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# Association Between Biological Lead Concentrations and Autism Spectrum Disorder (ASD) in Children: a Systematic Review and Meta-Analysis

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## Abstract

Studies have been conducted in different countries of the world to illustrate a link between autism spectrum disorder (ASD) and lead (Pb) in different specimens such as hair, blood, and urine. Therefore, we carried out a systematic review and meta-analysis to determine the association between Pb concentration in biological samples (blood, urine, and hair) and ASD in children through case–control and cross-sectional studies. In this systematic review, PubMed, Web of Sciences, Scopus, and Google Scholar were searched for relevant studies from January 2000 to February 2022. A random-effects model was used to pool the results. The effect sizes were standardized mean differences (proxied by Hedges' $g$) followed by a 95% confidence interval. Pooling data under the random effect model showed a significant difference between the children in the ASD group and the control group in blood lead level (Hedges' $g$: 1.12, 95% CI: 0.27, 1.97; $P = 0.010$) and hair level (Hedges' $g$: 1.25, 95% CI: 0.14, 2.36; $P = 0.01$)). For urine studies, pooling data under the random effect model from eight studies indicated no significant difference between the children in the ASD group and control group in urinary lead level (Hedges' $g$: 0.54, 95% CI: − 0.17, 1.25; $P = 0.137$). Moreover, the funnel plot and the results of the Egger test for the blood and urine samples showed no publication bias, while, for the hair samples, the funnel plot illustrated the existence of publication bias.

**Keywords** Autism spectrum disorder · Publication bias · Confidence interval · Children · Lead

## Introduction

Autism spectrum disorder (ASD) is a debilitating disease that impairs social interactions, verbal and nonverbal communication skills, the ability to learn, and some vital emotions [1, 2]. Over the past two decades, the increasing rate of ASD worldwide has caused great concern [3]. Epidemiologic findings have also shown that the prevalence of ASD has increased significantly in recent decades [1, 4–6]. It has been reported that the prevalence of ASD in boys is four to five times more common than in girls, but the disorder in girls is associated with more severe mental retardation [7]. Although the exact cause of ASD is unknown, multiple factors can contribute to the spread of the disease, such as those linked to environmental and genetic factors. Some studies have shown that there are several risk factors associated with the pathogenesis of ASD including obstetric problems, maternal or paternal age, fetal hypoxia, gestational diabetes, bleeding during pregnancy, diet, and medications used during the pregnancy [8, 9].

It has been reported that some environmental factors such as exposure to certain toxic metals in the environment and poor regulation of intracellular trace

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elements can result in human brain damage [3, 10]. Exposure to toxic metals including lead (Pb) and mercury is linked with various nervous system abnormalities [11, 12], although in some studies exposure to toxic elements such as lead, cadmium, arsenic, and aluminum have been mentioned as environmental factors related to ASD disorder [1, 13, 14].

Studies have been conducted in this regard in different countries of the world, but the results are inconsistent. In this regard, the findings of Grandjean and Landrigan (2006) showed that five toxins including lead, mercury, biphenyl polychlorinated, arsenic, and toluene can increase the incidence of ASD [15]. Furthermore, some studies on the relationship between the prevalence of ASD and the concentration of environmental elements as air pollutants have shown that lead along with exposure to mercury and arsenic, have synergistic effects on the prevalence of ASD [16, 17]. In contrast, Fuentes-Albero et al. (2015) conducted a study on 35 ASD children vs. 34 controls to assess the Pb concentration level in their urine. They found a not statistically relevant tendency to higher urine Pb levels in the ASD group [18]. However, due to the inconsistency between the different findings of the studies, the effect of lead on autism disorder has not been well clarified [1, 19]. Therefore, the current study aimed to comprehensively examine the current state of knowledge on the difference in lead concentration between patients with ASD and control subjects in different biological samples (blood, urine, and hair) and to identify research gaps.

## Materials and Methods

### Data Resources and Search Strategy

Data from the current systematic review study was collected using an advanced document protocol of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) instructions. This protocol provides a checklist for reporting systematic reviews (Fig. 1). Electronic searches were performed using PubMed, Web of Science, Scopus, and Google Scholar. Additional publications were identified through searching for references of selected studies, or through authors' awareness of published studies. After selecting the keywords in the form of mesh and text word, case–control and cross-sectional studies that were conducted between January 2000 and March 2022 were included. The following keywords were used to search for articles in various databases: Scopus: (TITLE-ABS-KEY ("autism spectrum disorder") OR TITLE-ABS-KEY ("autism") AND TITLE-ABS-KEY ("toxic heavy metal") OR TITLE-ABS-KEY ("toxic metal") OR TITLE-ABS-KEY ("trace element") OR TITLE-ABS-KEY ("non-essential element") OR TITLE-ABS-KEY ("lead") OR TITLE-ABS-KEY ("Pb") AND TITLE-ABS-KEY ("blood") OR TITLE-ABS-KEY ("urinary") OR TITLE-ABS-KEY ("urine") OR TITLE-ABS-KEY ("hair") AND TITLE-ABS-KEY ("children") OR TITLE-ABS-KEY ("child")); Web of science: TS = (autism spectrum disorder OR autism) AND TS = (toxic heavy metal OR toxic metal OR trace element OR non-essential element OR lead) AND TS = (blood OR urine OR hair) AND TS = (child OR children); and PubMed: "autism spectrum disorder"[Mesh]) AND "child"[Mesh]) AND "trace elements"[Mesh]) OR "metals, heavy"[Mesh]) OR "poisoning"[Mesh]) OR "toxicity" [Subheading]) OR "lead"[Mesh]) AND "blood"[Mesh]) OR "urine"[Mesh]) OR "hair"[Mesh].

### Selection Criteria

Inclusion criteria are all human studies that reported lead concentrations in blood, hair, and urine samples of children with ASD and the control group. Moreover, exclusion criteria in this study included studies with adults, with no history of chronic physical and psychiatric disorders, studies in which ASD was associated with other health situations, studies with highly abnormal values, and studies in overlapping conditions. Letter to editor, conference, and review and meta-analysis articles were removed from the list of articles.

### Data Extraction and Quality Assessment

The primary results were a list of publications with available titles, authors, and abstracts. The duplicate papers of different databases were removed. The titles and abstracts of retrieved studies were assessed by two independent researchers and items unrelated to the aim of this meta-analysis were not included. All full texts were then assessed to exclude items that did not meet the eligibility criteria defined in our study. A reference list of related studies was also screened to avoid missing related studies. The literature that fulfills all of our selection criteria underwent quality assessment using the Newcastle–Ottawa Scale (NOS). Disagreement about the inclusion or exclusion of papers was resolved by a third researcher. This study was conducted according to the requirements of PRISMA [20] (Table 1 and 2).

### Publication Bias

Publication bias was assessed first by visual evaluation of funnel plots, and then by Egger's Begg test [50]. Moreover, the trim-and-fill approach [51, 52] was applied to calculate the number of possible omitted studies.

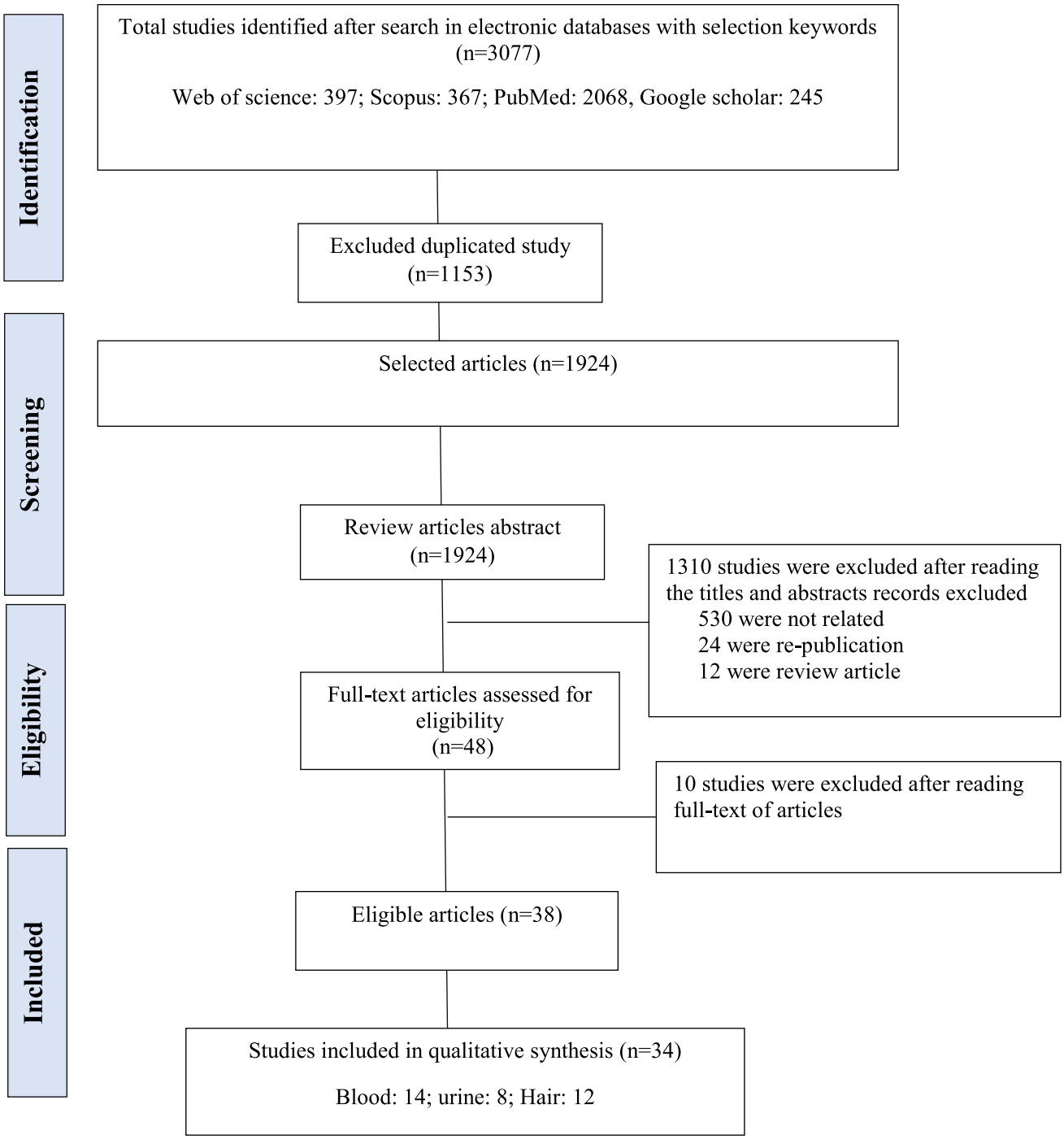


**Fig. 1** PRISMA flow diagram of study identification, inclusion, and exclusion

## Statistical Analysis

The heterogeneity of included studies was assessed using the *I*-squared ($I^2$) and chi-square-based *Q*-test. The *Q*-test results were considered significant at $p < 0.1$. The $I^2$ statistic was measured to show the total percentage of variation across different studies. When considerable heterogeneity (more than 70%) was detected, the pooled estimates were analyzed using a random-effects model; if heterogeneity was less than 70%, a fixed-effect model was applied. The publication bias possibility was identified using the funnel plot and the Egger test. Forest plots with Hedges' *g*, and 95% CIs were used to show pooled estimates (Table 3).

**Table 1** Characteristics of studies included in the meta-analysis

| Authors/year | Study population | Sample size and gender | Tissue | Mean age | Type of study | Diagnostic criterion for ASD | Outcome | Country |
|---|---|---|---|---|---|---|---|---|
| Albizzati et al. 2012 [21] | Children with ASD and control group | 17 children with ASD (15 boys and 2 girls) and 17 control subjects (15 boys and 2 girls) | Blood | Case: 11.52<br>Control:10.41 | Case–control study | DSM IV* | There was no statistically significant difference between the groups | Italy |
| Macedoni-Lukšič et al. 2015 [22] | Children with ASD and children with other neurological disorders | 52 children with ASD (7 girls, and 45 boys) and 22 children with other neurological disorders (10 girls, and 12 boys) | Blood | Case: 6.20<br>Control: 6.60 | Case–control study | DSM-IV-TR, Childhood Autism Rating Scale (CARS) | There was no statistically significant difference between the groups | Slovenia |
| Hawari et al. 2020 [23] | Children with ASD and control group | 31 children with ASD (5 girls and 26 boys) and 30 healthy children (10 girls and 20 boys) | Blood | - | Case–control study | DSM-V | Lead increase and manganese decrease may associate with the incidence of ASD | Syria |
| El Baz et al. 2016 [24] | Children with ASD and control group | Cases: 31 (23 boys and 8 girls)<br>Control: 20 (14 boys and 6 girls) | Blood | Case: 6.32<br>Control: 5.13 | Case–control | DSM IV | No significant differences were found | Egypt |
| Akinade et al. 2019 [25] | Children with ASD and controls | Eight with ASD and fifteen control subjects | Blood | Age range 2–12 years | Case–control | DSM IV | The lead level in ASD was slightly increased but not significant when compared with controls | Nigeria |
| Yassa et al. 2014 [26] | Children with ASD and control group | 45 children with ASD (13 girls, and 32 boys) and 45 healthy children (13 girls, and 32 boys) | Blood | - | Case–control study | DSM-V | There was a significant difference between the mean scores of lead between the groups | Egypt |
| Laura et al. 2011 [27] | Children with ASD and control group | 28 children with ASD (7 girls and 21 boys) and 32 healthy children (12 girls and 20 boys) | Blood | - | Case–control study | DSM | There was no statistically significant difference between the groups | Italy |
| Alawad et al. 2018 [28] | Children with ASD and control group | 60 children with ASD (16 girls and 44 boys) and 47 healthy children (11 girls and 36 boys) | Blood | - | Case–control study | Not mentioned | There was a significant difference between the mean scores between the groups | Iraq |
| El-Ansary et al. 2010 [29] | Children with ASD and control group | 60 children with ASD (16 girls and 44 boys) and 47 normal children (11 girls and 36 boys) | Blood | - | Case–control study | DSM-IV | There was a significant difference between the mean scores of lead between the groups | Saudi Arabia |
| Li et al. 2018 [30] | Children with ASD and control group | 180 children with ASD (30 girls and 150 boys) and 184 healthy children (38 girls and 146 boys) | Blood | Case: 5.06<br>Control: 6.12 | Case–control study | DSM-IV, ICD-10 | There was no statistically significant difference between the groups | China |

**Table 1** (continued)

| Authors/year | Study population | Sample size and gender | Tissue | Mean age | Type of study | Diagnostic criterion for ASD | Outcome | Country |
|---|---|---|---|---|---|---|---|---|
| Qin et al. 2018 [31] | Children with ASD and control group | 34 children with ASD (14 girls and 20 boys) and 38 normal children (17 girls and 21 boys) | Blood | Case: 4.19<br>Control: 4.32 | Cross-sectional study | DSM IV | The results showed that children with ASD had higher ($p < 0.01$, 0.05) Pb (ASD 31.9 µg/L, unaffected children 18.6 µg/L) | China |
| Alabdali et al. 2014 [32] | Children with ASD and control group | Cases: 58, all boys<br>Controls: 32, all boys | Blood | Ages: 3–12 years | Cross-sectional | DSM IV. CARS | patients with ASD had significantly higher Pb compared with the controls | Saudi Arabia |
| Rahbar M.H. et al. 2015 [33] | Children with ASD and control group | Cases: 100<br>Controls: 100 | Blood | Age 2–8 years | Case–control | DSM IV, ADI-R, CARS | there were no significant differences between the two groups | USA |
| Tian et al. 2011 [34] | Children with ASD and control group | Cases: 37 (32 boys, 5 girls)<br>Controls: 15 (11 boys, 4 girls) | Blood | Case:44.2 months<br>Control:41.2 months | Case–control | DSM IV, ADI-R, ADOS | there were no significant differences between the two groups | USA |
| Metwally et al. 2015 [35] | Children with ASD and control group | 55 children with ASD (16 girls and 39 boys) and 75 normal children (18 girls and 57 boys) | Urine | Case: 4.01<br>Control: 4.02 | Case–control study | DSM-V | The results of this study revealed that the mean level of lead was higher among the group suffering from ASD compared to their controls | Egypt |
| Adams et al. 2013 [36] | Children with ASD and neurotypical children | 55 children with ASD (6 girls, and 49 boys) and 44 neurotypical children (5 girls, and 39 boys) | Urine | Case: 10.00<br>Control: 11.00 | Case–control study | Not mentioned | The ASD group had higher levels of lead in RBC and had higher urinary levels of lead | USA |
| Albiaazti et al. 2012 [21] | Children with ASD and normal child | 17 children with ASD (2 girls, and 15 boys) and 20 normal children (5 girls, and 15 boys) | Urine | Case: 11.52<br>Control: 10.41 | Case–control study | DSM-IV, ADOS | There was no statistically significant difference between the groups | Italy |
| Yorbik et al. 2010 [37] | Children with ASD and typically developing children | 30 children with ASD (6 girls, and 24 boys) and 20 typically developing children (7 girls, and 13 boys) | Urine | Case: 6.9<br>Control: 5.6 | Case–control study | DSM-IV, ABC | There was a significant difference between the mean scores of lead between the groups | Turkey |
| Khaled et al. 2016 [38] | Children with ASD and control group | 40 children with ASD (8 girls, and 32 boys) and 40 healthy children (12 girls, and 28 boys) | Hair | Case: 4.11<br>Control: 5.23 | Case–control study | DSM-IV-TR, ADI-R, CARS | There was a significant difference between the mean scores of lead between the groups | Egypt |
| Domingues et al. 2016 [39] | Children with ASD and control group | 19 children with ASD (4 girls, and 15 boys) and 21 healthy children (4 girls and 17 boys) | Urine | Case: 6.9<br>Control: 0.7.4 | Case–control study | DSM IV-TR, ADOS | There was no statistically significant difference between the groups | Italy |

**Table 1** (continued)

| Authors/year | Study population | Sample size and gender | Tissue | Mean age | Type of study | Diagnostic criterion for ASD | Outcome | Country |
|---|---|---|---|---|---|---|---|---|
| Blaurock-Busch et al. 2011 [40] | Children with ASD and control group | 25 children with ASD (3 girls, and 22 boys) and 25 normal children (6 girls, and 19 boys) | Urine | Case: 6.24<br>Control: 6.80 | Cross-sectional study | DSM-IV, ABC | The mean levels of lead in the hair and urine of the ASD group were significantly higher than in the controls | Saudi Arabia |
| Adams et al. 2017 [41] | Children with ASD and control group | 67 children and adults with ASD and 50 neurotypical controls | Urine | 2.5–60 years | Case–control | DSM IV. CARS | mean excretion rates of lead were greater for the ASD group than the control group | USA |
| Albiaazti et al. 2012 [21] | Children with ASD and normal child control group | 17 children with ASD (2 girls, and 15 boys) and 20 normal children (5 girls, and 15 boys) | Hair | Case: 11.52<br>Control: 10.41 | Case–control study | DSM-IV, ADOS | There was no statistically significant difference between the groups | Italy |
| Fiłon et al. 2020 [42] | Children with ASD and control group | 30 children with ASD (5 girls, and 5 boys) and 30 healthy children (5 girls, and 25 boys) | Hair | Case: 5.25<br>Control: 5.09 | Case–control study | Not mentioned | There was a significant difference between the mean scores of lead between the groups | Poland |
| El-Ansary et al. 2017 [12] | Children with ASD and control group | 35 boy children with ASD 30 normal boy child | Hair | Case: 7.00<br>Control: 7.2 | Case–control study | DSM-IV-TR | The obtained data demonstrate a significant elevation of Pb in the hair of children with ASD when compared to the healthy controls | Saudi Arabia |
| Elsheshtawy et al. 2011 [43] | Children with ASD and control group | 32 children with ASD (8 girls, and 24 boys) and 32 healthy children (8 girls and 24 boys) | Hair | Case: 4.1<br>Control: 4.0 | Case–control study | DSM-IV | Results showed highly significant differences between the studied cases and the controls | Egypt |
| Yassa et al. 2014 [26] | Children with ASD and control group | 45 children with ASD (13 girls and 32 boys) and 45 healthy children (13 girls and 32 boys) | Hair | - | Case–control study | DSM-V | There was a significant difference between the mean scores of lead between the groups | Egypt |
| Kern et al. 2007 [44] | Children with ASD and control group | 45 children with ASD (10 girls, and 35 boys) and 45 healthy children (10 girls and 35 boys) | Hair | Case: 3.0<br>Control: 3.0 | Cross-sectional study | DSM-IV, clinical judgment | Lead was significantly lower in the hair of children with ASD than in matched controls | USA |
| El Baz Mohamed et al. 2015 [45] | Children with ASD and control group | 100 children with ASFD (16 girls and 84 boys) and 100 healthy children (26 girls and 74 boys) | Hair | Case: 6.24<br>Control: 6.80 | Cross-sectional study | DSM-IV-TR | The mean Levels of lead, in the hair of the ASD group were significantly higher than controls | Egypt |
| Blaurock-Busch et al. 2011 [40] | Children with ASD and control group | 25 children with ASD (3 girls and 22 boys) and 25 normal children (6 girls and 19 boys) | Hair | Case: 6.24<br>Control: 6.80 | Cross-sectional study | DSM-IV, ABC | The mean levels of lead in the hair and urine of the ASD group were significantly higher than controls | Saudi Arabia |

**Table 1** (continued)

| Authors/year | Study population | Sample size and gender | Tissue | Mean age | Type of study | Diagnostic criterion for ASD | Outcome | Country |
|---|---|---|---|---|---|---|---|---|
| Rashaid et al. 2021 [46] | Children with ASD and control group | 57 children with ASD (11 girls and 46 boys) and 50 normal children (9 girls and 41 boys) | Hair | Case: 7.33<br>Control: 7.60 | Case–control study | DSM-V | Exposure to Pb may contribute to ASD etiology | Jordan |
| Skanly et al. 2017a [47] | Children with ASD and control group | 16 children with ASD and 16 normal children | Hair | Case: 5.0<br>Control: 5.0 | Case–control study | DSM-IV-TR | Pb level was significantly lower in ASD patients than in controls | Russia |
| Skanly et al. 2017b [48] | Children with ASD and control group | 74 children with ASD and 74 normal children | Hair | Case: 5.12<br>Control: 5.11 | Case–control study | DSM-IV-TR | Pb level was lower in ASD patients than in controls | Russia |
| Zhai et al. 2019 [49] | Children with ASD and control group | 78 children with ASD (22 girls and 56 boys) and 58 normal children (27 girls and 31 boys) | Hair | Case: 4.96<br>Control: 4.90 | Case–control study | DSM-IV-TR | Concentrations of Pb was significantly higher in the ASD group than in the control group | China |

**DSM-IV*, Statistical Manual of Mental Disorders, 4th Edition, *DSM-V*, Statistical Manual of Mental Disorders, 5th Edition; *ICD-10*, International Classification of Diseases-10; *ADOS*, Autism Diagnostic Observation Schedule; *ABC*, Autism Behavior Checklist; *DSM-IV-TR*, Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision; *ADI-R*, Autism Diagnostic Interview-Revised; *CARS*, Childhood Autism Rating Scale

## Results

### Blood Lead Levels in Children with ASD and Control Group

In this section, 14 studies with a total sample size of 692 autistic children and 607 typical children were included (Table 1). For two studies the mean age of subjects was not reported. For the remaining studies (12 studies), the mean age of ASD and typical children were 6.91 and 6.74 years respectively. In the ASD group, 590 were boys and 102 were girls. In the control group, 532 were boys and 75 were girls. Pooling data from 14 studies under the random effect model showed a significant difference between the mean levels of lead between the two groups (Hedges' $g$: 1.12, 95% CI: 0.27, 1.97; $P = 0.010$) (Fig. 2). Heterogeneity was 95% ($I^2 = 95.3$, $Q(13) = 277.39$, $p < 0.001$) indicating a high heterogeneity between true mean effects ($\tau^2 = 2.54$). In other words, almost all variability of the observed variance comes from real differences between studies. The funnel plot (Fig. 3) and the results of the Egger test ($t = 3.13$, $P = 0.009$) indicated publication bias.

### Urinary Lead Levels in Children with ASD and Control Group

In this review, eight studies with a total sample size of 308 autistic children and 292 normal children were included (Table 1). For two studies the mean age of subjects was not reported. In six studies, the mean age of ASD and typical children was 8.64 and 8.47 years respectively. In the ASD group, 227 were boys and 81 were girls. In the control group, 203 were boys and 89 were girls. Pooling data from eight studies under the random effect model showed no significant difference between the mean levels of lead between the two groups (Hedges' $g$: 0.54, 95% CI: − 0.17, 1.25; $P = 0.137$) (Fig. 4). Heterogeneity was 93% ($I^2 = 93$, $Q(7) = 99.40$, $p < 0.001$) indicating a high heterogeneity between true mean effects ($\tau^2 = 0.975$). In other words, almost all variability of the observed variance comes from real differences between studies. The funnel plot (Fig. 5) and the results of the Egger's test ($t = 1.37$, $P = 0.203$) indicated no publication bias.

### Hair Lead Levels in Children with ASD and Control Group

Studies included for in-hair lead concentration levels were 12 studies with a total sample size of 537 autistic children and 512 healthy children (Table 1). The mean age of ASD and typical children were 6.67 and 6.61 years, respectively. Pooling Hedges's g effect sizes from 12

**Table 2** Quality assessment of studies included in the meta-analysis of lead (Pb) in ASD children: Based on the Newcastle–Ottawa Scale for cross-sectional studies

| Papers | Selection | | | | Comparab | Outcome | | Score |
|---|---|---|---|---|---|---|---|---|
| | Represen-tativeness | Size | Non-re-spondents | Ascertain-ment | Design/analysis | Ascertain-ment | Stat. test | |
| Blood | | | | | | | | |
| Albizzati et al. 2012 [21] | a | a | a | a | a | a | a | 7 |
| Macedoni-Lukšič et al. 2015 [22] | a | a | b | a | a | a | a | 6 |
| Hawari et al. 2020 [23] | a | a | b | a | b | a | a | 5 |
| El Baz et al. 2016 [24] | a | a | b | a | a | a | a | 6 |
| Akinade et al. 2019 [25] | a | a | a | a | a | a | a | 7 |
| Yassa et al. 2014 [26] | a | a | b | a | a | a | a | 6 |
| Laura et al. 2011[27] | a | a | b | a | a | a | a | 6 |
| Alawad et al. 2018 [28] | a | a | a | a | b | a | a | 6 |
| El-Ansary et al. 2010 [29] | a | a | a | a | a | a | a | 7 |
| Li et al. 2018 [30] | a | a | a | b | a | a | a | 6 |
| Qin et al. 2018 [31] | a | a | b | b | a | a | a | 5 |
| Alabdali et al. 2014 [32] | a | a | b | a | b | a | a | 5 |
| Rahbar et al. 2015 [33] | a | a | a | a | a | a | a | 7 |
| Tian et al. 2011[34] | a | a | b | a | a | a | a | 6 |
| Urine | | | | | | | | |
| Metwally et al. 2015 [35] | a | a | a | a | a | a | a | 7 |
| Albizzati et al. 2012 [21] | a | a | a | a | a | a | a | 7 |
| Adams et al. 2013 [36] | a | a | b | a | a | a | a | 6 |
| Adams et al. 2017 [41] | a | a | a | a | a | a | a | 7 |
| Yorbik et al. 2010 [37] | a | a | a | a | b | a | a | 6 |
| Khaled et al. 2016 [38] | a | a | a | a | a | a | a | 7 |
| Domingues et al. 2016 [39] | a | a | b | a | a | a | a | 6 |
| Blaurock-Busch et al. 2011 [40] | a | a | b | a | b | a | a | 5 |
| Hair | | | | | | | | |
| Albizzati et al. 2012 [21] | a | a | a | a | a | a | a | 7 |
| Albizzati et al. 2012 [21] | a | a | a | a | a | a | a | 7 |
| El-Ansary et al. 2017 [12] | a | a | a | a | a | a | a | 7 |
| Elsheshtawy et al. 2011 [43] | a | a | b | a | a | a | a | 6 |
| Yassa et al. 2014 [26] | a | a | b | a | a | a | a | 6 |
| Kern et al. 2007 [44] | a | a | b | a | a | a | a | 6 |
| El Baz Mohamed et al. 2015 [45] | a | a | a | a | a | a | a | 7 |
| Blaurock-Busch et al. 2011 [40] | a | a | b | a | b | a | a | 5 |
| Rashaid et al./2021 [46] | a | a | a | a | a | a | a | 7 |
| Skanly et al./2017a [47] | a | a | a | a | a | a | a | 7 |
| Skanly et al./2017b [48] | a | a | a | a | a | a | a | 7 |
| Zhai et al./2019 [49] | a | a | a | a | a | a | a | 7 |

Selection: (1) Representativeness of the sample, *a*, truly representative of the average in the target population; *b*, somewhat representative of the average in the target population; *c*, selected group of users; *d*, no description of the sampling strategy. (2) Sample size, *a*, justified and satisfactory; *b*, not justified. (3) Non-respondents, *a*, comparability between respondents and non-respondent characteristics is established, and the response rate is satisfactory; *b*, the response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory; *c*, no description of the response rate or the characteristics of the responders and the non-responders. (4) Ascertainment of exposure, *a*, validated measurement tool; *b*, non-validated measurement tool, but the tool is available or described; *c*, no description of the measurement tool. Comparability, (1) Comparability of subjects based on the design or analysis, *a*, the study controls for the most important factor; *b*, the study control for any additional factor. Outcome: (1) ascertainment of the outcome, *a*, independent blind assessment; *b*, record linkage; *c*, self-report; *d*, no description. (2) Statistical test, *a*, the statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (*p*-value); *b*, the statistical test is inappropriate, not described or incomplete

**Table 3** The effect size (SMD and OR) and their 95% respective confidence of primary studies. An estimate of the pooled effect size was calculated by averaging over primary effect sizes weighted by fixed and random effects model

| Study | Effect size | | % weight | |
|---|---|---|---|---|
| | Hedges | 95% CI | Fixed | Random |
| Blood lead level | | | | |
| Hawari et al. 2020 [23] | 0.41 | −0.99, 0.91 | 4.11 | 5.93 |
| Akinade A et al. (2019) [25] | 1.10 | 0.22, 1.99 | 1.31 | 5.56 |
| El-baz et al. (2018) [45] | 1.15 | 0.49, 1.81 | 2.39 | 5.80 |
| Alawad et al. (2018) [28] | 1.78 | 1.33, 2.23 | 5.15 | 5.96 |
| Qin et al. (2018) [31] | 1.26 | 0.76, 1.76 | 4.09 | 5.92 |
| Li et al. (2017) [30] | −0.09 | −0.30, 0.11 | 24.52 | 6.08 |
| Rahbar et al. (2015) [33] | −0.23 | −0.51, 0.04 | 13.44 | 6.06 |
| Yassa et al. (2014) [26] | 6.35 | 5.34, 7.37 | 1.00 | 5.41 |
| Macedoni-Lukšič et al. (2015) [22] | 0.41 | −0.08, 0.91 | 4.17 | 5.93 |
| Alabdali A (2014) [32] | 2.33 | 1.78, 2.88 | 3.45 | 5.89 |
| Albiaazti et al. (2012) [21] | 0.55 | −0.12, 1.22 | 2.30 | 5.79 |
| Laura et al. (2011) [27] | 0.26 | −0.24, 0.77 | 4.08 | 5.92 |
| Tian Y et al. (2011) [34] | 0.00 | −0.59, 0.59 | 2.96 | 5.86 |
| El-Ansary et al. (2010) [29] | 1.82 | 1.37, 2.27 | 5.08 | 5.96 |
| Urinary lead level | | | | |
| Metwally et al. (2015) [35] | 0.77 | 0.41, 1.13 | 8.03 | 6.02 |
| Adams J (2017) [41] | 2.41 | 1.93, 2.89 | 17.87 | 14.44 |
| Domingues et al. (2016) [39] | 0.25 | −0.36, 0.86 | 10.91 | 14.18 |
| Adams et al. (2013) [36] | 0.19 | −0.21, 0.58 | 26.18 | 14.58 |
| Albizzati et al. (2012) [21] | −0.07 | −0.72, 0.59 | 9.44 | 14.08 |
| Khaled et al. (2016) [38] | 1.0 | 0.54, 1.46 | 4.30 | 4.38 |
| Blaurock-Busch et al. (2011) [40] | 0.84 | 0.27, 1.41 | 12.52 | 14.27 |
| Yorbik et al. (2010) [37] | −1.18 | −1.79, −0.58 | 11.18 | 14.20 |
| Hair lead level | | | | |
| Rashaid et al. (2021) [46] | 1.05 | 0.59, 1.52 | 4.25 | 4.38 |
| Fiłon et al. (2020) [42] | 2.64 | 1.95, 3.33 | 1.93 | 4.36 |
| Zhai et al. (2019) [49] | 2.16 | 1.74, 2.59 | 5.07 | 4.38 |
| El BazMohamed et al. (2015) [45] | 0.38 | 0.10, 0.66 | 11.78 | 4.39 |
| Skanly et al. (2017) b [48] | 0.66 | −0.03, 1.36 | 1.89 | 4.35 |
| Skanly et al. (2017) a [47] | −0.77 | −1.10, −0.44 | 8.28 | 4.39 |
| El-Ansary et al. (2017) [12] | 0.47 | −0.02, 0.96 | 3.83 | 4.38 |
| Yassa et al. (2014) [26] | 6.90 | 5.81, 7.98 | 0.77 | 4.29 |
| Albizzati et al. (2012) [21] | −0.12 | −0.78, 0.54 | 2.12 | 4.36 |
| Blaurock-Busch et al. (2011) [40] | 0.0 | −0.55, 0.55 | 3.07 | 4.37 |
| Elsheshtawy et al. (2011) [43] | 2.07 | 1.46, 2.67 | 2.52 | 4.37 |
| Kern et al. (2007) [44] | −0.02 | −0.43, 0.39 | 5.45 | 4.38 |

primary studies under the random effect model showed a significant difference between the mean levels of lead between ASD and typical children (Hedges' $g$: 1.25, 95% CI: 0.14, 2.36; $P = 0.01$) (Fig. 6). Heterogeneity was 96.5% ($I^2 = 96.5$, $Q(11) = 316$, $p < 0.001$) indicating a high heterogeneity between true mean effects ($\tau^2 = 3.75$). In other words, almost all variability of the observed variance comes from real differences between studies. The funnel plot (Fig. 7) and the results of the Egger test showed ($t = 2.39$, $p = 0.038$) indicated the existence of publication bias.

## Discussion

In the current study, we performed meta-analyses to compare lead levels in different tissues between children with ASD and control subjects. The results of our study demonstrated that compared with the corresponding values in the control group, the ASD group had elevated lead levels in blood, and hair. In terms of urinary lead concentrations, the current study does not reinforce the view that ASD is associated with the alteration of urinary lead concentrations. A previous meta-analysis study failed to evidence significant

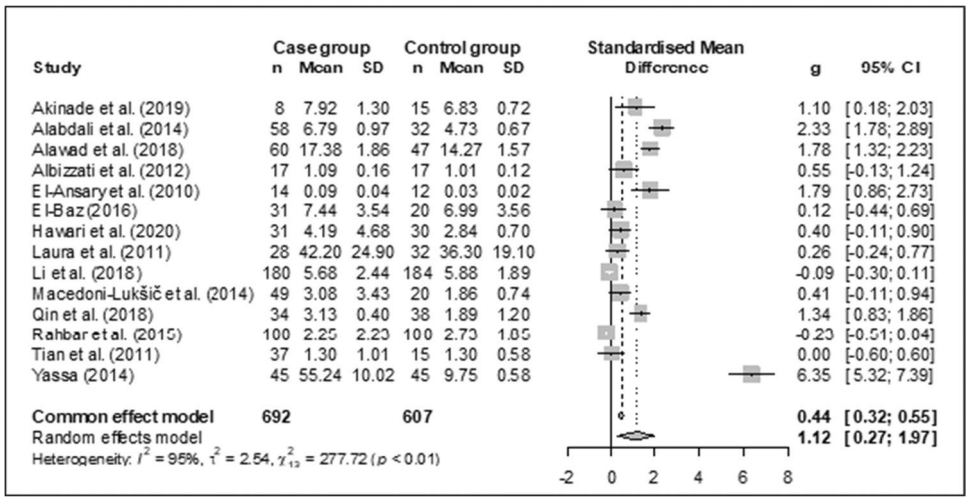


**Fig. 2** The forest plot of the meta-analysis addressing the mean concentration levels of lead in autistic children (case group) compared to typical children (control group) using blood samples. For primary studies, the sample size ($n$), mean, and standard deviation (SD), standardized mean difference (SMD), and its corresponding Hedge's $g$ effect size with 95% CI are also shown. The horizontal line represents 95% CI of SMD and the vertical line denotes SMD = 0 (no difference between the two groups). Studies on the left side of the vertical line had $SMD < 0$ suggesting higher concentration levels of lead in typical children as compared to ASD children. Studies on the right side of the vertical line had SMD > 0 and favored higher levels of lead in autistic children. Pooled results from all studies are shown at the bottom with the random-effect model. Heterogeneity indices, as well as the $p$ value for Cochran's $Q$-test of heterogeneity, are also presented

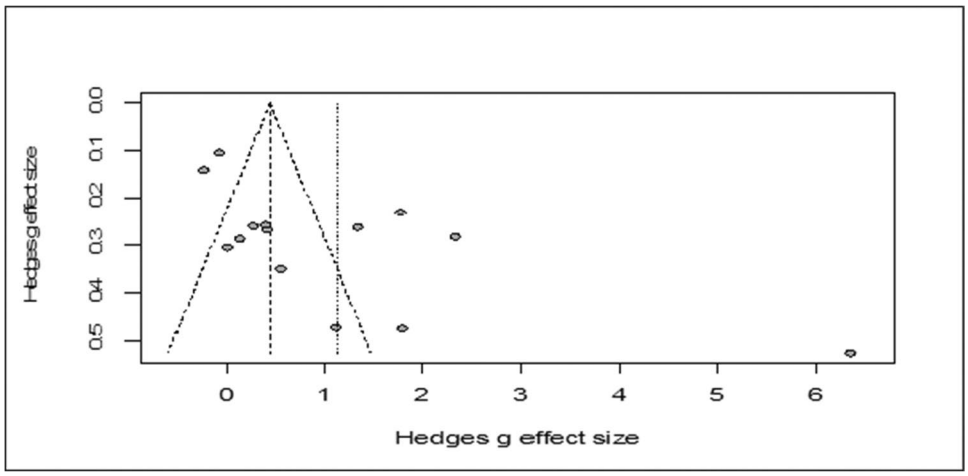


**Fig. 3** Funnel plots of Hedges' g effect sizes for blood lead levels

differences in urinary lead concentrations between their studied groups but they found a significant association between hair and blood lead levels [1]. It is noteworthy that lead concentrations in blood and urine are short-term and recent exposure criteria instead of past cumulative state during brain development of children. The hair measurement is more associated with long-term exposures and is, therefore, a more appropriate criterion of accumulative exposures. For this reason, more strengths were considered for the evidence obtained from hair samples than the documents from urine and blood assessments [1]. Another meta-analysis study documented an association between the severity of ASD and heavy metal concentration (mostly mercury and lead) [53]. Based on the results of a Cochrane systematic review suggest no evidence for the beneficial effect of pharmaceutical chelation therapy for ASD children [54]. The inconsistent evidence for the link between Pb exposure and the risk of ASD supports the need for further studies.

The previous literature implicated heavy metals in causing negative neurodevelopmental effects [1]. Pb is one of the most abundant heavy metals in the Earth's crust. The main sources of exposure in the environment are paint, soil, dust, and dirt. Occupational and environmental exposures leave people vulnerable to lead poisoning, especially in industrial areas [55]. Lead is known as a developmental toxin that adversely affects different organs including the nervous

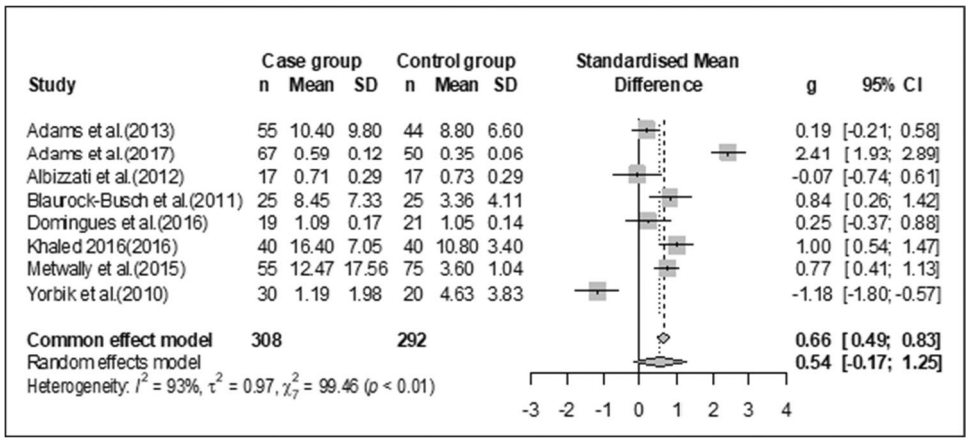


**Fig. 4** The forest plot of the meta-analysis addressing the mean concentration levels of lead in autistic children (case group) compared to typical children (control group) using urinary samples. For primary studies, the sample size ($n$), mean, and standard deviation (SD), standardized mean difference (SMD), and its corresponding Hedge's $g$ effect size with 95% CI are also shown. The horizontal line represents 95% CI of SMD and the vertical line denotes SMD = 0 (no difference between the two groups). Studies on the left side of the vertical line had SMD < 0 suggesting higher concentration levels of lead in typical children as compared to ASD children. Studies on the right side of the vertical line had SMD > 0 and favored higher levels of lead in autistic children. Pooled results from all studies are shown at the bottom with the random-effect model. Heterogeneity indices, as well as the $p$ value for Cochran's $Q$-test of heterogeneity, are also presented

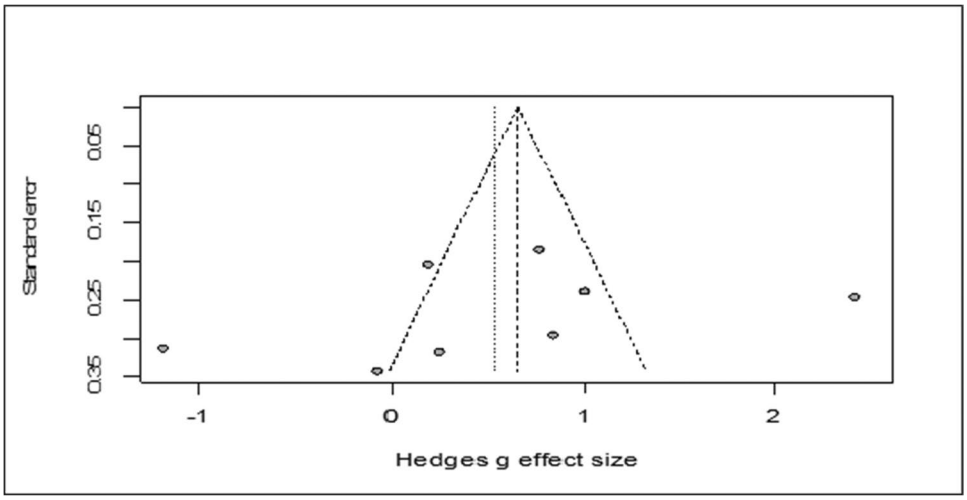


**Fig. 5** Funnel plots of Hedges' $g$ effect sizes for urinary lead levels

system and impairs cognitive and intellectual skills even at low exposure levels [1, 56]. This toxic heavy metal has evidence of damage to the developing nervous system, leading to disorders such as IQ loss and behavioral problems [57]. Lead exposure in children can impair mental development and normal growth and cause serious neurological disorders, such as brain damage, mental retardation encephalopathy, increased intracranial pressure, and cerebral edema [55, 58]. These health effects are not limited to high-exposure settings but have been seen with low exposure concentrations, resulting in "silent toxicity" [59]. Children are highly susceptible to lead poisoning due to their specific oral behaviors [55]. Children with neurodevelopmental problems such as ASD are also more likely to be exposed to lead via increased pica and hand-mouth behaviors, thereby increasing the risk of high lead accumulation [60]. One proposed mechanism of Pb is its ability for mimicking zinc and calcium, resulting in lead crossing the blood–brain barrier (BBB) through the same vector and interfering with neurotransmissions at the synapses [61, 62]. It can pass the placenta and the BBB, accumulate in the growing brain, and directly interact at the cellular level using different mechanisms, including the interference with key cellular receptors and producing reactive oxygen species [57, 61, 63] Experimental studies have shown that lead in the CNS can apply dose-dependent effects such as apoptosis and synaptogenesis dysregulations [64].

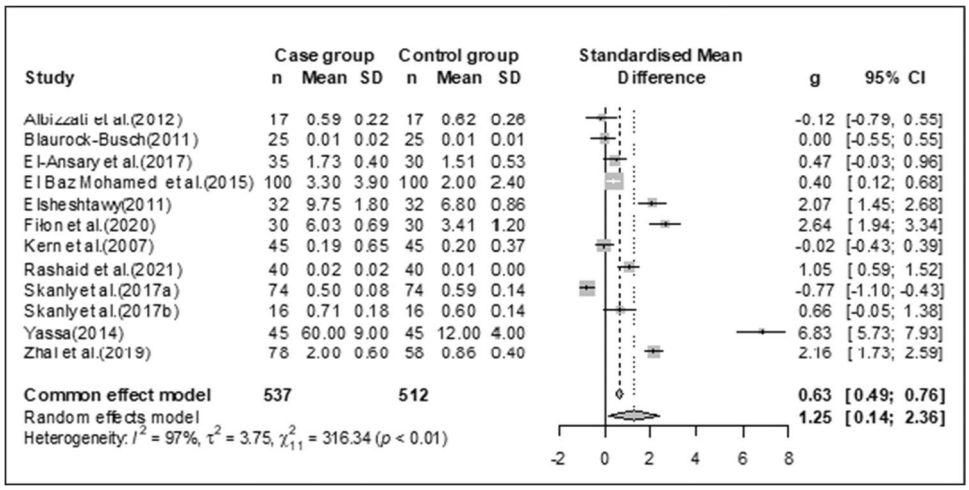


**Fig. 6** The forest plot of the meta-analysis addressing the mean concentration levels of lead in autistic children (case group) compared to typical children (control group) using hair samples. For primary studies, the sample size ($n$), mean, and standard deviation (SD), standardized mean difference (SMD), and its corresponding Hedge's $g$ effect size with 95% CI are also shown. The horizontal line represents 95% CI of SMD and the vertical line denotes SMD = 0 (no difference between the two groups). Studies on the left side of the vertical line had SMD < 0 suggesting higher concentration levels of lead in typical children as compared to ASD children. Studies on the right side of the vertical line had SMD > 0 and favored higher levels of lead in autistic children. Pooled results from all studies are shown at the bottom with the random-effect model. Heterogeneity indices, as well as the $p$-value for Cochran's $Q$-test of heterogeneity, are also presented

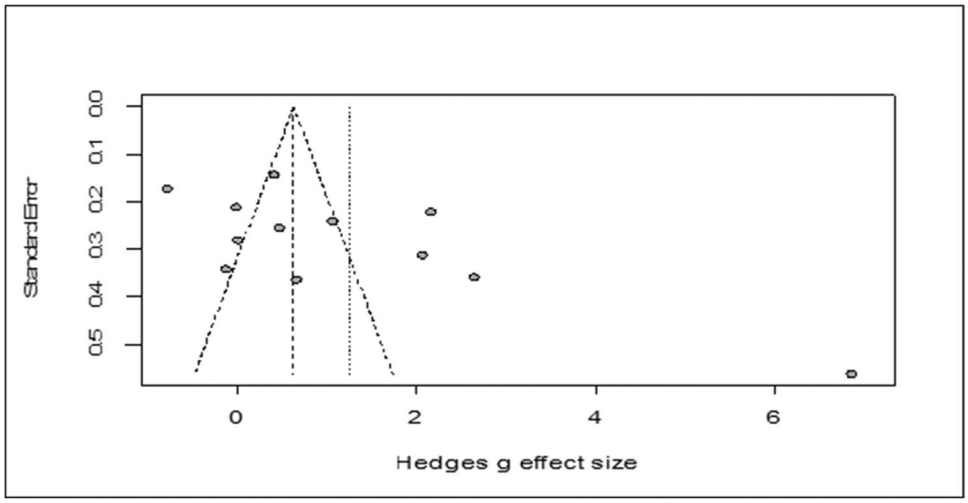


**Fig. 7** Funnel plots of Hedges' $g$ effect sizes for hair lead levels

## Limitations

The current meta-analysis study has some limitations that should be considered in interpreting our results. The included studies have different study population characteristics including the family history, ethnicity, education, duration of exposure, assessment of independent variable, and the method for ASD confirmation. For example, some documents contain information not only on lead levels but also on other trace elements. Some other heavy metals are also associated with a higher risk of ASD. We were unable to adjust the potentially confounding effects of other trace elements on ASD risk.

Some studies employed different versions of the Diagnostic and Statistical Manual of Mental Disorders (DSM) as a diagnostic tool for ASD. Moreover, some of them applied various auxiliary scales simultaneously. Also, there were considerable differences in laboratory operating processes and reliability of the experimenter among the included studies. Such uncertain elements make it hard to know how they affect the results of our meta-analysis. The included studies were not specified the isotopic composition of lead. Lead

isotope ratio measurements provide analytical information relating to the source of lead contamination in the samples. Concentration measurements cannot provide this information. The difference in the site of sampling may affect the results of the analysis. Guo et al. (2019) reported that the hair Pb levels were higher in children with ASD in the studies that collected samples from the back of the neck (but not in samples from other areas). These showed that the amount of accumulated Pb in the hair may alter by the hair growth position. They also reported that larger sample sizes of children will make smaller differences between ASD cases and control subjects [65]. Therefore, these factors should be considered in future research examining hair samples in children. Also, most studies had a case–control design and compared the differences in metal concentration between the ASD group and controls. So, the occurrence and causality could not be determined according to the case–control and cross-sectional study design. Furthermore, future investigations are needed to assess the effects of toxic metals exposure in a cohort study to determine the casual relationships. The sources of heterogeneity at the trace element level through these limited studies were not assessed in the current study.

The mentioned limitations in this body of evidence point to gaps in the scientific literature and pave the way for optimizing future studies related to the effects of environmental factors on ASD risk.

## Conclusion

The results of the current meta-analysis study showed that, compared to controls, children with ASD had increased lead levels in blood and hair. This study does not reinforce the view that ASD is associated with altered urinary lead concentrations. Our study highlights the need for large-scale human research to accurately measure and determine the long-term body load of lead exposure to identify the impact of lead exposure on ASD risk.

### Clinical and Environmental Implications

The scientific literature can help in making policy decisions. Our research findings can help researchers, physicians, families, policymakers, and funding agencies in accelerating the scientific discovery in this field as well as developing evidence-based decision-making on how to take action for preventing future harm to children. Accurate measurement of human exposure to harmful metals during the developmental period is crucial. Identification of environmental factors in ASD and neurodevelopment is an important unmet public health and clinical need, which introduces the risk of environment for neurodevelopment. There is a need for finding the sources and ways of lead exposure for children and their mothers during pregnancy. In the clinical setting, the use of chelation therapy in ASD is not well understood [66]. The lack of clear clinical evidence to support the use of chelation therapy and its potential side effects confirms the need for risk–benefit assessments.

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**Author Contribution** VF and BM generated the idea and design of the study. BM and FR searched the literature in databases and wrote some parts of the manuscript. AA and NA participated in statistical analyses and edited the result part. SN, AA, VF, NA, BM, and FR reviewed the manuscript. SN and BM wrote the discussion section. All authors have read and approved the final version of the manuscript. BM acted as the corresponding author.

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**Data Availability** The datasets used and analyzed during the current research are available from the corresponding author on request.

## Declarations

**Ethics Approval and Consent to Participate** This study was approved by the Research and Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1399.700).

**Consent for Publication** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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