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Lead and Mercury Levels in Preterm Infants Before and After Blood Transfusions

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Abstract

Very low birth weight (VLBW) infants usually receive packed red blood cell unit (pRBC) transfusions. Heavy metal transfer via pRBCs is not widely discussed before. This study aimed to determine pre-/post-transfusion erythrocyte lead and mercury levels in infants and to correlate these levels to heavy metal concentrations in pRBCs. VLBW infants ($n = 80$), needing pRBC transfusion for the first time, were enrolled. Erythrocyte heavy metal levels were determined in pre-/post-transfusion blood samples and also in pRBC units. Mean lead and mercury levels in the pRBCs were found to be 16.3 ± 10.8 and 3.75 ± 3.23 μg/L, respectively. Of the infants, 69.7% received lead above reference dose. Erythrocyte lead levels increased significantly after transfusions (10.6 ± 10.3 vs. 13 ± 8.5, $p < 0.05$) with significant correlated to amount of lead within pRBCs ($r = 0.28$). Mean pre-/post-transfusion erythrocyte mercury levels were 3.28 ± 3.08 and 3.5 ± 2.83 μg/L, respectively ($p > 0.05$). There was a significant correlation between mean difference of mercury levels after transfusion and amount of mercury delivered by pRBCs ($r = 0.28$). Infants can be subject to high levels of lead and mercury through pRBC transfusions.

Keywords Blood transfusion · Lead · Mercury · Neonate · Prematurity

Abbreviations

ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CL	Confidence level
CPDA-1	Citrate-phosphate-dextrose-adenine
ELBW	Extremely low birth weight
EPA	Environmental Protection Agency
IQ	Intelligence quotient
JECFA	the Joint Food and Agriculture Organization/ World Health Organization Expert Committee
LOD	Limit of detection
NHANES	National Health and Nutrition Examination Survey
NICU	Neonatal intensive care unit
OSHA	the US Occupational Safety and Health Administration
pRBC	packed red blood cell unit
RfD	Reference dose
VLBW	Very low birth weight
WHO	World Health Organization

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Introduction

The frequency of anemia is high in very low birth weight (VLBW) infants in the neonatal intensive care units (NICU). Almost 80% of infants require at least one packed red blood cell (pRBC) transfusion because of repeated blood samplings for laboratory analysis, anemia of prematurity, infections, and bone marrow suppression [1]. In acute and long term, pRBC transfusion has various adverse effects, like immunologic/ non-immunologic modifications, metabolic changes, and increased frequency of infections. In addition to several

unfavorable effects, heavy metal overload via pRBC transfusions is not usually well-noted and can be regarded as a hidden danger [1].

Lead and mercury are heavy metals that are highly present in the environment, food, and water. Lead is present in dyes, paints, crayons, air, water (in the water directly or in water carrying pipes, old plumbing), batteries, semiconductors, pottery (ceramics), PVC coating of electrical cords, glass, textiles, automobile exhaust, food (especially canned food), batteries, and cosmetics (e.g., mascaras). All these sources cause high lead overload in the environment. If severe cautions are not taken, lead can be abundant in the environment and in the air [2]. Humans can be exposed to mercury through anthropogenic sources (from hydroelectric, mining, pulp, and paper industries; incineration of municipal and medical waste and emissions from coal-using power plants). Mercury is also present in different sources, including water, thermometers, amalgams, food (particularly fish and seafood), vaccines (thiomersal, a preservative), pharmaceuticals, and cosmetics (mascaras) [3]. Although placenta is a good barrier for a vast variety of toxic elements in fetal life, early-life exposure to heavy metals (i.e., lead and mercury) has long to be known neurotoxic. In particular, very preterm infants have increased vulnerability to neurotoxic agents, due to the immature blood-brain barrier and ongoing neurological developmental processes [4–6].

The blood of adult donors may contain both lead and mercury depending upon their occupations, diets, and lifestyles. As blood transfusions are administered usually within the first 2 weeks of life, early exposure of VLBW and very preterm infants to heavy metals via transfusion may exert serious long-term neurotoxic effects. No blood lead or mercury threshold has been identified for children. Regulatory authorities have not currently set safe lead and mercury levels for infants. Centers for Disease Control and Prevention (CDC) case management guidelines are designed to keep children's blood lead levels below 10 μg/dL. However, studies have found neurobehavioral impairment in children with blood lead levels even below this limit [7–9]. It is not clear whether elevated blood lead levels is related to low intelligence scores, behavioral problems, and diminished school performance or not [7]. On the other hand, fetal exposure to mercury is associated to neuropsychological dysfunctions such as language, attention, and memory deficits and visuospatial and/or motor dysfunctions [10]. Neurodevelopmental disabilities are even evident in infants with low mercury exposure [11]. In general population, mercury blood levels should be below 10 μg/dL while some researchers suggest as low as < 5 μg/dL [12].

Taking into account all the available data, the aim of this study was to determine pre-and post-transfusion erythrocyte lead and mercury levels in VLBW infants receiving blood transfusions and to correlate these levels to the lead and mercury concentrations in pRBCs.

Material and Methods

This study was carried out prospectively over 21 months (July 2011–March 2013) at Hacettepe University İhsan Doğramacı Children's Hospital, NICU. The study was approved by Hacettepe University Human Ethics Committee (HEK 11/18-7). An informed consent was obtained from all families before the infants were recruited to the study. Premature infants born ≤ 1500 g and in need of blood transfusion for the first time (for any reason) were enrolled. Demographic variables were recorded for each infant.

Web-based software (Survey Software, 2016 Creative Research Systems) was used to calculate the sample size. There are almost 1500 cases of blood transfusion per year in the hospital. Considering confidence level (CL) of 95% and confidence interval (CI) of 10%, the sample size needed for the study, the sample size needed was 90. Due to several reasons beyond control (coagulation, not getting parents' approval, etc.), our sample size was limited to 80.

Chemicals

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO), or Merck (Darmstadt, Germany), except ERA 100 mg/L mercury SRM 3133 was purchased from Waters Corporation (Milford, MA) and Seronorm Trace Elements Whole Blood was obtained from Sero (Billingstad, Norway).

Transfusion Practices

All the pRBCs used in the transfusions were obtained from healthy adults. Eighty VLBW neonates (29 male/51 female) were consecutively enrolled in the study. Birth weights ranged from 600 to 1500 g with the gestational age from 24.4 to 34 weeks. First, pRBC transfusion was performed within 2–22 days (mean 8.5 ± 6.6 day). Thirty-three (41.2%) infants received their first transfusion in the first week of life while 34 (42.5%) infants in the second week, 8 (10%) infants in the third week, and 5 (6.3%) infants in the fourth week of life. Each infant received 1 to 9 transfusions (3.5 ± 1.7 times) during their NICU stay. Blood transfusions were carried out according to our institutional guideline, and for each infant, 10 to 20 ml/kg pRBCs were administered. All of the pRBCs were irradiated and filtrated before use. The preservative was citrate-phosphate-dextrose-adenine (CPDA-1). Total amount of pRBC volume ranged between 17 and 200 ml per infant with a mean of 69.5 ± 35.6 ml/kg (Table 1).

Sample Collection, Lead and Mercury Analysis

About 0.5 ml of blood was drawn before transfusion and approximately 6 h after the transfusion to analyze the erythrocyte

Table 1 The characteristics pRBCs administered to infants

Mean transfusion count	3.5 ± 1.7
Median (minimum-maximum)	3 (1–9)
Mean pRBC volume given per infant (ml)	17.50 ± 2.70
Median (minimum-maximum)	17.50 (10–20)
Mean total pRBC volume given per infant (ml/kg)	69.50 ± 35.60
Median (minimum-maximum)	60.50 (17–200)
Mean lead concentration in pRBC units (μg/L)	16.30 ± 10.80
Median (minimum-maximum)	14.30 (2.20–62.80)
Mean mercury concentration in pRBC units (μg/L)	3.75 ± 3.23
Median (minimum-maximum)	3 (1.30–13.70)
Mean lead dose given per infant (μg/kg)	0.30 ± 0.21
Median (minimum-maximum)	0.30 (0.04–1.26)
Mean mercury dose given per infant (μg/kg)	0.08 ± 0.05
Median (minimum-maximum)	0.07 (0.03–0.27)

pRBC packed red blood cell

lead and mercury levels. Lead and mercury concentrations in the pRBC transfusions were also determined. The samples were collected to heparinized glass tubes and were centrifuged at 3000 rpm for 15 min. Erythrocytes were washed with phosphate-buffered saline (PBS) two more times and centrifuged again. Later, aliquoted erythrocytes were stored in − 80 °C until analysis.

Erythrocyte lead levels were analyzed with a Perkin-Elmer Analyst 800 atomic absorption spectrophotometer (AAS, Norwalk, CT) equipped with a graphite furnace and Zeeman background correction. Briefly, 0.2% (*v*/*v*) nitric acid (0.6 ml) and 0.2% (*v*/*v*) Triton-X (0.3 ml) were added on erythrocyte packages (0.1 mL) before analysis. Nitric acid (0.2%, *v*/*v*) was used for the dilution of the samples and was also used as blank. Standards contained between 1 and 50 μg/L of lead. Light source was lead discharge lamb (without electrode). The limit of detection (LOD) for lead was 1 μg/L.

Erythrocyte total mercury levels (methyl, ethyl, and other organic forms) were analyzed with a Perkin-Elmer FIAS-Analyst 100 Hydrur System AAS (Waltham, MA) in Düzen Laboratories (Ankara, Turkey). Briefly, potassium persulfate (0.2 mL), nitric acid (82 mL), and sulfuric acid (0.5 ml) were added on erythrocyte packages (0.3 ml) before analysis and hydrolyzed at 100 °C. Samples were later cooled and sample volume was brought to 2.5 mL. Calibrators and controls were also prepared accordingly. ERA 100 mg/L mercury SRM 3133 was used as calibrator and Seronorm Trace Elements Whole Blood was used as control. The LOD for mercury was 2 μg/L.

Lead and Mercury Reference Doses

Reference Lead Dose Dose response analysis, performed by The Joint Food and Agriculture Organization/World Health Organization Expert Committee (JECFA), showed that even at low doses of 1.9 μg/kg/day, a decrease of 3 in intelligence quotient (IQ) scores can be observed in populations. Such decreases are considered to be very important as they may cause a shift in the general population's IQ scores. As described in the previous study of Elabiad and Hook, we used the higher intake dose of 1.9 μg/kg/day as the reference dose (RfD) to compare lead levels administered by blood transfusions [13]. Thus, an "intravenous (iv) reference dose" would be the one tenth of the oral reference dose [14]. Therefore, the corresponding daily iv RfD was considered as 0.19 μg/kg [15].

Reference Mercury Dose Three major agencies [Agency for Toxic Substances and Disease Registry (ATSDR), World Health Organization (WHO), and Environmental Protection Agency (EPA)] have considered the safe mercury intake for a life span [16–18]. The current oral RfDs given by ATSDR, WHO, and EPA are 0.3, 0.23, and 0.1 μg/kg/day, respectively [16–18]. Considering about the 95% oral mercury is absorbed, the daily equivalent iv RfDs were calculated as 0.29, 0.22, and 0.095 μg/kg/day by ATSDR, WHO, and EPA, respectively [16–18].

Calculating Lead and Mercury Doses

A load of transfused lead and mercury per given transfusion was calculated as follows: volume of blood transfusion (mL) × pRBC mercury or lead levels (μg/L)/weight (kg). The result was compared with daily iv RfDs.

Statistical Analysis

The differences between the pre-transfusion and post-transfusion lead and mercury concentrations were analyzed with paired samples *t* test. Repeated measures for ANOVA were used to determine the effect of lead and mercury dose on the difference of pre- and post-transfusion lead and mercury levels. Pearson's correlation coefficient was used to determine the relation between continuous variables. Descriptive statistics were expressed as mean ± standard deviations (SDs) and/or medians (minimum-maximum). The significance level was set at $p < 0.05$.

Results

Pre-transfusion and post-transfusion mean hemoglobin (Hb) levels were found to be 9.70 ± 1.00 and 12.50 ± 1.40 g/dL, respectively ($p = 0.000$). Pre- and post-transfusion hematocrit (%) levels were 29.10 ± 3 and 38.40 ± 4.20%, respectively ($p = 0.000$).

In a total of 80 pRBCs, 76 samples were eligible for lead analysis. Four samples were inappropriate due to hemolysis and/or coagulation. Pre- and-post transfusion lead levels were

measured in 68 erythrocyte samples of infants. Lead was detected in 76 pRBCs and 68 infants. Mean pRBC erythrocyte lead level was 16.30 ± 10.80 μg/L (range 2.20–62.80 μg/L) (Table 1). The mean lead dose received per infant in the first transfusion was 0.30 ± 0.21 μg/kg (range 0.04–1.26 μg/kg) (Table 1). Fifty-three (69.7%) infants received lead above the RfD according to JECFA (Fig. 1). Mean pre-transfusion and post-transfusion erythrocyte lead levels were 10.60 ± 10.30 and 13.00 ± 8.50 μg/L, respectively ($p = 0.000$) (Table 2). Mean erythrocyte lead difference before and after the transfusion was 2.39 ± 5.4 μg/L. There was a significant correlation between the mean difference of lead levels of pre-transfusion and post-transfusion in infants and mean lead load of pRBCs administered ($r = 0.28$, $p = 0.02$) (Fig. 2). The highest lead concentration was 60 μg/L in an infant before transfusion; after transfusion, it was found to be 60.50 μg/L. The concentration of lead was 36 μg/L in pRBCs given to this infant as 0.60 μg/kg lead was calculated to be given via pRBC.

In a total of 80 pRBCs, 78 samples were eligible for mercury analysis. In 24 samples (30.80%), mercury levels were below the detectable limits. Pre- and post-transfusion mercury levels were measured in erythrocytes of the infants ($n = 76$) as well as in pRBC transfusions. Twenty-four (31.60%) of pre-transfusion samples and 22 (28.90%) of post-transfusion samples were below the detectable limit for mercury. Mean erythrocyte mercury levels of pRBCs was 3.75 ± 3.23 μg/L (range 1.30–13.70 μg/L) (Table 1). The mean mercury load received per infant in first transfusion ranged from 0.03 to 0.27 μg/kg with a mean of 0.08 ± 0.05 μg/kg. Twenty-one infants (27%) received mercury above the RfD set by EPA while one infant (1.3%) received mercury above the RfD set by WHO. None of the infants received mercury above the RfD set by ATSDR (Fig. 2). Mean pre-transfusion and post-transfusion mercury levels were 3.28 ± 3.08 and 3.42 ± 2.83 μg/kg, respectively ($p = 0.712$) (Table 2). The highest value of pre-transfusion mercury level was 12.4 μg/L, and it was 10.9 μg/L after transfusion. Although there was not a statistically significant difference between pre-transfusion and post-transfusion erythrocyte mercury levels, there was a significant correlation between the pre-transfusion and post-transfusion levels and the dose of mercury levels administered through pRBCs to each infant ($r = 0.28$, $p = 0.016$) (Fig. 3).

Discussion

Blood transfusion is a critical part of NICU stay for very preterm infants, and it is considered to be life-saving for neonates with severe conditions. Due to iatrogenic blood loss (immaturity of the hematopoietic system, shorter erythrocyte life span) or blood loss because of medical conditions (sepsis, hemolysis or bleeding disorders, and surgery), preterm infants particularly those with VLBW, often need multiple blood transfusions during hospitalization [19]. However, transfusion has been associated with significant morbidity. One of the causes of morbidity can be increase in iron or heavy metal content of blood [13, 20].

In this prospective study, we investigated the effects of blood transfusion on the erythrocyte lead and mercury levels with a relatively large sample size. Ankara, the capital of Turkey, is an industrialized city and Turkey is a developing country, with several environmental issues that are yet to be solved. These are the main reasons that lead and mercury are still highly present in the environment. Currently, we have also evaluated the mercury and lead levels of pRBC transfusions given the small preterm infants and determined the correlations between the increases in blood lead and mercury levels of these infants along with the levels of lead and mercury in the pRBCs. Measurement of mercury and lead levels in pRBCs with pre- and post-transfusion heavy metal levels strengthened the findings of the current work. Lead and mercury levels were measured in erythrocytes instead of whole blood as the main aim of the study was to determine whether the increases in lead and mercury levels after transfusion were ingenerated directly by the administration of pRBCs or not. Therefore, the erythrocyte lead and mercury levels, rather than whole-blood levels, enabled us to obtain more accurate data.

It is known that most of the pRBC transfusions are given in the first 2 weeks of life [21, 22]. Depending on the NICU's transfusion policy, as many as 80% of infants with a birth weight < 1250 g receive pRBC transfusion at least once during their NICU period [23]. However, this time period is very critical for brain development of premature infants. Exposure to heavy metals, i.e., lead and mercury, during this early stage of life poses neurodevelopmental toxic effects in vulnerable infants. Such exposures are suggested to cause the acceleration of decline in the central nervous system functions and late-life neurotoxic effects [6, 24]. These suggestions may not possibly be very realistic, considering low amount of intake and short exposure time. However, the critical timing of the exposure and certain factors related to the prematurity needs the scientific community to pay more attention on lead and mercury intake via pRBC transfusions. We have shown that blood transfusions can be potential sources of lead and mercury exposure for VLBW infants, herein.

Most of VLBW infants are not stable during the first weeks of life, and the blood-brain barrier tend to have a higher permeability due to several unfavorable factors like hypoxia-ischemia, infections, inflammation, intra-ventricular hemorrhage, blood pressure alterations, mechanical ventilation, and oxidative stress [21, 22]. In the current work, first blood transfusion was performed with a mean 8.5 days and 42.2% of the infants received blood in the first week of life. Mean total pRBC volume given per infant was 69.5 ml/kg which is comparable with previous studies, ranging from approximately 40

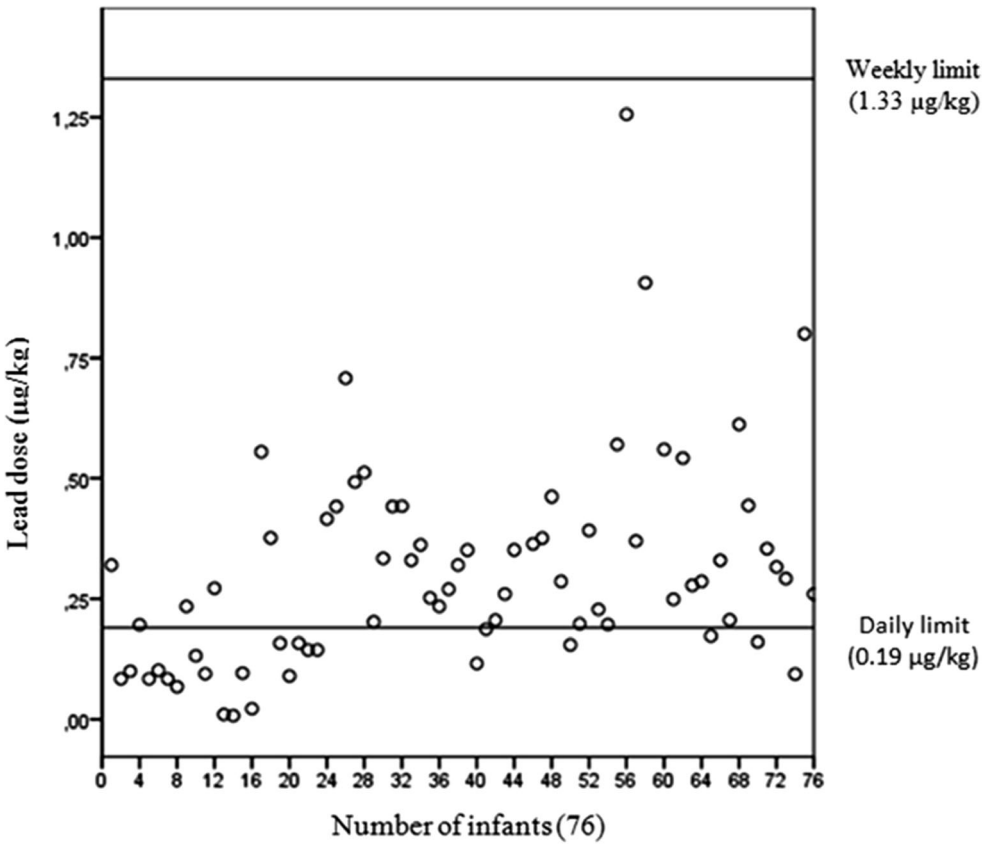

Fig. 1 Lead levels in pRBC samples. Fifty-three infants (69.7%) received lead above the daily RfD set by JECFA. JECFA: The Joint Food and Agriculture Organization/World Health Organization Expert Committee; RfD: Reference Dose

to 90 ml/kg, depending on the NICU policy and inclusion criteria to the study [13, 25, 26]. Although our transfusion policy seems to be in accordance with the current transfusion practices throughout the world, applying "lower total transfusion volume" strategies to prevent heavy metal exposure should be considered. Delayed clamping or milking of the umbilical cord at birth, limiting blood loss by phlebotomy (such as using micro methods for laboratory analysis), adequate nutrition, erythropoietin and using of standardized transfusion guidelines are now highly discussed issues in contemporary medicine [27].

Lead exposure can affect nearly every system in the body and often shows no obvious symptoms at first sight. Neurodevelopment impairment in children may occur even in low lead levels [9]. In the present study, mean erythrocyte lead level in pRBCs was found to be 16.30 µg/L [median 14.30 (min 2.20; max 62.80)]. pRBC lead levels were 18.3 and 15.6 µg/L in two previous studies in literature [13, 28]. In addition, our results are consistent with a previous study investigating blood lead levels in healthy Turkish population [29]. According to "Adult Blood Lead Epidemiology and Surveillance Program" set by The United States National Institute for Occupational Safety and Health (NIOSH), lead exposure at low doses can lead to adverse outcomes. The US Department of Health and Human Services recommends that blood lead levels among all adults be reduced to < 10 µg/dL. The US Occupational Safety and Health Administration (OSHA) Lead Standards require workers to be removed from lead exposure when blood lead levels are ≥ 50 µg/dL (construction industry) or 60 µg/dL (general industry) and allow workers to return to work when the blood lead level is below 40 µg/dL. In the current study, the highest lead levels observed in pRBCs was 62.80 µg/L (6.28 µg/dL), which was lower than the limits given by NIOSH and OSHA [30].

Table 2 Mean lead and mercury levels before and after pRBC transfusions

	Number	Pre-transfusion	Post-transfusion	*p*
Lead (µg/L)	68	10.60 ± 10.30	13.00 ± 8.50*	0.000
Mercury (µg/L)	76	3.28 ± 3.08	3.42 ± 2.83	0.712

*Significantly different from pre-transfusion values

pRBC packed red blood cell transfusion

Erythrocyte lead levels increased significantly after transfusions (10.60 ± 10.30 µg/L vs. 13.00 ± 8.50, $p = 0.00$), and these alterations were significantly correlated to the dose of lead given with pRBCs (0.30 ± 0.21 µg/kg) herein ($r = 0.28$, $p = 0.022$). Our findings were supported by the study of Elabiad and Christensen (2014), who also observed significant increases in blood lead and mercury levels after blood transfusion in premature infants, although they did not

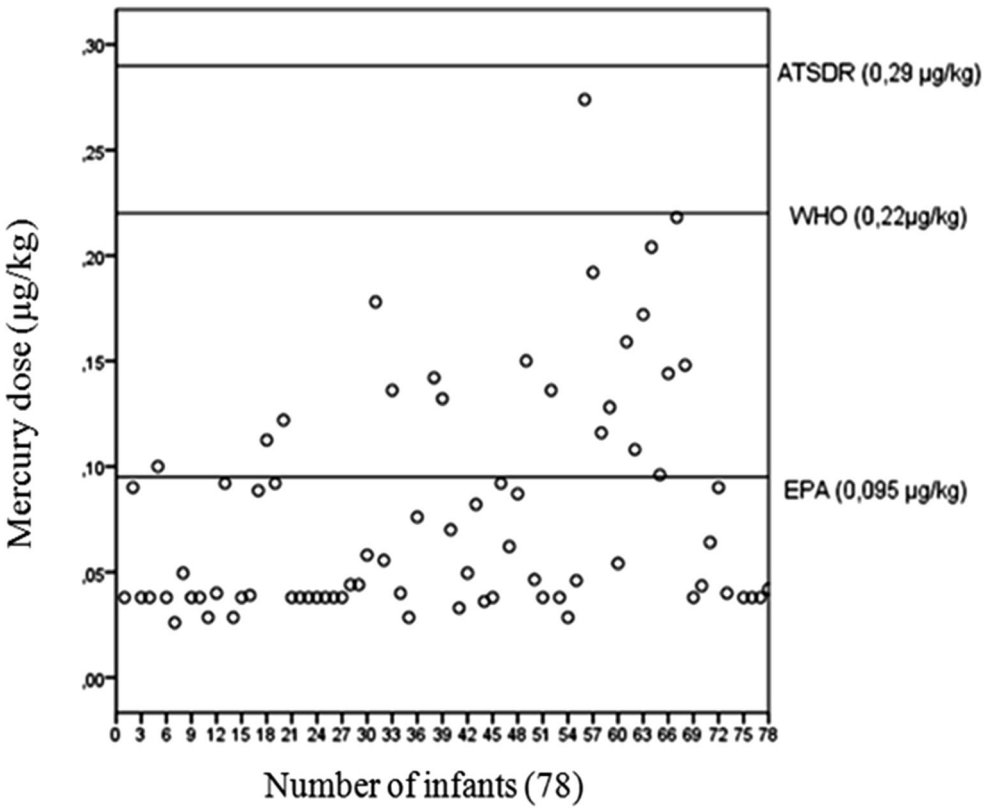

Fig. 2 Mercury levels in pRBC samples. Twenty-one (27%) infants received mercury above the RfD set by EPA, and one infant (1.3%) received mercury above the RfD set by WHO. EPA: Environmental Protection Agency; RfD: Reference dose; WHO: World Health Organization

measure the levels of these heavy metals in pRBCs [20]. Recently, Zubairi et al. (2015) also showed a significant correlation between the post-transfusion premature infant blood lead levels and lead levels in transfused pRBCs. Post-transfusion mean erythrocyte lead level was increased by 22.6% in our study when compared to pre-transfusion lead levels [31]. In the study of Bearer et al. (2000), the researchers found 27% increase in blood lead levels in premature infants under 30 weeks of gestation [28]. Recently, CDC released a reference value of 50 µg/L. This value was based on the data

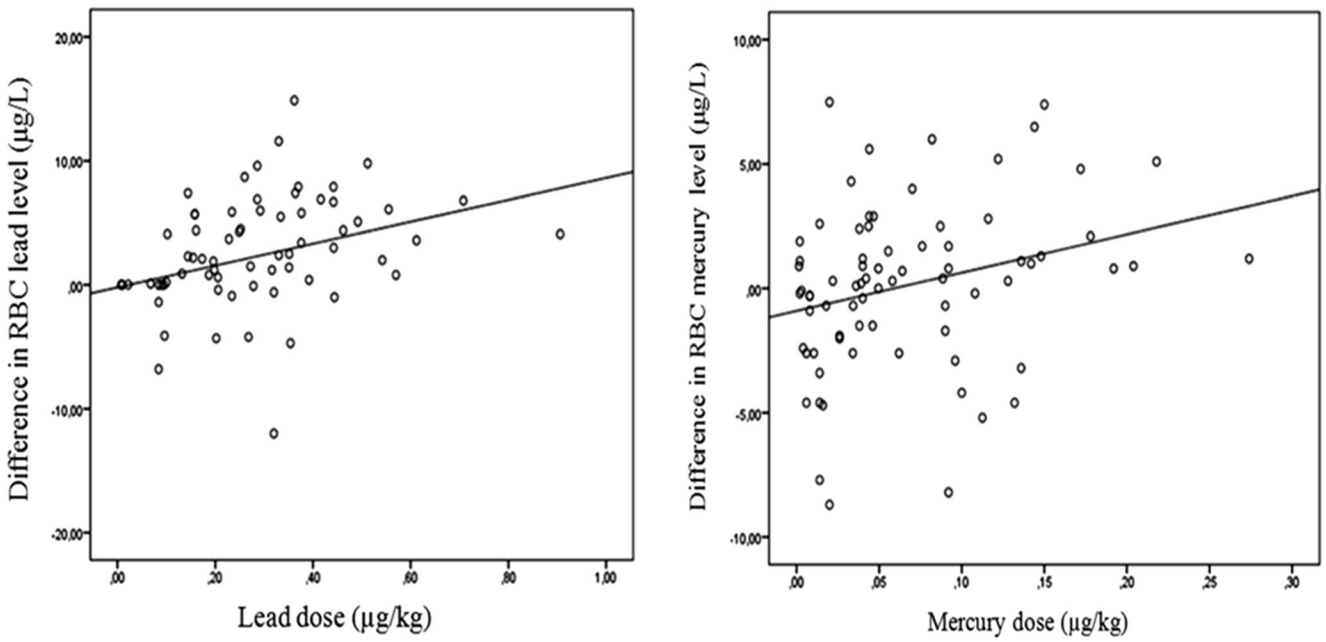

Fig. 3 The correlation between the mean difference of lead and mercury levels of pre-transfusion and post-transfusion in infants and mean lead load of pRBCs administered ($r = 0.28$, $p < 0.05$ for both lead and mercury). pRBC: packed red blood cell

obtained from the 97.5th percentile of the National Health and Nutrition Examination Survey (NHANES)'s blood lead distribution in children [9]. In our study population, the highest erythrocyte lead concentration was 60 μg/L in an infant before transfusion; after transfusion, it was 60.5 μg/L.

As in study of Elabiad and Hook, the cut of value of tolerable daily iv intake of lead was 0.19 μg/kg. Fifty-three (69.7%) infants received lead above this limit set by JECFA within the single blood transfusion [13]. The researchers investigated 322 pRBC units given to extremely low birth weight (ELBW) infants and found 42% of all transfusions exceed the daily limit. The average lead load from each transfusion was 0.21 ± 0.13 μg/kg in that study [11]. Previously, it was reported as 1.56 ± 1.77 μg/kg in another study [30]. We found the average lead amount given by single transfusion slightly higher than the results of Elabiad and Hook (2013) as 0.3 ± 0.21 μg/kg.

Mercury is a ubiquitous metal in the environment. It bioaccumulates in the food chain and can affect neurodevelopment, particularly in the perinatal period [6]. Study showed that pRBC transfusions may also be a source of mercury. Mercury was detected 69.2% of the pRBC units and mean erythrocyte mercury concentration was 3.75 μg/L. However, high cut-off value for mercury detection was a limitation for our study. Before transfusion, mean erythrocyte mercury level was 3.3 ± 3.1 μg/L and after transfusion, mercury levels raised to 3.4 ± 2.8 μg/L. pRBCs mean mercury level was found to be 3.7 ± 3.1 μg/L [median 3 μg/L (min 1.3 μg/L-max 13.7 μg/L)]. Although the difference between pre-transfusion and post-transfusion levels was not statistically significant ($p = 0.712$), there was a significant correlation between the difference of pre-transfusion and post-transfusion mercury levels and the amount of mercury in pRBCs ($r = 0.28$, $p = 0.016$).

On the basis of current levels set by EPA, 21 infants (27%) received mercury above the RfD. The EPA appointed the estimated mercury levels below 5.8 μg/L as without perceptible harm based on measures of mercury in cord blood [18]. In the study of Elabiad and Hook, it was reported that the average mercury level in pRBCs was 1.9 μg/L and 6.8% transfusions were above the RfD according to EPA [13, 16]. This difference may be attributed to the high single transfusion volume (mean 17.5 ml/kg) in our study. We can suggest that mercury levels may be decreased by using more restricted transfusion criteria and by applying decreased volume of transfusion.

The major route of mercury excretion is through stool, followed by urine whereas absorbed lead is excreted primarily in urine followed by stool. VLBW infants have prolonged intestinal transit time and very few bowel movements during the first days of life [16, 32]. Urinary excretion may be considered negligible because of low glomerular filtration rate in this population [33]. After thiomersal containing hepatitis B vaccine, mean mercury level was three times higher in the preterm infants than those in full-term infants, suggesting elimination of mercury is limited in premature babies [34]. Mercury and lead accumulations are possible via repeated blood transfusions in VLBW infants.

One should also consider placental transport of lead and mercury. Cord blood and maternal blood lead and mercury levels were found to be highly correlated and in some studies, cord blood lead levels were determined to be higher than maternal blood lead levels [6, 35]. Our pre-transfusion lead and mercury levels reflect cord blood level because of early mean sampling time (8.5 days). Lead is lifelong stored in bone. During pregnancy, calcium requirements increase and give rise to bone turn over. Therefore, calcium supplementation of the maternal diet was suggested to prevent mobilization of maternal bone lead stores [36]. In addition, some sources of lead exposure such as smoking, occupational lead exposure, exposure to lead paint and gasoline should be avoided during pregnancy. Pregnant women should also avoid excessive consumption of seafood and dental amalgam fillings in order to prevent early mercury exposure of their babies [6, 37]. Infants may also receive heavy metals by breast milk or infant formulas; however, intake via enteral route may be negligible due to small volumes of enteral feeding in VLBW infants in the first week of life.

When transfusing blood to a preterm infant, some authors recommend screening the donated blood, specifically for lead, whereas the others do not [31, 38–41]. In conclusion, a cost-effective analysis of this procedure should be elaborately discussed. Particularly, in infants requiring excessive amount of blood transfusions (such as exchange transfusion or Extra Corporeal Life Support), pRBCs may be tested for lead and mercury. Thus, more simply, pre-blood transfusion checklist can be enhanced or a scoring system, which can cover the occupation, demographics, living area, nutritional habits, amalgam fillings, tobacco use, alcohol consumption, and other factors (that possess risk for lead and mercury exposure) of the blood donor, may be settled. In a well-designed cohort study, evaluating donated blood for lead levels, sex, and age were found to be independent risk factors for high blood lead levels. The authors suggested a viable policy alternative to systematic determination of blood lead levels to eliminate burden of additional laboratory testing for pRBCs [11]. Finally, blood transfusions can be suggested to be sources of lead and mercury and therefore, several factors should be taken into account when administering blood transfusions to infants, particularly to very preterm babies.

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Compliance with Ethical Standards

The study was approved by Hacettepe University Human Ethics Committee (HEK 11/18-7).

Conflict of Interest The authors declare that they have no conflicts of interest.

References

1. Fabres J, Wehrli G, Marques MB, Phillips V, Dimmitt RA, Westfall AO, Schelonka RL (2006) Estimating blood needs for very-low-birth-weight infants. Transfusion 46(11):1915–1920
2. Sources of Lead. New York State. April 2010. Available from: https://www.health.ny.gov/environmental/lead/sources.htm. Last accessed: 26th June, 2018
3. Mercury in the Environment and Water Supply. Sources of Mercury. Available from: https://people.uwec.edu/piercech/hg/mercury_water/sources.htm. Last accessed: 26th June, 2018
4. Davidson PW, Palumbo D, Myers GJ, Cox C, Shamlaye CF, Sloane-Reeves J, Cernichiari E, Wilding GE, Clarkson TW (2000) Neurodevelopmental outcomes of Seychellois children from the pilot cohort at 108 months following prenatal exposure to methylmercury from a maternal fish diet. Environ Res 84(1):1–11
5. Hu H, Téllez-Rojo MM, Bellinger D, Smith D, Ettinger AS, Lamadrid-Figueroa H, Schwartz J, Schnaas L, Mercado-García A, Hernández-Avila M (2006) Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. Environ Health Perspect 114(11):1730–1735
6. Dorea JG, Donangelo CM (2006) Early (in uterus and infant) exposure to mercury and lead. Clin Nutr 25(11):369–376
7. Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J, Roberts R (2005) Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 113(7):894–899
8. Canfield RL, Henderson CR, Cory-Slechta DA, Cox C, Juski TA et al (2003) Intellectual impairment in children with blood lead concentrations below 10 μg per deciliter. New Engl J Med 348(16):1517–1526
9. Centers for Disease Control and Prevention (2002) Response to advisory committee on childhood lead poisoning prevention recommendations in "low level lead exposure harms children: a renewed call of primary prevention". US Department of Health and Human Services, Atlanta Available from: URL: https://www.cdc.gov/nceh/lead/acclpp/cdc_response_lead_exposure_recs.pdf
10. Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, SØRENSEN N, Dahl R, JØRGENSEN PJ (1997) Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 19(6):417–428
11. Jedrychowski W, Jankowski J, Flak E, Skarupa A, Mroz E, Sochacka-Tatara E et al (2006) Effects of prenatal exposure to mercury on cognitive and psychomotor function in one-year-old infants: epidemiologic cohort study in Poland. Ann Epidemiol 16(6):439–447
12. Clifton JC 2nd (2007) Mercury exposure and public health. Pediatr Clin N Am 54(2):237–269
13. Elabiad MT, Hook RE (2013) Lead content of blood transfusions for extremely low-birth-weight infants. Am J Perinatol 30(9):765–770
14. Sundararajan S, Blatz AM, Dearborn DG, Varnes AW, Bearer CF, El Metwally D (2015) Toxic metal contamination of banked blood designated for neonatal transfusion. J Clin Toxicol 5(5):1–5
15. Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA). 73rd meeting; Geneva; June 8–17, 2010. Available from: URL: www.who.int/ foodsafety/publications/chem/ summary73.pdf. Last accessed December, 2017
16. ASTDR. Toxicological profile for mercury: Update Atlanta, GA: ASTDR. Available from: URL: www.atsdr.cdc.gov/toxprofiles/tp46.pdf. Last accessed December, 2017
17. World Health Organization. Joint FAO/WHO expert committee on food additives: 61st Meeting, Rome, 10–19 June. Available from: URL: ftp://ftp.fao.org/es/esn/jecfa/jecfa61sc.pdf
18. US Environmental Protection Agency. Methylmercury (MeHg) (CASRN 22967–92-6). Available from: URL: www.epa.gov/iris/subst/0073. Last accessed December, 2017
19. Bain A, Blackburn S (2004) Issues in transfusing preterm infants in the NICU. J Perinat Neonatal Nurs 18(2):170–182
20. Elabiad MT, Christensen M (2014) Changes in premature infant mercury and lead blood levels after blood transfusions. Am J Perinatol 31(10):863–868
21. Ozment CP, Turi JL (2009) Iron overload following red blood cell transfusion and its impact on disease severity. Biochim Biophys Acta 1790(7):694–701
22. Widness JA, Seward VJ, Kromer IJ, Burmeister LF, Bell EF, Strauss RG (1996) Changing patterns of red blood cell transfusion in very low birth weight infants. J Pediatr 129(5):680–687
23. Strauss RG (1997) Practical issues in neonatal transfusion practice. Am J Clin Pathol 107(4 Suppl 1):S57–S63
24. Moretti R, Pansiot J, Bettati D, Strazielle N, Ghersi-Egea JF et al (2015) Blood-brain barrier dysfunction in disorders of the developing brain. Front Neurosci 9(1):40
25. Kirpalani H, Whyte RK, Andersen C, Asztalos EV, Heddle N, Blajchman MA, Peliowski A, Rios A, LaCorte M, Connelly R, Barrington K, Roberts RS (2006) The premature infants in need of transfusion (PINT) study: a randomized, controlled trial of a restrictive (low) versus liberal (high) transfusion threshold for extremely low birth weight infants. J Pediatr 149(3):301–307
26. Chen HL, Tseng HI, Lu CC, Yang SN, Fan HC, Yang RC (2009) Effect of blood transfusions on the outcome of very low body weight preterm infants under two different transfusion criteria. Pediatr Neonatol 50(3):110–116
27. Bell EF (2008) When to transfuse preterm babies. Arch Dis Child Fetal Neonatal Ed 93(6):F469–F473
28. Bearer CF, O'Riordan MA, Powers R (2000) Lead exposure from blood transfusion to premature infants. J Pediatr 137(4):549–554
29. Kirel B, Akşit MA, Bulut H (2005) Blood lead levels of maternal-cord pairs, children and adults who live in a central urban area in Turkey. Turk J Pediatr 47(2):125–131
30. The National Institute for Occupational Safety and Health (NIOSH). Adult blood lead epidemiology and Surveillance (ABLES). Available from: URL: http://www.cdc.gov/niosh/topics/ables/description.html. Last accessed December, 2017
31. Zubairi H, Visintainer P, Fleming J, Richardson M, Singh R (2015) Lead exposure in preterm infants receiving red blood cell transfusions. Pediatr Res 77(6):814–818
32. Centers for Disease Control and Prevention (2004) Blood mercury levels in young children and childbearing-aged women—United States, 1999—2002. MMWR 53:1018–1020
33. Kher K, Mistry K (2014) Assessment of glomerular and tubular function. Curr Pediatr Rev 10(2):142–150
34. Stajich GV, Lopez GP, Harry SW, Sexson WR (2000) Iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants. J Pediatr 136(5):679–681

35. Harville EW, Hertz-Picciotto I, Schramm M, Watt-Morse M, Chantala K, Osterloh J, Parsons PJ, Rogan W (2005) Factors influencing the difference between maternal and cord blood lead. Occup Environ Med 62(4):263–269
36. Ettinger AS, Hu H, Hernandez-Avila M (2007) Dietary calcium supplementation to lower blood lead levels in pregnancy and lactation. J Nutr Biochem 18(3):172–178
37. Wu J, Ying T, Shen Z, Wang H (2014) Effect of low-level prenatal mercury exposure on neonate neurobehavioral development in China. Pediatr Neurol 51(1):93–99
38. Bulleova S, Rothenberg SJ, Manalo MA (2001) Lead levels in blood bank blood. Arch Environ Health 56(4):312–313
39. Bearer CF, Linsalata N, Yomtovian R, Walsh M, Singer L (2003) Blood transfusions: a hidden source of lead exposure. Lancet 362(9380):332
40. Hillyer C, Goldman M, Dzik S (2002) Journal club. Transfus Med Rev 16(4):325–333
41. Delage G, Gingras S, Rhainds M (2015) A population-based study on blood lead levels in blood donors. Transfusion 55:633–2640