.12 ± 2.36	5.11 ± 2.34	Male & female	Hair	ICP-MS
Skalny et al. [62]	Russia	8	33	33	5.0 ± 1.7	5.0 ± 1.7	Male	Hair	ICP-MS

Abbreviations; AAS, Atomic absorption spectrometry; AFS, Atomic fluorescence spectroscopy; CV-AAS, Cold vapor-Atomic absorption spectrometry; HG-AAS, Hydride generation-Atomic absorption spectrometry; ICP-MS, Inductively coupled plasma-Mass spectrometry; ICP-OES, Inductively coupled plasma-Optic emission spectrometry; RBC, Red blood cells.

^a Study quality was assessed based on the Newcastle-Ottawa scale [17].

^b Minimum-maximum value (year).

urine and whole blood did not differ between ASD patients and healthy subjects in America as well as the other continents.

4. Discussion

The relationship between mercury and ASD has always been a topic of controversy among researchers. Concerns about the relationship between mercury and ASD began in the 1990s when investigators reported on a suspected relationship between vaccines containing mercury and the risk of autism [64]. In 2001, the Institute of Medicine (IOM) [65] also stated that it was biologically plausible for exposure to mercury to be as a risk factor for neurodevelopmental disorders such as autism.

Increased level of mercury in the body of patients with autism compared to healthy subjects has been proven in many studies; however, some studies demonstrated no statistically significant difference. Results of current meta-analysis revealed that the concentrations of mercury were significantly higher in the blood, RBC, and brain tissue of ASD patients compared to healthy subjects. There are powerful detoxification mechanisms to deal with toxic agents and protect the organs from their damages. Glutathione-S-transferase (GST) is an important example of these mechanisms. GST detoxifies the xenobiotics, drugs, and toxins and inhibits accumulation of toxic agents in the organs. It also activates some mitogen-activated protein kinases which are involved in the differentiation and development of cells [47]. The other mechanism for excreting mercury involves metal binding to glutathione

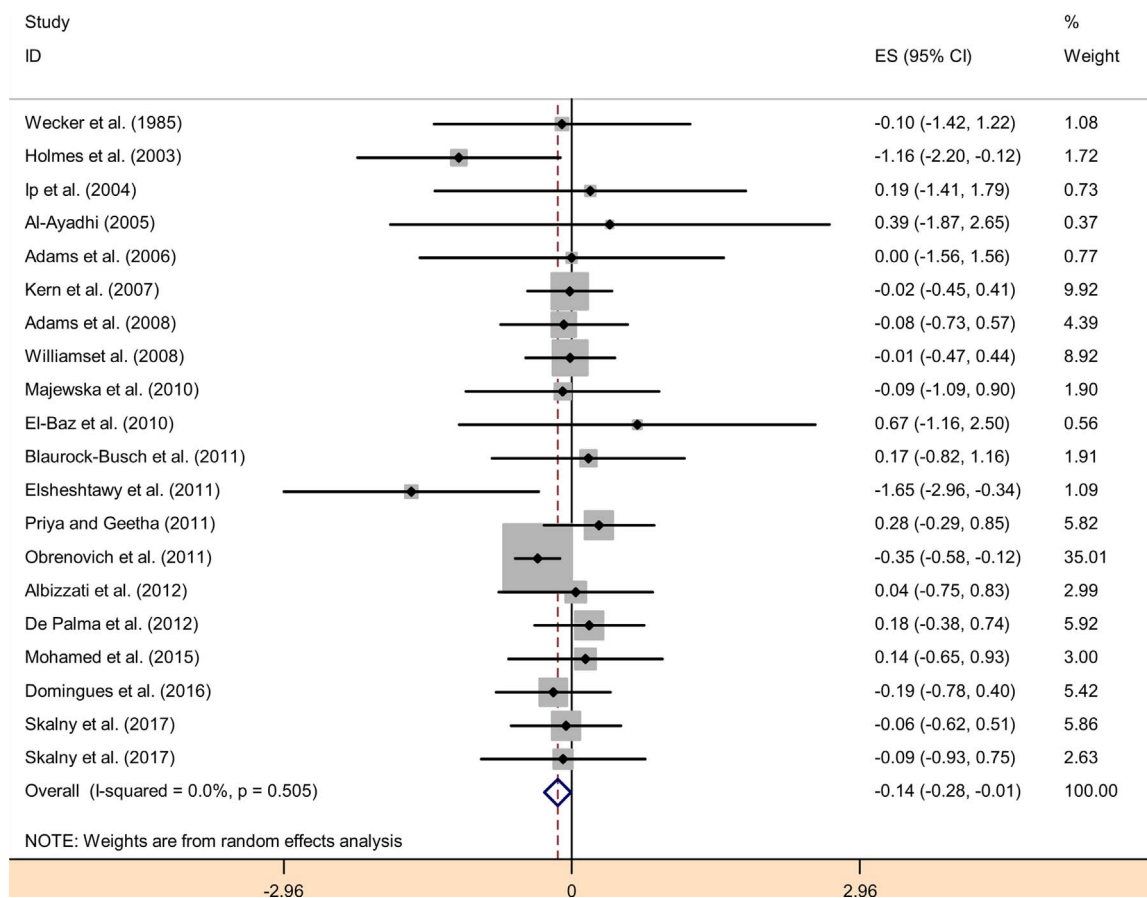


Fig. 2. Forest plot of the association between hair mercury level and ASD without studies of Fido and Al-Saad [29], Hodgson et al. [48], and Yassa [50].

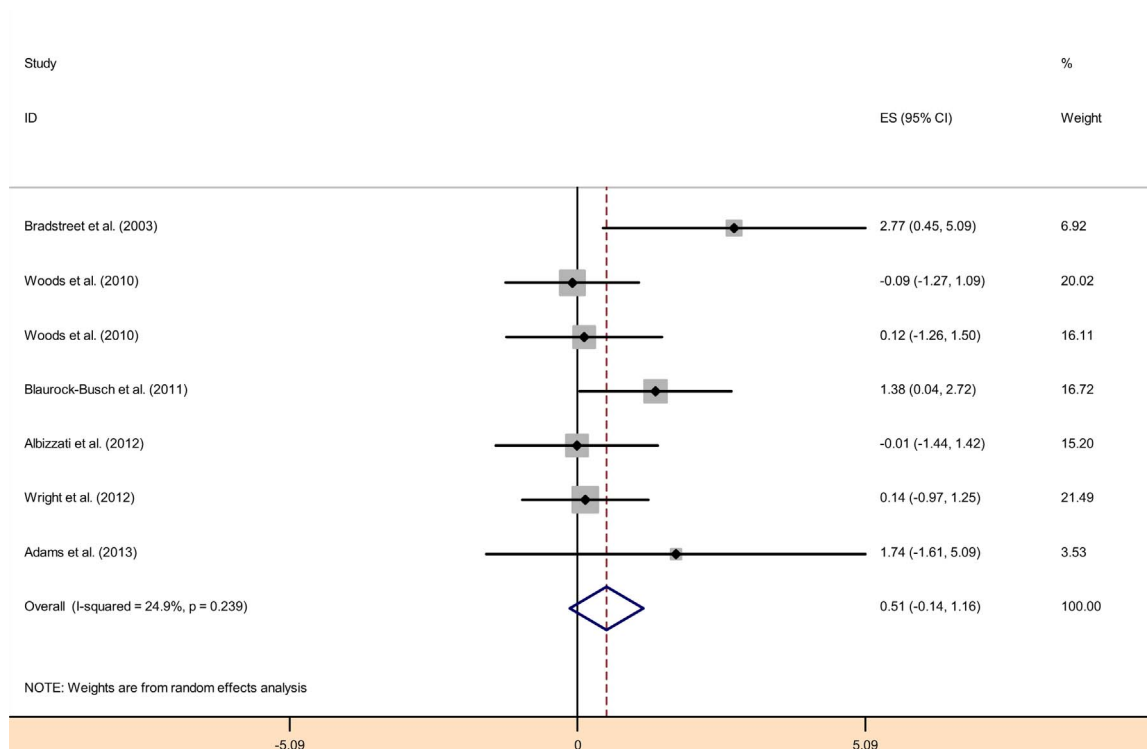


Fig. 3. Forest plot of the association between urine mercury level and ASD without study of Metwally et al. [53].

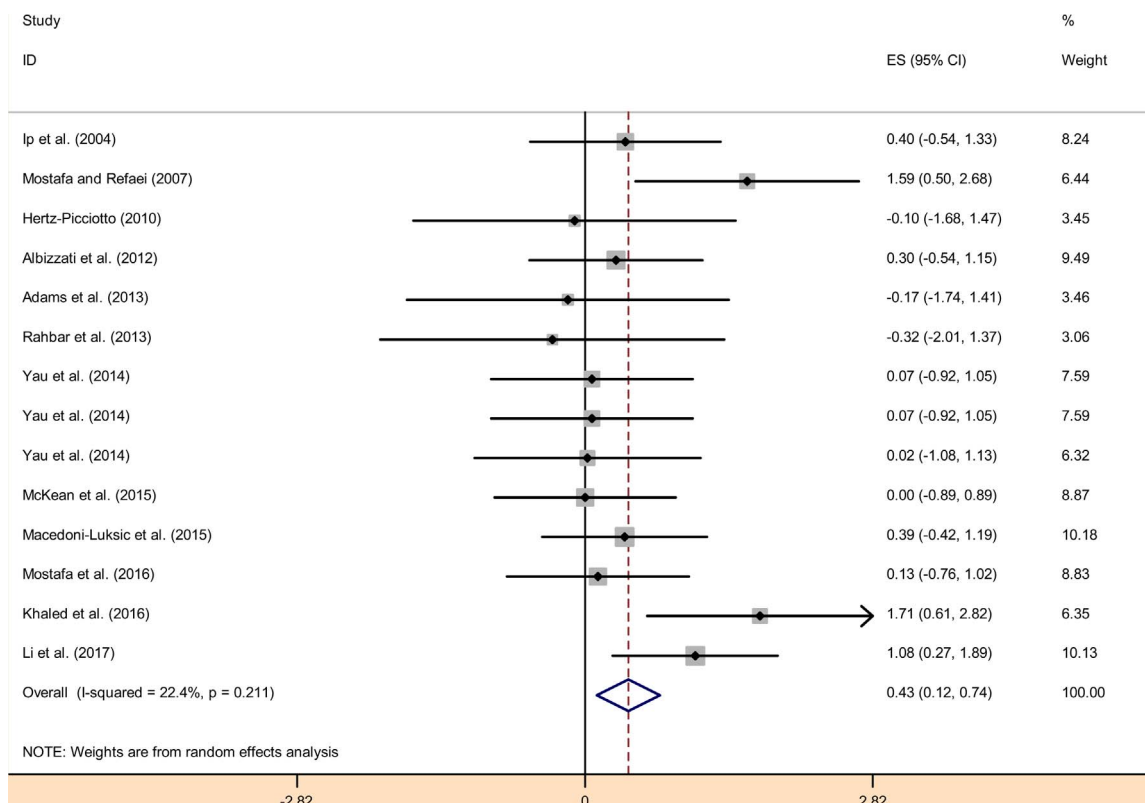


Fig. 4. Forest plot of the association between whole blood mercury level and ASD without studies of Yassa [50] and Mostafa and Al-Ayadhi [55].

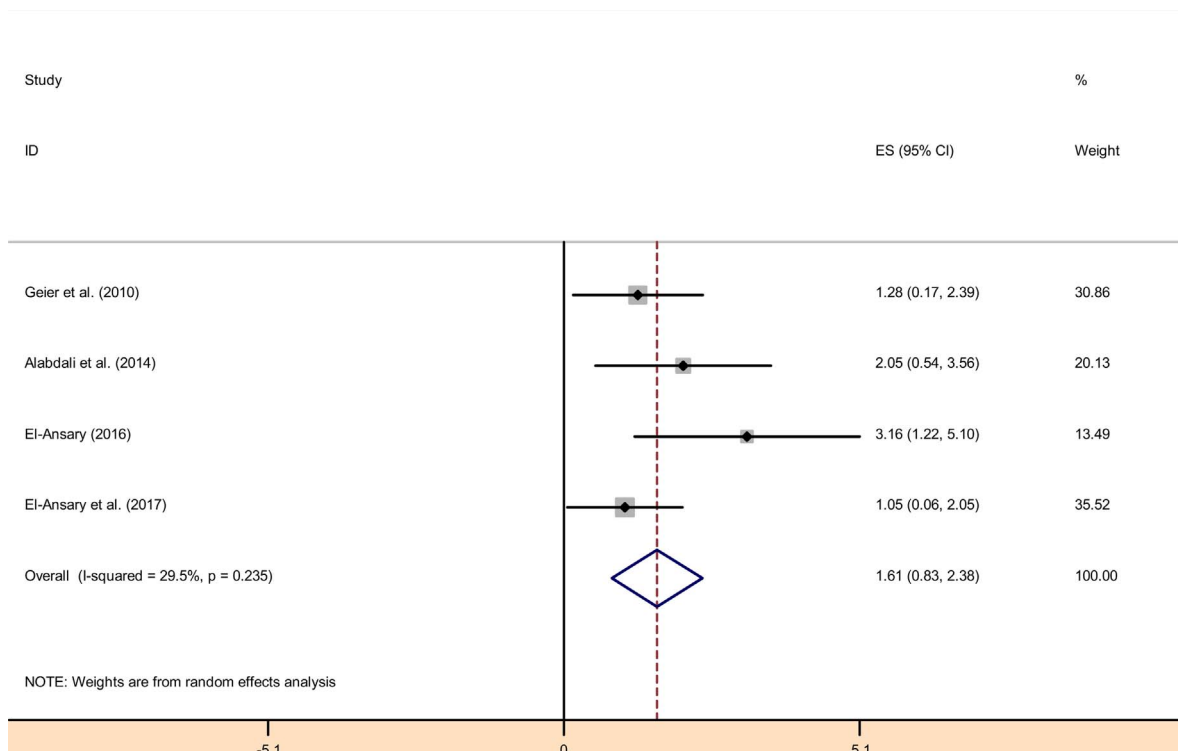


Fig. 5. Forest plot of the association between RBC mercury level and ASD without study of Adams et al. [45].

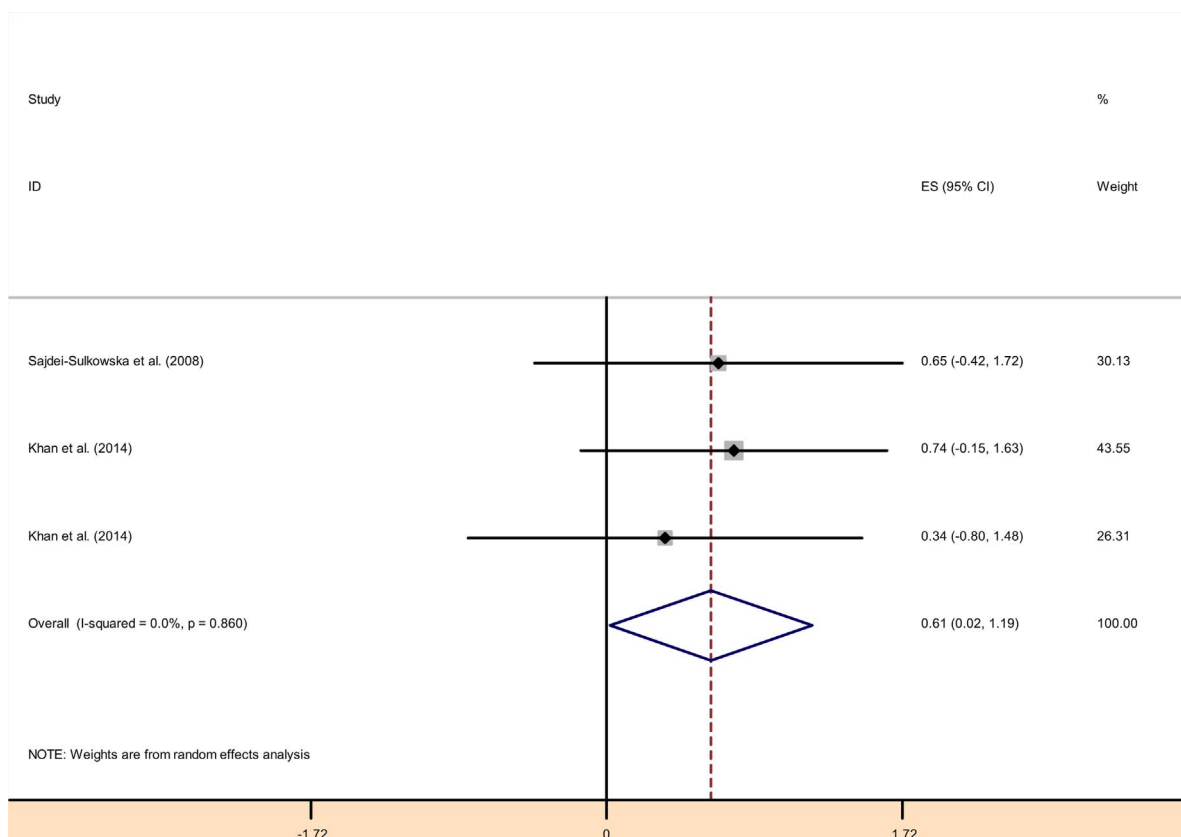


Fig. 6. Forest plot of the association between brain mercury level and ASD.

and then excretion in the bile. It seems that in ASD patients, detoxification mechanisms are impaired and these patients have lower concentration of glutathione which lead to retention of toxins in the body [32,61]. Considering the developmental process of the brain in children and vulnerability of the blood-brain barrier, mercury can accumulate in the brain and initiate neuro-inflammatory and oxidative stress process in the brain as well as increasing the levels of brain tissue auto-antibodies which are important factors in the pathophysiology of autism and other neurodevelopmental disorders [5,66].

Mercury level in the hair of autistic patients and healthy subjects is one of the most controversial topic among the researchers. Studies showing higher mercury level in the hair of ASD patients than the healthy subjects conclude that mercury concentration in the hair is in compliance with its concentration in the body [28,35,43]. In contrast, other studies representing lower mercury concentration in ASD subjects compared to healthy ones infer that ASD patients could not excrete the

toxic agents like mercury from the body [27,41,56]. Our meta-analysis demonstrated that the concentration of mercury in hair of autistic patients was significantly lower than healthy ones. The low levels of mercury in the hair of ASD patients may be due to the retention of mercury inside the cells. A portion of mercury is retained in the cells of central nervous system. It has been found that cellular thiol groups and their availability, playing important role in sequestration or detoxification of mercury, are limited in ASD patients compared to healthy individuals. This limitation can increase the susceptibility of ASD patients to adverse effects of mercury such as its role in neurodevelopmental disorders [5,27]. These findings and also the results observed in this study lend support to the hypothesis that as the body tries to get rid of toxic agents like mercury by excretion of them through the stool, urine, hair, nail, baby teeth, and other waste materials, there is significant dysfunction in such excretory pathways/mechanisms in ASD patients [13,27,63].

Table 2
Results of the association between autism and mercury level based on continent.

Tissue	Continent	No. of studies	Effect size (95% CI)	P value	I ² (%)	Q-statistics (P)
Hair	America	7	-0.239 (-0.42, -0.058)	0.010	2.30	0.408
	Asia ^a	6	0.096 (-0.233, 0.425)	0.567	0	0.965
	Europe	4	-0.002 (-0.341, 0.338)	0.993	0	0.840
	Africa ^b	3	-0.317 (-1.611, 0.976)	0.631	47.2	0.076
Urine	America	4	0.706 (-0.504, 1.917)	0.253	44.9	0.142
	Others ^c	3	0.482 (-0.352, 1.315)	0.257	21.1	0.281
Blood	America	7	-0.011 (-0.446, 0.423)	0.959	0	1.00
	Others ^d	7	0.737 (0.290, 1.183)	0.001	40.3	0.123

^a Analysis performed without studies of Fidio and Al-Saad [29], and Hodgson et al. [48].

^b Analysis performed without study of Yassa [50].

^c Analysis performed without study of Metwally et al. [53].

^d Analysis performed without studies of Yassa [50] and Mostafa and Al-Ayadhi [55].

In India, Priya and Geetha [43] evaluated the mercury level of nails in 45 ASD patients and 50 healthy subjects and showed that the concentration of mercury was significantly higher in the nails of ASD patients compared to healthy subjects. In USA, Adams et al. [63] conducted a study to compare the concentration of mercury in baby teeth of 15 ASD patients and 11 healthy subjects. They represented that autistic children have higher level of mercury in their teeth compared to healthy ones. However, in another study in USA, Abdullah et al. [9] found no significant difference between the concentration of mercury in the teeth of ASD subjects and healthy ones. Regarding the lack of enough data about the concentration of mercury in the nail and baby teeth, we did not perform the analyses in these tissues. It seems that more studies should be performed on the concentration of mercury in hair, nail, and other excretory materials of ASD patients worldwide.

Yoshimasu et al. [67] conducted a meta-analysis to evaluate the association between prenatal or early infancy exposures to mercury and autism. They concluded that exposure to thimerosal vaccines (contained ethyl mercury) did not associate with ASD, while there was a significant association between environmental exposure to mercury and ASD. To our knowledge, our meta-analysis is the first study aimed to assess the relationship between mercury levels in the different tissues and ASD. We infer that exposure to mercury increase the risk of ASD and critical care is necessary to reduce the incidence of ASD.

The current study has also some limitations:

- The major problem with case-control studies is the temporal relationship between exposure and outcome. It is possible, for example, that older children with ASD may exhibit more mouthing behavior than healthy controls, leading to increased levels of mercury (and other pollutants) in their biological tissues.
- Lack of enough studies for nail and teeth
- Differences in the diagnostic criteria for ASD in the studies.
- Measurement of total mercury but not inorganic or organic forms separately in the studies: it is believed that exposure to organic forms of mercury such as ethylmercury, used as vaccine preservative thimerosal, and methylmercury, found primarily in seafood products, are mostly implicated in the ASD.
- Few number of studies performed outside the America continent.

5. Conclusion

Results of the current meta-analysis revealed that mercury is a causal factor in the etiology of ASD. The higher levels of mercury in the blood, RBC, and brain tissue of ASD patients observed in this study significantly support this conclusion. Furthermore, significant evidence supports impaired mercury detoxification and excretory mechanisms in ASD patients. Finally, future worldwide studies should be conducted to examine the concentration of mercury in urine, hair, nail, baby teeth, and other excretory materials of ASD patients.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jtemb.2017.09.002>.

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