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Hair, serum and urine chromium level in children with cognitive defect: A systematic review and meta-analysis of case control studies

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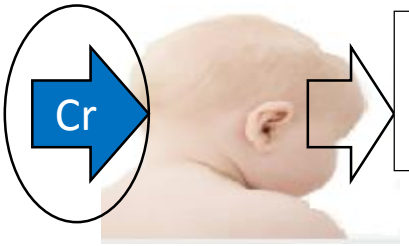
Graphical Abstract



Articles identified through database search (n=61)
CINAHL: 17, Embase 18, Web of Science 1, PubMed 25

Articles remaining after duplicate removal n=39

Records excluded after title and abstract search (n=27)

Case control study includes:
Only hair Cr level (n=7)
Only serum Cr level (n=2)
Only urine (n=1)
Hair and serum (n=1)
Hair and urine (n=1)

Hair

Serum

Urine

Overall SMD: -0.01 µg/g
95% CI: –0.04, 00
p=0.27
I^2 = 98.64%
$p_{heterogeneity}$ < 0.00001

Overall SMD: 0.32 µg/g
95% CI: -0.78, 1.42
p=0.32
I^2 =99.33 %,
$p_{heterogeneity}$ < 0.00001

Overall SMD: 0.64 µg/g
CI: -0.07,1.36
p=.08
I^2 =66.42%
$p_{heterogeneity}$ < 0.05

Hair

Serum

Urine

Pooled standardized mean difference Cr level low in cases vs. controls

Cr level low in cases vs. controls

Cr level high in cases vs. controls

Revised manuscript Clean version (double-spaced and continuously LINE and PAGE numbered)

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Hair, serum and urine chromium level in children with cognitive defect: A systematic review and meta-analysis of case control studies

Abstract

Environmental chromium exposure may cause impaired development of children. We conducted a systematic review and meta-analysis. Electronic databases including PubMed, Embase, Web of Science and CINAHL were searched to identify case-control studies that reported childhood Cr exposure and cognitive development. The Newcastle-Ottawa Scale (NOS) was used to ensure the quality of the included studies. Cr levels were compared in cases and controls, and a random effect meta-analysis was performed using Stata version 16. Twelve of 61 studies identified in the literature search were eligible for this analysis. Hair, serum and urine Cr measurements were reported by seven, two and one studies, respectively. In addition, one study reported both serum and hair Cr exposure and another reported urine and hair Cr exposure. The pooled standard mean differences (SMD) showed that hair Cr levels were non-significantly lower among children with cognitive defects (-0.01 µg/g, 95% CI: –0.04, 00, *p*=0.27). In serum and urine, the pooled SMD was higher in children with cognitive deficits compared with healthy control children (0.32 µg/g, 95% CI: -0.78, 1.42, p=0.56 and 0.64 µg/g, CI: -0.07,1.36, p=0.08; respectively). In summary, this systematic review found no significant differences in hair, serum and urine Cr levels between children with cognitive deficits and healthy control children when all study data were pooled in the meta-analysis. Larger studies using standardized criteria and longitudinal assessment of cognitive development are needed to determine whether there is a dose response effect of childhood Cr exposure on cognitive development of children.

Key words: Chromium, children, cognitive development, systematic review, meta-analysis

Introduction

Globally, concerns regarding exposure to environmental pollutants have been raised due to the adverse influences of pollutant exposure on public health. Various environmental chemicals may have an adverse effect on neurodevelopment (Adams et al., 2006; Zeng et al., 2019). Millions of children have never had an opportunity to reach their full neurodevelopmental potential due to metal and trace element exposures during pregnancy

and childhood (Grandjean and Landrigan, 2014; Wang et al., 2016; Ye et al., 2017). Children are particularly vulnerable to exposure to environmental chemicals owing to inadequate development of metabolic pathways and a higher degree of exposure per body weight (Rodríguez-Barranco et al., 2016; Choi et al., 2017; Ghassabian et al., 2018). For this reason, children are at increased risk of adverse neuropsychological and cognitive outcomes (González-Alzaga et al., 2015; Ye et al., 2017; Liu et al., 2019).

Chromium (Cr) is present in numerous oxidation forms, but the most stable and usual states are the trivalent Cr (III) and hexavalent Cr (VI) groups. Cr is present widely in nature and Cr (VI) is used in various industrial processes, for example, tanning of animal hides, alloying, plating, textile dyes and mordants, inhibition of water erosion, pigments, ceramic glazes and many more uses (Kimbrough et al., 1999; Keegan et al., 2008; ATSDR, 2012). The harmful effects of Cr(VI) on mammals were found in some animal model studies, for example: Cr(VI) may contribute to the development of different types of cancer (McCarroll et al., 2010; Thompson et al., 2014), may cause DNA damage, hepatic oxidative stress and hepatocyte apoptosis (Wang et al., 2006). In a rat model, Cr(VI) promoted oxidative stress and toxicity in cultured cerebellar granule neurons (Dashti et al., 2016). Furthermore, while Cr(VI) has harmful effects, Cr(III) has beneficial effects and is used as part of dietary supplements for weight loss, increasing muscle mass, and decreasing body fat as well as to control diabetes mellitus (Ghosh et al., 2002; Lukaski et al., 2007; Panchal et al., 2017). In addition ingested Cr(VI) may reduce to Cr(III) (Petrilli et al., 1986; De Flora et al., 1987; Suzuki and Fukuda, 1990). Nevertheless, any consistent dose-response relationships between Cr(III) and beneficial health outcomes in humans have not yet been established (EFSA Panel on Dietetic Products and Allergies, 2014; Vincent, 2018). However, the United State Environmental Protection Agency (US EPA) classifies Cr(VI) as a known carcinogen and the acceptable oral reference dose (RfD) for chromium (VI) is $\leq$0.003 mg/kg/d (USEPA, 1998).

While it is evident that exposure to certain heavy metals may interfere with neurodevelopment of children (Bao et al., 2009; Rodríguez-Barranco et al., 2016; Ye et al., 2017), yet to the best of our knowledge the association between postnatal Cr(VI) exposure and neuropsychological and cognitive outcome in children has not been addressed conclusively to date (ATSDR, 2012). Several biomarkers have been suggested to be useful for diagnosis and monitoring of children's neuropsychological development and its association with exposure to heavy metal and trace element (Ray et al., 2011; Saghazadeh and Rezaei, 2017; Caparros-Gonzalez et al., 2019; Zhou et al., 2020). The molecular

mechanism through which trace elements including Cr(VI) might affect child development is poorly understood. The aim of this systematic review and meta-analysis is to investigate Cr exposure as measured in different biological samples (e.g., in hair, serum, urine) in children with cognitive deficit compared with normal children.

2. Materials and methods

2.1 Search strategy

We conducted a systematic literature review and meta-analysis prepared according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement(Page et al., 2021). We searched the PubMed, Embase, Web of Science and CINAH databases for relevant literature up until December 14, 2020. The following search terms were used: chromium (chromium* or Cr) AND cognitive development (neurodevelopment* OR mental OR intelligence OR cognition* OR brain OR memory OR iq OR 'intelligence quotient' OR neurocognitive* OR psychomotor* OR sensorimotor* OR motor* 'executive function' OR attention* OR memory* OR learning* OR emotion* OR emotional* OR behavior problem) AND offspring (offspring OR children OR child* OR infant OR school OR youth OR preschool* OR kindergarten OR adolescent* OR student* OR teen*). We did not apply any language restrictions during the search. Our search strategies consisted of a combination of free-text words, words in titles and abstracts and key words.

2.2. Eligibility criteria

We included only case-control studies that addressed childhood Cr exposure as measured in hair, urine and blood/serum or any other biomarkers used to reflect childhood exposure to Cr that affected any kind of cognitive development (case). Case–control studies are often considered one of the first approaches in an etiological study of a disease or health condition. Though longitudinal studies are particularly useful for evaluating exposure–response relationships in our search, we did not find any such a study in our search. In our searches we did not impose any language, date, or study design limitations. To manage all the searched studies, EndNote software was used.

2.3. Assessment of quality of studies included in the systematic review and data extraction

Two reviewers (GMRI and MMR) independently screened titles and abstracts and then reviewed full texts of selected studies to assess eligibility. The references of selected article were also screened to avoid the missing of potential article. Newcastle-Ottawa Scales (NOS) were used to assess methodological quality of the studies. The NOS suggest using a checklist to judge the quality of studies across three areas: selection, comparability, and outcomes, using a "star" rating system to assess the quality of included studies (Wells GA 2011). Scores range from zero to nine where zero star is used for worst quality and nine stars is used for the best quality study. In our analysis, studies with scores ≤ 5 were considered to be of relatively high quality. No foreign language articles were found that needed to be translated into English. Data from these articles were compiled in Microsoft Excel and included the authors' names, publication years, countries, sample sizes, Cr exposure levels, and the specific assessments of neurodevelopment. We resolved any inconsistency that arose during the activity through a consensus process.

2.4 Statistical analysis

To compare hair, urine and serum Cr levels in cases compared with control children, a random effect meta-analysis was performed using STATA version 16 with standardized mean difference (SMDs) and 95% confidence intervals (CIs). As the Cr levels were different in the studies, we used SMD and 95% CI in the forest plot. Using Q and I^2 statistics the heterogenicity among the studies was assessed. In this meta-analysis, we consider heterogenous for p <0.10 or I^2>50 % and p-value (2 sided) <0.05 was statistically significant (Higgins and Thompson, 2002). After conducting sensitivity analyses for hair chromium levels, we removed two studies as highly influenced on the basis of SMD and the rest were analyzed to evaluate whether the results were statistically significant. Further, we assessed publication bias by visual inspection of funnel plots and formal testing using and Egger's tests (Egger et al., 1997). The units of measurement of hair, serum and urine levels were μg/g and μg/L. To detect sources of heterogeneity, further subgroup analysis was performed according to region of the study (e.g., Asia, America and Europe) and age less than five, greater than five, and mixed (studies including children both less than and greater than five years old were considered as mixed).

3. Results

3.1 Literature search and study characteristics

A total of 61 studies were identified from the different databases (viz., CINAHL: 17, Embase: 8, Web of Science: 1, PubMed: 25); however, after deleting duplication studies, the title and abstract of 39 studies were screened (Figure 1). Twenty-seven studies were rejected because they did not have relevant data and therefore the full texts of 12 studies were considered eligible for analysis. Among them seven studies reported hair Cr measurements (Kracke, 1982; Wecker, 1985; Al-Ayadhi, 2005; Munakata et al., 2006; De Palma et al., 2011; Skalny et al., 2016; Alqhazo and Rashaid, 2018), two studies serum (Wojciak et al., 2013; Skalny et al., 2020) and one study urine Cr level only (Yorbik et al., 2010). The report of Kracke (1982) encompassed two comparisons, psychotic vs. control and neuro-group vs. normal (Kracke, 1982) from the hair Cr level. Tinkov et al. reported hair and serum Cr levels and Blaurock-Bush reported urine and hair Cr levels (Blaurock-Busch et al., 2011; Tinkov et al., 2019). Results of quality assessment by NOS are shown in Supplementary table 1. Most included studies were high quality and the scores of included studies comprised of 5 or more. The studies were published from 1982 to 2020 (Table 1). We considered any kind of cognitive disability (e.g., autism, autism spectrum disorder, psychotic, stutter, epilepsy etc.) as the outcome. The summary of the Cr exposure and study characteristics are given in table 1.

<Insert Fig.1 >; <Insert Table 1>

3.2 Standard mean differences of Cr levels

For the hair levels the pooled SMD between cases and controls for the ten studies was -0.04 µg/g (95% CI: –0.09, 00; I^2 = 99.74%; $p_{\text{heterogeneity}} < 0.00001$). The findings imply that hair Cr level was significantly lower in cases compared with controls ($p \leq 0.05$, Fig. 2). We performed a sensitivity analysis to determine in summary effects by dropping two studies (Wecker, 1985; Al-Ayadhi, 2005) that we determined as highly influenced on the basis of SMD. After their removal, the overall pooled SMD became -0.01 µg/g (95% CI: –0.04, 00; I^2 = 98.64%; $p_{\text{heterogeneity}} < 0.00001$) and thus the overall effect size did not significantly differ (p=0.27; Fig. 3).

<Insert Fig.2 >; <Insert Fig.3 >

The subgroup analysis for hair Cr level between case and control children also showed a significantly lower amount of Cr in cases compared with controls in all three regions, e.g., Asia, America, and Europe (Table 2, see supplementary Fig. 1) but no consistent regional variation was observed (p= 0.66, supplementary Fig. 1). Similarly, the result of subgroup analysis for hair Cr level illustrated a significantly lower Cr level in the children with

cognitive defect (Table 2, supplementary Fig. 2) and did not reveal any consistent significant variation in the three age groups (supplementary Fig. 2, p=0.33).

<Insert Table 2>

In case of serum levels, the pooled effect size was 0.32 µg/g (95% CI: -0.78, 1.42); I^2 =99.33 %, *pheterogeneity* < 0.00001) and the effect size did not represent significant differences between cases and controls (p=0.56; Fig. 4). Similarly, from the urine sample, the pooled SMD was 0.64 µg/g (CI: -0.07,1.36); I^2 =66.42 %; $p_{\text{heterogeneity}} < 0.05$) indicating that the Cr level was higher in cases compared with the controls, but the effect size did not show significant differences between children with any cognitive deficits (cases) and children with normal cognition (controls) (p=0.08; Fig. 5).

<Insert Fig 4>; < Insert Fig 5>

3.3 Publication bias

The funnel plot of all the meta-analyses is presented in supplementary figure 3 (A-D, 4 and 5). Using Egger's test to evaluate publication bias, we found significant evidence of publication bias among the 10 studies of hair Cr levels (p=<0.0001) (supplementary Fig. 3A); on sensitivity analysis after removing two studies (Wecker, 1985; Al-Ayadhi, 2005) (Fig. 3), Egger's tests did not reveal significant evidence of publication bias among the included studies (supplementary Fig. 3B, p=0.50). Supplementary figures 4 and 5 show the funnel plot for serum and urine Cr level. We did not perform the Eggers test for serum and urine Cr level as there was an insufficient number of selected studies.

4. Discussion

This study is the first to address the standardized mean difference (SMD) of hair, serum and urinary chromium exposure between children with adverse cognitive outcomes and children with normal development. Because the child cognitive development measure in different studies used different kinds of instruments or disability syndromes (e.g., stuttering, psychotic, motor disabilities, epilepsy etc.), it was difficult to pool and compare outcomes. Our findings nevertheless show that the pooled SMD was low in the hair of children with cognitive defects compared with healthy children whereas the serum and urine Cr levels were high among children with autism though the pooled SMD was not significant.

Out of ten studies of hair Cr level in the meta-analysis, two studies (Munakata et al., 2006; De Palma et al., 2011) showed higher hair levels of Cr in children with cognitive defects whereas the other studies (Kracke, 1982; Wecker, 1985; Al-Ayadhi, 2005; Blaurock-Busch et

al., 2011; Skalny et al., 2016; Alqhazo and Rashaid, 2018; Tinkov et al., 2019) showed lower levels. Alqhazo and Rashaid reported that the Cr level was significantly lower among children who stutter (Alqhazo and Rashaid, 2018). Kracke made a comparison of chromium levels among psychotic vs. control and neurotic vs. normal children and reported that in both cases the Cr level was significantly lower among the affected children in comparison with the control group (Kracke, 1982). From the study of Munakata et al. it appears that the Cr level was higher among children with severe motor disabilities (Munakata et al., 2006). Wecker et al., Al- Ayadhi, Skalny et al. De Palma et al. measured the hair Cr level between autistic and normal children and the results illustrated that Cr level was significantly lower among autistic children (Wecker, 1985; Al-Ayadhi, 2005; De Palma et al., 2011; Skalny et al., 2016). Although Tinkov et al. reported low Cr levels among children with autism spectrum disorder, this was not a significant difference (Tinkov et al., 2019).

To understand the difference in Cr concentrations in hair between normal children and those with cognitive deficits, we performed several meta-analyses. The result showed that the pooled SMD is low among children with cognitive deficits and the overall effect size is significant ($p \leq 0.05$). The sensitivity analyses showed a similar trend, but the overall effect size is insignificant (p=0.27). The subgroup analysis for different regions and age groups showed significant lower hair Cr levels in case compared with control subjects but did not reveal consistent variation for different regions and age groups. This result suggested that the development status of different regions and age groups have an influence on the overall effect size of mean difference in hair Cr levels between children with cognitive defect and healthy controls. These findings strongly imply that children with cognitive deficits in different regions and age groups should be further evaluated to understand the dose-response association of Cr with cognitive deficits. However, these results should be treated with caution because of small number of studies in USA (n=3) and Europe (n=2) as well as those with children less than 5 years old (n=2).

From the literature search we found three studies that evaluated the serum Cr level in children with or without cognitive deficits (Wojciak et al., 2013; Tinkov et al., 2019; Skalny et al., 2020). We included these three studies for the meta-analysis and no significant differences were observed. One study reported that the serum Cr level was significantly lower among children with attention deficit hyperactive disorder (Skalny et al., 2020). Similarly, the study of Wojciak et al. illustrated that the Cr level in the serum was also significantly lower in

epileptic children (Wojciak et al., 2013). In contrast, from the findings of Tinkov et al. it appears that the serum Cr level was significantly higher in the children with autism spectrum relative to normal children (Tinkov et al., 2019).

Among the two studies that were included in this meta-analysis to evaluate the association between urine Cr level and cognitive deficit, both reported that Cr level was significantly higher in urine of children with autism in comparison to typically control children (Yorbik et al., 2010; Blaurock-Busch et al., 2011), although the meta-analysis showed no significant differences between the case and control groups.

Some studies have presented evidence that chromium may have adverse results on neuropsychological development (Suades-González et al., 2015; Caparros-Gonzalez et al., 2019). Indeed Cr(VI) can easily cross the blood-brain barrier and reach neural cells where it is reduced to Cr (V, IV, and III) reactive intermediates by intracellular reducing agents such as glutathione, leading to oxidative stress and oxidative DNA damage (Kart et al., 2016). From animal models it was evident that chromium may eventually cause cell apoptosis and hypoxia, which may be linked to cognitive impairments (Clark et al., 2014). Some other studies have reported the function of chromium as an endocrine disruptor and thereby interfere with insulin and testosterone that play a role in brain development (Shobana et al., 2017; Tang et al., 2018). Whereas generally the elimination of chromium from plasma takes place rapidly (within hours), elimination from tissues occurs slowly. Usually, around 80% of absorbed chromium is discarded in the urine, where its biological half-life is less than 2 days (ATSDR, 2012). On the other hand, approximately 10% of an absorbed dose is eliminated by biliary excretion, and small amounts are emitted in nail, hair and sweat (Kiilunen et al., 1983). Thus, urine chromium levels can be considered a biomarker of recent environmental or dietary exposure to chromium whereas for long-term exposure the best biomarker can be hair chromium level. With time the absorbed chromium is generally accumulated into the hair matrix, thus it offers evidence on a larger exposure window that may be more applicable for evaluating the impact of prolonged exposures on neurodevelopmental outcomes. Based on the lack of sufficient toxicokinetic evidence, it was of value to assess the pooled SMD difference between children with cognitive development defect and normal growth function using the serum urine and hair chromium level, since these might have different impact on neuropsychological outcomes to chromium due to short versus long-term exposure. However, our study encompasses some shortcomings. First, in this meta-analysis we consider only

case-control studies, therefore temporal relationship between a risk factor and an outcome cannot be inferred properly. Thus, the study findings cannot be used to define causation. Usually, longitudinal studies are particularly useful for evaluating exposure–response relationships, especially exposures that influence the development of disease. Such study designs allow data collection among individuals within a predefined group and application of appropriate statistical testing to analyses change over time for the group, as a whole, or for particular individuals. But in our search, we do not find such a study (Van Belle et al., 2004). Second, we do not consider prenatal exposure to metals, so the probable impact of such exposure during pregnancy cannot be inferred from the effect of childhood exposure. Third, we do not consider sex-related differences in this study due to limitation of data although the sex-related differences have been reported elsewhere in respect of the detrimental effects of toxic elements on health for example differences in patterns of exposure, metabolism or susceptibility which are the potential causes of these sex-related variation (Mergler, 2012; Caparros-Gonzalez et al., 2019). Fourth, we did not find any study that had evidence of chemical speciation to separate and quantify the different chemical forms of chromium. Cr(III) is not toxic because it cannot cross cell membranes. Recent evidence from an animal model suggests that Cr(III) picolinate can reverse the attention defect whereas Cr(VI) is highly toxic (Chiu et al., 2010; ATSDR, 2012; Akhtar et al., 2020). Fifth, there is lack of information on Cr(III)-containing supplements, their use by children in many of the studies that we evaluated, and childhood cognitive outcomes. Sixth, since we only looked at one heavy metal, we cannot assess the concurrent impact of other toxins on cognitive development and how they might be additive or synergistic.

5. Conclusion

Our findings provide little evidence of the detrimental effect of potential chromium exposure in hair, urine and serum and its association with cognitive outcomes as discussed above. Further research is needed to determine the most suitable biomarker for chromium exposure and the impact of chromium on cognitive development. Moreover, studies that separate and quantify the different chemical forms of chromium are needed. Moreover, further longitudinal studies should be performed in environmentally contaminated areas to understand potential dose–response relationships of Cr exposure with neurocognitive outcomes while controlling for exposure to Cr through micronutrient supplements.

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Fig. 1: Study flow chart

Fig. 2: Forest plot of random effects of hair Cr levels in cases compared with controls

Fig. 3: Forest plot of random effects of hair Cr levels in cases compared with controls (sensitivity analysis)

Fig. 4: Forest plot of random effects of serum Cr levels in cases compared with controls

Fig. 5: Forest plot of random effects of urine Cr levels in cases compared with controls

Graphical Abstract

Highlights

- The aim of this analysis as to evaluate the relationship among hair serum and urine chromium levels and child cognitive defects
- The pooled standardized mean difference (SMD) of hair Cr levels was lower in cases (N=326) than controls (N=486)
- The pooled SMD of serum Cr levels was lower in cases (N=123) vs. controls (N=123)
- The pooled SMD of urine Cr levels was lower in cases (N=55) than controls (N=45)
- No consistent variation was observed for different age group and region

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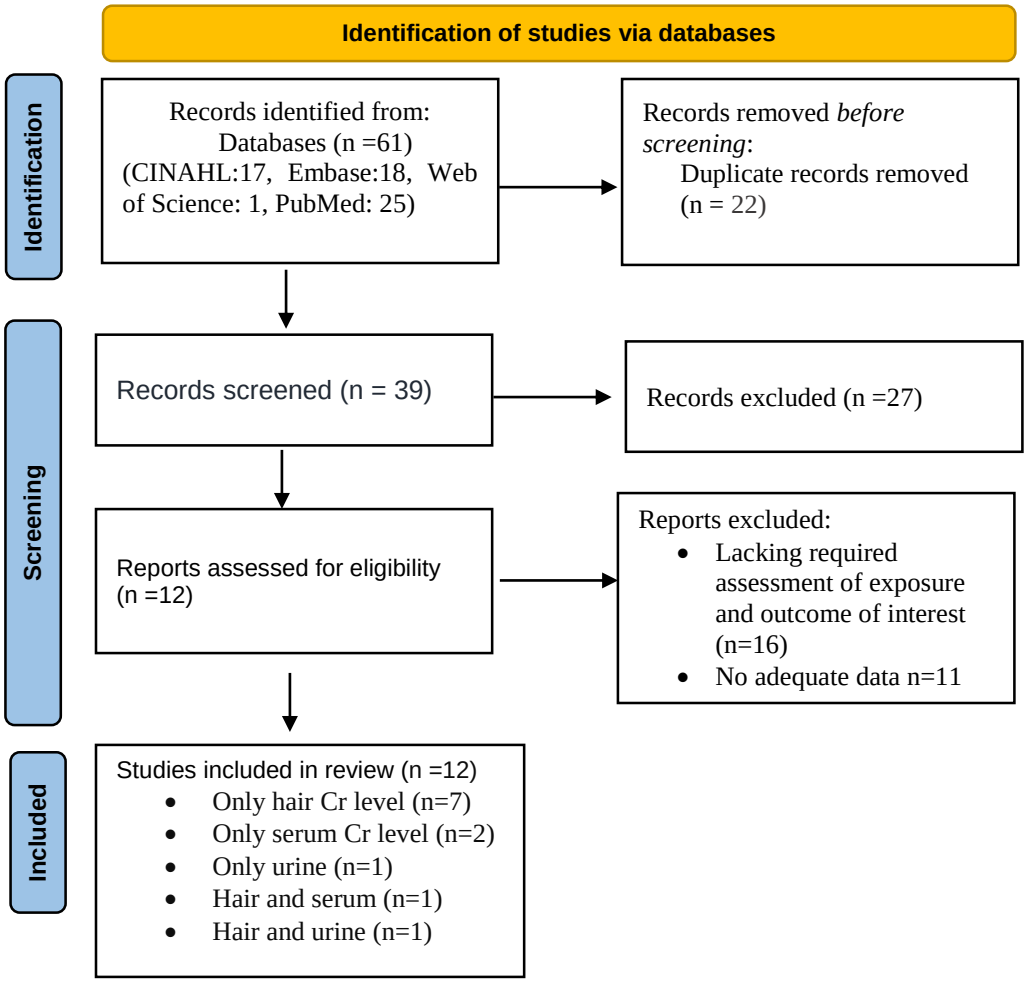

Fig. 1: Study flow chart

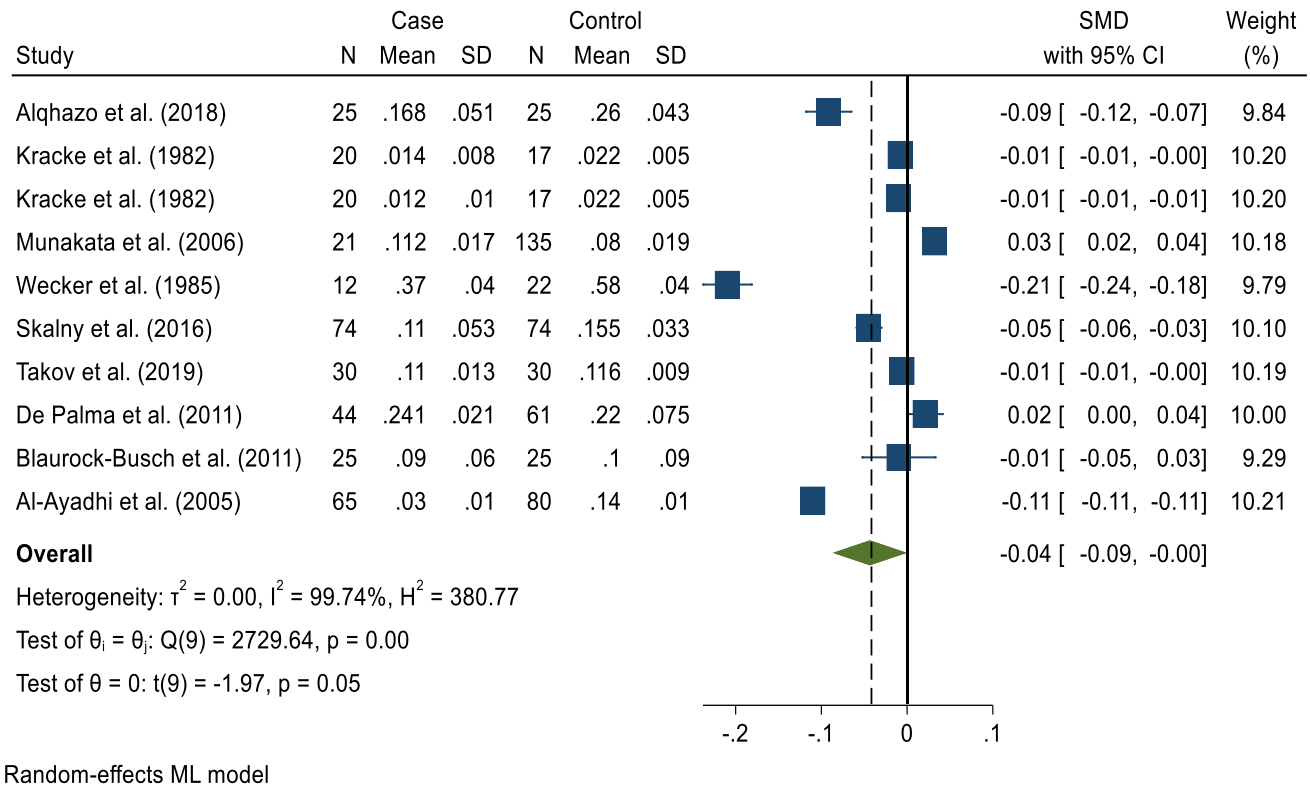

Fig. 2: Forest plot of random effects of hair Cr levels in cases compared with controls

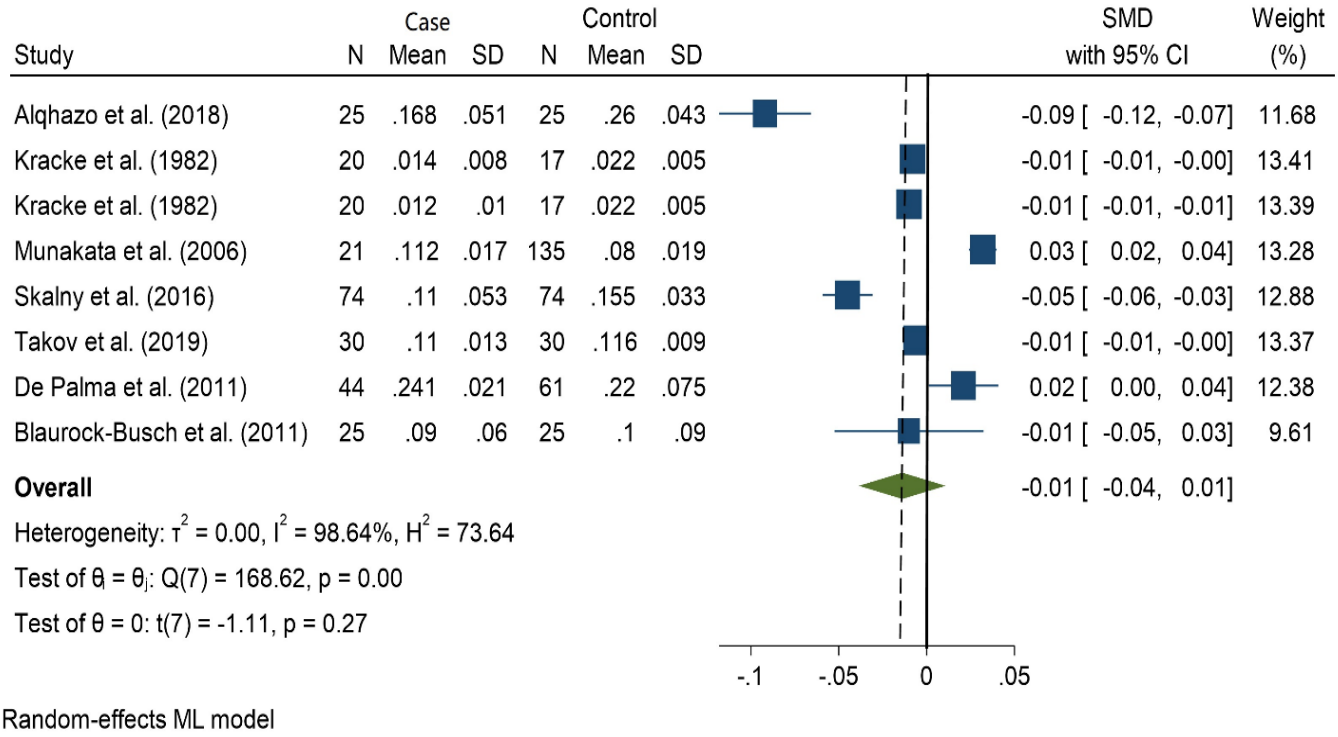

Fig. 3: Forest plot of random effects of hair Cr levels in cases compared with controls (sensitivity analysis)

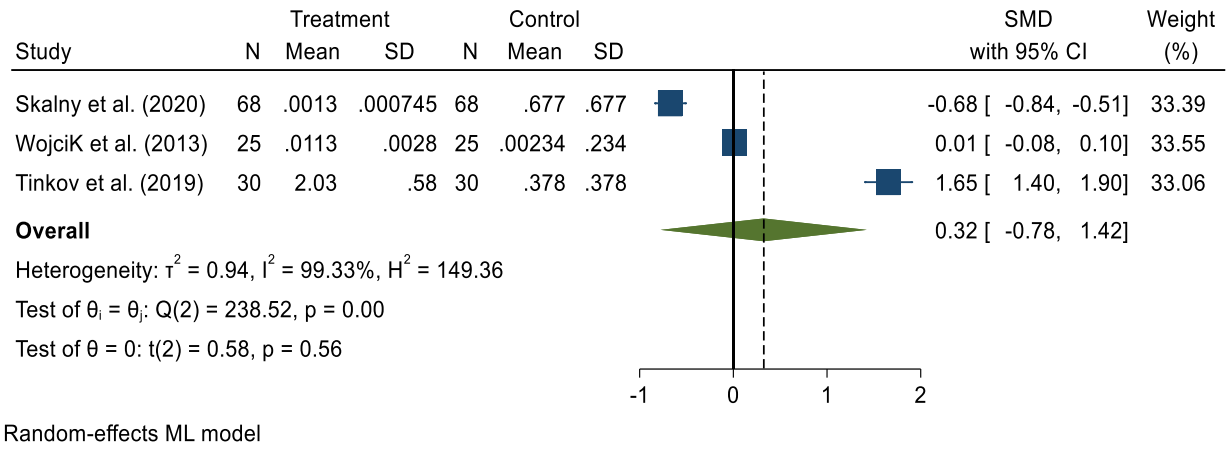

Fig. 4: Forest plot of random effects of serum Cr levels in cases compared with controls

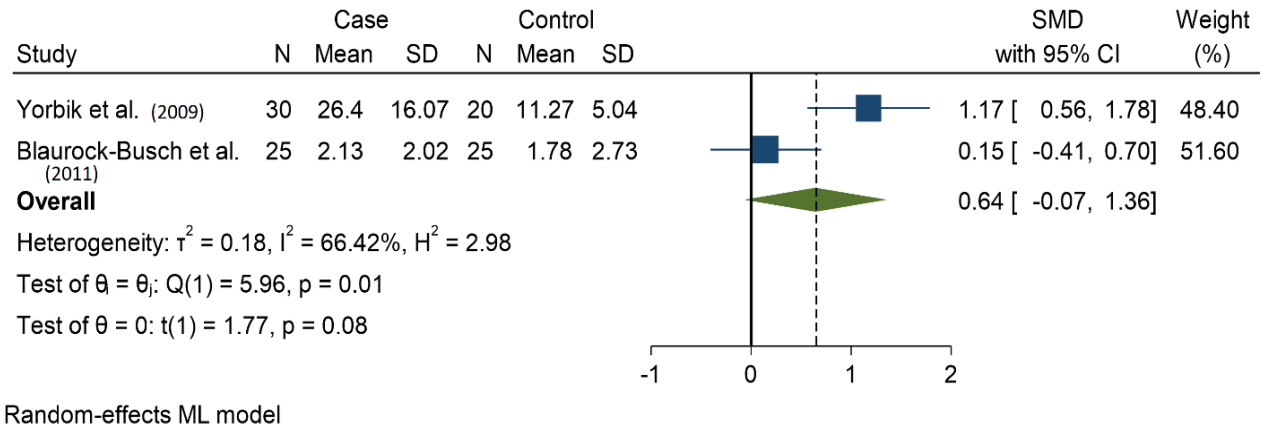

Fig. 5: Forest plot of random effects of urine Cr levels in cases compared with controls

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Table 1: Characteristics and quality assessment of included studies

Author	Country	Specimen	Settings	age	Scale	Health effect	NOS score
Alqhazo and Radhaid, 2018	Jordan	Hair	Stuttering s. normal children	3-8	Stuttering Severity Instrument version 4 (SSI-4)	Chromium (Cr) significantly lower among the stuttering children (p=0.014)	8
Karacke, 1979	USA	Hair	Psychoti vs. normal; and nuro-group vs. normal	7-12	Child behavior checklist	Both in psychotic and neuro group, Cr level significant lower compared with normal group (p=0.005, p=0.005)	6
Munakata et al., 2006	Japan	Hair	Severe motor disabilities vs. control	1-5	Motor-disabilities patient	Cr level low in children with motor disabilities but not statistically significant (p>0.05)	8
Wicker et al., 1982	USA	Hair	Autistic vs Normal; and Childhood-Onset pervasive disorder (COPD) Vs. Normal	2-11	Diagnostic and Statistical Manual of Mental Disorders version III (DSM III)	-Cr level significantly low among the autistic children (p=.004) -COPD vs. normal: No significant differences (p>0.05)	6
Skalny et al., 2016	Russia	Hair	Autism spectrum disorder vs. normal	2-8	Diagnostic and Statistical Manual of Mental Disorders	Cr level significantly low among autistic children (p=0.003)	7
Tinkov et al., 2019	Russia	Hair and serum	Autism spectrum disorder vs. normal	4-7	Childhood Autism Rating Scale (CARS) and Clinical Global Impression-Severity scale (CGI-S)	-Hair Cr level low among the children with ASD but not significantly differ (p>0.05) - Serum Cr level significantly high among children with ASD (p<0.05)	8
De Palma et al., 2011	Italy	Hair	Autism vs. control	9-14	Diagnostic and Statistical Manual of Mental Disorders version iv (DSM iv)	Cr level low among the children with autism but not significantly different (p>0.05)	8
Blaurock-Bush et al., 2011	Saudi Arabia	Hair and urine	Autism spectrum disorder vs. control	3-9	Diagnostic and Statistical Manual of Mental Disorders version iv (DSM-iv)	-Cr level low among the children with ASD, but the differences not significant (p=.64); -Cr level high among the children with ASD, but the differences not significant (p=0.6)	6
Al-Ayadhi et al., 2005	Saudi Arabia	Hair	Autism vs. control	0-14	Diagnostic and Statistical Manual of Mental Disorders version iv (DSM-iv)	Cr level significantly low among the children with autism (p<0.05)	6

Skalny et al., 2019	USA	Serum	Attention deficit hyperactivity disorder (ADHD) vs. control	4-9	Attention deficit hyperactivity disorder (ICD-10:F90) was diagnosed using ICD-10 criteria including intension, hyperactivity; impulsivity	Chromium level significantly lower among children with ADHD (p=0.01)	8
Wojciak et al., 2012	Poland	Serum	Epilepsy vs. control	13-16	Epileptic children with idiopathic generalized tonic-clonic seizures	Cr level significantly lower among epileptic children (p<0.001)	7
Yorkib et al. 2009	Turkey	Urine	Autism vs. control	3-12	Diagnostic and Statistical Manual of Mental Disorders version iv (DSM iv)	Chromium level significantly higher among children with autism (p<0.001)	7

Table 2: Subgroup Analysis of Hair Cr levels

Stratification group	N	SMD (95% CI)	Heterogeneity test		
			Q	*p*	I^2
Region					
Asia	5	-0.03 (-0.08 to 0.02)	1178.12	<0.0001	99.13%
USA	3	-0.07 (-0.18 to 0.03)	194.19	<0.0001	99.88%
Europe	2	-0.02 (-0.05 to 00))	24.93	<0.0001	91.95%
Combined	10	-0.04(-0.09 to 0.00)	2729.64	<0.0001	99.74%
Age					
> 5 years	2	-0.01 (-0.06 to 0.05)	85.79	<0.0001	97.67%
< 5years	4	-0.03 (-0.08 to 0.02)	1922.01	<0.0001	99.74 %
Mixed	4	-0.08 (-0.16 to 00)	226.99	<0.0001	98.12 %
Combined	10	-0.04(-0.09 to 0.00)	2729.64	<0.0001	99.74%

Credit author statement

G.M. Rabiul Islam: Conceptualization, Screening- Titles & Abstracts; Reviewing- Full texts of selected studies, Data analysis; Drafting- Original manuscript

Mohammad Meshbahur Rahman: Screening- Titles &Abstracts; Reviewing- Full texts of selected studies, Data analysis

Mohammed Imrul Hasan: Revieing- Manuscript

Amare Worku Tadesse: Review- Manuscript

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