Science of the Total Environment 592 (2017) 51–59

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Elevated lead levels and changes in blood morphology and erythrocyte CR1 in preschool children from an e-waste area

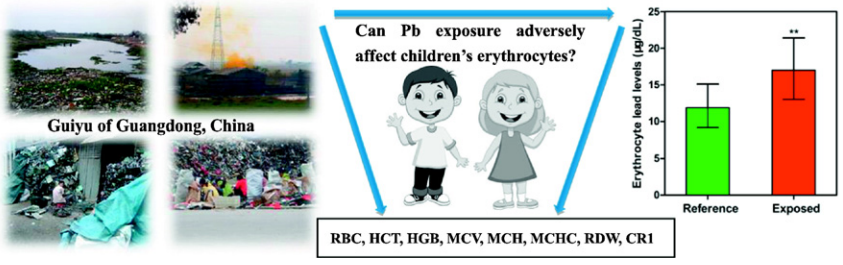
Yifeng Dai [a,b], Xia Huo [a,b,c], Yu Zhang [a,b], Tian Yang [a,b], Minghui Li [a,b], Xijin Xu [a,b,d,*]

[a] Laboratory of Environmental Medicine and Developmental Toxicology, Shantou University Medical College, Shantou, Guangdong 515041, China
[b] Guangdong Provincial Key Laboratory of Infectious Diseases, Shantou University Medical College, Shantou, Guangdong 515041, China
[c] School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou, Guangdong 510632, China
[d] Department of Cell Biology and Genetics, Shantou University Medical College, Shantou, Guangdong 515041, China

HIGHLIGHTS

- Blood and erythrocyte Pb levels of children from the e-waste recycling area were significantly higher.
- The Pb toxicity of erythrocytes is more significant in high Pb-exposed preschool children.
- The elevated erythrocyte Pb levels adversely affected CR1 expression in all children.
- There was no association between Pb exposure and CR1 immune adherence in children.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 September 2016
Received in revised form 8 March 2017
Accepted 8 March 2017
Available online 29 March 2017

Editor: D. Barcelo

Keywords:
Erythrocyte complement receptor type 1
Lead
Erythrocyte immunity
Preschool children
Electronic waste

ABSTRACT

Improper dismantling and combustion of electronic waste (e-waste) may release persistent organic pollutants and heavy metals that possess potential risk for human health. Lead (Pb) is carried through the circulatory system by erythrocytes and is known to alter the functions of hematopoietic and immune systems. The aim of the study was to investigate the effect of Pb exposure on blood morphology and erythrocyte complement receptor 1 (CR1) levels as related to immunologic function in preschool children. We recruited 484 preschool children, 2- to 6-years of age, among whom 332 children were from Guiyu, a typical and primitive e-waste processing area, and 152 children from Haojiang (reference area). Results showed that the blood Pb level (BPb) and erythrocyte Pb level (EPb) of exposed children were significantly higher, but, the mean corpuscular hemoglobin concentration (MCHC) and erythrocyte CR1 levels were significantly lower than reference children. Elevated EPb and BPb was related to disadvantageous changes in hematocrit (HCT), mean corpuscular volume (MCV), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), and MCHC, respectively, in children from the e-waste recycling area. Furthermore, in the high Pb-exposed group, the Pb toxicity of erythrocytes was more significant compared to the low Pb-exposed group in e-waste-exposed children. Combine with the BPb and EPb would be better to evaluating the Pb toxicity of erythrocytes. Compared to low Pb exposure, high BPb and EPb were associated with lower erythrocyte CR1 expression in all children. Our data suggests that elevated Pb levels result in adverse changes in blood morphology, hemoglobin synthesis and CR1 expression, which might be a non-negligible threat to erythrocyte immunity development in local preschool children. It is therefore imperative for any intervention to control the Pb exposure of children and actively educate adults to raise their environmental awareness of potential e-waste pollution during the recycling process.

* Corresponding author at: Laboratory of Environmental Medicine and Developmental Toxicology, Shantou University Medical College, 22 Xinling Rd., Shantou 515041, Guangdong, China.
E-mail address: xuxj@stu.edu.cn (X. Xu).

http://dx.doi.org/10.1016/j.scitotenv.2017.03.080
0048-9697/

1. Introduction

The shortened useful life expectancy of electrical and electronic equipment has led to a major increase in the accumulation of electronic waste (e-waste) (Breivik et al., 2014). The recycling of e-waste is regarded as an effective approach to relieving the necessity of acquiring scarce mineral resources and to continuing local economic and social development. At the same time, the improper dismantling and burning of e-waste releases persistent organic pollutants and heavy metals that can cause a potential risk for the health of local residents (Zeng et al., 2016a). Among the multiple contaminants, lead (Pb) is a ubiquitous and persistent environmental toxicant known to bioaccumulate in the human body and has no safe limit (Coelho et al., 2016). Guiyu, one of the largest e-waste destinations and recycling areas in the world, has a 30-year history of e-waste dismantling (Huo et al., 2007). Pb pollution comes mostly from prevalent and inappropriate e-waste recycling activities in Guiyu. Our previous studies showed that the soil and plants in Guiyu have been severely contaminated with Pb (road side soil Pb concentration of 540.9 mg/kg, and road side plant Pb concentration of 18.7 mg/kg.), and the levels of placental Pb in pregnant woman (median 301.4 ng/g, range 6.5–3465.2 ng/g.) from this area are significantly elevated and associated with fetal growth restriction (Alabi et al., 2012; Guo et al., 2010; Xu et al., 2016). Preschool children are more likely to contact e-waste when playing outdoors, and their hand to mouth activity is frequent, as well as their immature immune system, which is considered more susceptible to hazardous metal substances compared to adults for the reason that children have a higher ventilation rate and much larger surface area in relation to body size than adults (Cao et al., 2016; Heacock et al., 2016).

The interaction of Pb with the immune system, leading to immunosuppression or immunodysregulation, and Pb poisoning may be cause the disruption of erythrocyte heme synthesis (Aguilar-Dorado et al., 2014; Kathleen, 2010; Zeng et al., 2016b). In our prior studies, we observed that Pb exposure is associated with hematotoxicity, decreases in immune response to hepatitis B vaccination and declines in the function of natural killer (NK) cells in preschool children from Guiyu (Lin et al., 2016; Liu et al., 2015; Xu et al., 2015; Zhang et al., 2016). Furthermore, erythrocytes are not just a primary target of Pb toxicity, but also play an important role in innate immunity. When Pb reaches the systemic circulation, >95% of blood Pb accumulates in erythrocytes. Therefore, erythrocytes are regarded as an early and primary target for Pb-induced toxicity in the blood circulatory system (Jang et al., 2011). It is well-established that Pb has a direct negative effect on hematocrit or hemoglobin, which may be due to increased erythrophagocytosis, hemolysis and splenic sequestration of erythrocytes, or by impaired erythropoiesis (Jang et al., 2011; Kasten-Jolly et al., 2010).

Complement receptor 1 (CR1/CD35) is a type 1 transmembrane glycoprotein that is differentially expressed on erythrocytes, neutrophils, eosinophils, monocytes, B-lymphocytes, a subpopulation of $CD4^+$ T cells, dendritic cells and kidney podocytes, but not platelets (Fearon, 1980; Java et al., 2015; Katyal et al., 2004; Tedder et al., 1983). However, about 90% of the total CR1 is expressed on erythrocytes, with <10% being expressed on leukocytes (Siegel et al., 1981), and the total number of CR1 clusters per erythrocyte can be as high as ~350 (Lapin et al., 2012). Siegel et al. (1981) firstly reported the erythrocyte immune system and immune function are predominantly exerted through erythrocyte CR1. As an important adhesion molecule of erythrocytes, the major function of CR1 is to clear immune complexes and transport complement-opsonized particles from the blood circulation to the liver and spleen, and to mediate the phagocytosis of opsonized complexes (Brouwers et al., 2012; Dai et al., 2015). It also serves as a co-factor in factor I-mediated cleavage of C3b to C3bi, and accelerates the decay of C3 and C5 convertases (Awandare et al., 2011; Zipfel and Skerka, 2009). In erythrocyte immune function, CR1 is a vital component which is closely linked with the development and pathologic processes of various diseases, such as autoimmune, hematologic, and respiratory diseases, and inflammation (Barros et al., 2009; Beck et al., 2011; Marzocchi-Machado et al., 2005; Wang et al., 2005). In addition, the expression of CR1 on erythrocytes is influenced by a variety of factors, such as gene polymorphisms, age of the erythrocyte and host, health status and environmental factors (Pham et al., 2010; Waitumbi and Stoute, 2004). Animal studies suggest that exposure to metal and/or persistent organic pollutants influence immune function associated with erythrocytes (Li et al., 2014; Zhao et al., 2014; Zhu et al., 2011). Moreover, Fyfe et al. (1987) indicate that the numerical expression of erythrocyte CR1 is governed by environmental, rather than genetic factors in normal individuals. In this respect, we consider the possibility that erythrocytes and/or immune function of Pb-exposed children could be impaired.

To our knowledge, although erythrocyte CR1 function is well understood, there are no prior studies to elucidate the effect of Pb exposure on erythrocyte CR1 expression and immunologic function in the population. On the basis of available evidence, the main goal of the present study is to provide evidence to further understand the association between long-term Pb exposure and blood morphology, erythrocyte CR1 levels and immunologic function in preschool children.

2. Materials and methods

2.1. Study population

The children from two different kindergartens were chosen as research subjects from Guiyu and Haojiang, in Shantou southern China. We performed a cross-sectional study using stratified random sampling from the two kindergartens where voluntary participants were chosen from their classes and classified by their age. Finally, a total of 484 preschool children, 2- to 6-years of age, were recruited from the Guiyu ($n = 332$) and Haojiang ($n = 152$) kindergartens in November 2015. To be eligible, children could not have an infectious disease within one month before sample collection, and hematological or autoimmune diseases. Haojiang was selected as the reference area based on similarities to Guiyu (e-waste-exposed area) in population, residential lifestyle, cultural background and socioeconomic status, but lacks e-waste pollution. Prior to enrollment, we obtained a signed informed consent from parents or guardians and the study protocol was approved by the Human Ethics Committee of Shantou University Medical College, China. All parents completed a questionnaire administered by a trained research staff. The questionnaire acquired information about general characteristics of the children and parents, such as child dieting habits, nutrition, physical activity, medical and health history, parent occupation and education level, residential environment and socioeconomic status.

2.2. Blood sample preparation

A 4 mL sample of venous blood was obtained from each child and collected in two Pb-free tubes, containing EDTA or sodium citrate as anticoagulants, by well-trained nurses at the kindergartens. All blood samples were transported to the laboratory. The blood sample in the EDTA tube was used for routine blood examination, and then stored at −20 °C until Pb quantitation. The other blood-containing tube, with sodium citrate as the anticoagulant, was centrifuged at room temperature for 5 min at 200 ×g, then separated erythrocytes that were used to determine the surface CR1 expression level as related immune adherence activity.

2.3. Measurement of hematological parameters

Hematological parameters were estimated using an automated Sysmex XT-1800i hematology analyzer (Sysmex Corporation, Kobe, Japan). Red blood cell parameters, such as the red blood cell (RBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular

hemoglobin concentration (MCHC) and red cell distribution width (RDW), were assessed. At the same time, monocyte counts were also determined. Calibrators and controls were obtained from the manufacturer, and analysis of samples was performed within 8 h of blood collection.

2.4. Measurement of blood Pb level and calculation of erythrocyte Pb concentration

The blood Pb level (BPb) was determined by digestion of 100 μL whole blood sample in 900 μL 0.5% nitric acid (analytical reagent), followed by quantification of the Pb level by graphite furnace atomic absorption spectrophotometry (Jena Zeenit-650, Germany). The details of procedure for measuring BPb have been previously described (Liu et al., 2011). Erythrocyte Pb concentration was calculated according to the formula suggested by deSilva, i.e. erythrocyte Pb level (EPb) is equal to the BPb divided by the HCT as a fraction of the whole blood (deSilva, 1981).

2.5. Determination of erythrocyte CR1 surface expression by flow cytometry

The mean erythrocyte CR1 expression level on fresh blood samples was determined using a modification of a previously published method (Senbagavalli et al., 2008). Whole blood (500 μL) was washed three times in PBS (Shanghai BioScience Co., Ltd. China) and ten microliters of packed erythrocytes was taken and diluted with 2% BSA in PBS, to a final concentration of 2×10^6 cells/mL, after which 100 μL suspension was stained with 5 μL of anti-human CD235a-APC conjugate (Becton Dickinson) and 20 μL of anti-human CR1-PE conjugate (BD Bioscience, USA), followed by incubation at room temperature in the dark for 25 min. Excess antibody was removed by washing once with 1 mL BSA-PBS, and cells were resuspended in 500 μL of BSA-PBS for analysis by flow cytometry (Accuri C6, BD Bioscience, USA). Non-specific staining of the cells was excluded by running a parallel set of cells stained with irrelevant isotype-matched antibody (BD Bioscience, USA). The RBC population, based on positive staining of CD235a, a sialoglycoprotein on human erythrocytes, and the CR1 mean fluorescence intensity (MFI) of 10,000 erythrocytes were measured. Both the percentage of positive cells and the MFI for the antibody were measured as an indication of the receptor density. Fluorescence histograms were obtained on a logarithmic scale.

2.6. Determination of erythrocyte immune adherence in children

Erythrocyte immune adherence was measured by an erythrocyte yeast rosette formation test for examination of the erythrocyte C3b receptor rosette rate (E-C3bRR) and erythrocyte immune complex rosette rate (E-ICRR). Erythrocytes were washed three times with normal saline and the supernatant was discarded. Erythrocytes were resuspended with normal saline to a concentration of 1.25×10^7 cells/mL. Novelty yeast (Angel Yeast Co., Ltd. China) was dissolved in normal saline and inactivated at 100 °C for 20 min. After filtration, half of the yeast suspension was adjusted to 1×10^8 cells/mL and served as the unsensitized yeast sample. The remainder of the yeast suspension was opsonized with an equal volume of healthy guinea pig serum at 37 °C in a water bath for 25 min and then washed with normal saline. The sensitized yeast was resuspended in normal saline at a concentration of 1×10^8 cells/mL. In the assay, 50 μL sensitized yeast suspension and 50 μL erythrocyte suspension were combined in a tube and incubated at 37 °C for 40 min with periodic shaking, then 25 μL of 0.25% glutaraldehyde was added to each tube, and smears were then prepared on microscope slides. Slides were fixed and stained with Wright's stain, and 200 erythrocytes were counted from each smear under a microscope. The E-C3bRR and E-ICRR were equal to the number of erythrocytes binding ≥2 yeast cells/200 × 100%. The average percentage of two smears was calculated to obtain the results for each blood sample (Li et al., 2014).

2.7. Statistical analysis

All statistical analyses were performed using Stata 13.0 software (Stata, College Station, TX, USA) and Statistical Package for the Social Sciences (SPSS) 19.0 package (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD for normally distributed data, or median and interquartile range (IQR) for non-normally distributed data. The independent-sample *t*-test was used for two-group comparisons of normally distributed data. The Mann-Whitney *U* test was applied to two group comparisons of non-normally distributed data. In addition, we deleted the outliers and extreme values during analyses to ensure the match and comparability between two groups. The U.S. Center for Disease Control and Prevention (CDC) revised the threshold value for childhood Pb exposure downward from 10 to 5 μg/dL in 2012 (Betts, 2012). Therefore, child BPb concentration data were dichotomized at 5 μg/dL, and then Pearson's chi-square test was used to compare the over-limit ratio of BPb concentrations and other categorical data. Pearson's correlations (two-tailed) were used to test the associations between different normally distributed variables. Spearman's correlation test was used for the non-normally distributed data, and correlation coefficients (r_s) with *p*-values were presented. Considering the collinearity of these independent variables, we conducted multiple stepwise regression analyses to investigate the relevant factors contributing to BPb. Multiple linear regression analyses were performed to evaluate the associations between BPb or EPb levels and hematological parameters. In order to examine the associations of BPb and EPb concentrations with erythrocyte CR1 expression, separate regression models were used for each exposure-outcome association. We categorized BPb and EPb into quarters and estimated differences in mean values for the 2nd, 3rd and 4th quarters compared with the first quarter. The significance level was set at $\alpha = 0.05$ for two-sided tests. All regression models were adjusted for potential confounders as covariates. Regression diagnostics were conducted for all models, including examination of fit, influence and heteroscedasticity. All statistical tests requiring assumption of normality were conducted on natural logarithmic-transformed concentrations.

3. Results

3.1. Demographic characteristics of the study population

The demographic characteristics of the 484 participants are summarized in Table 1. The gender distribution of children was significantly different between exposed and reference groups ($p < 0.05$). The median BPb and EPb of e-waste-exposed children was 6.5 μg/dL and 17.0 μg/dL respectively, which were significantly higher than the 4.5 μg/dL and 11.9 μg/dL for reference children (all $p < 0.001$). >76.2% of children from the e-waste-exposed group exceeded the threshold value of BPb (5 μg/dL), in contrast to 38.8% for the reference group ($p < 0.001$). Furthermore, significant differences between the two groups were observed for child contact with e-waste, paternal education levels and monthly household income (all $p < 0.01$).

3.2. Factors contributing to Pb concentrations in child blood

Multiple stepwise regression analysis was performed to assess whether specific factors were related with BPb in preschool children from the e-waste recycling area. After adjusting for confounding factors, including gender and age, we observed that BPb was negatively associated with monthly household income ($\beta = -0.191$, $p < 0.01$) and paternal education level ($\beta = -0.160$, $p < 0.05$), as well as positively associated with sucking/biting toys ($\beta = 0.139$, $p < 0.05$) and child contact with e-waste ($\beta = 0.211$, $p < 0.01$) (Table 2). Meanwhile, we also used the multiple stepwise regression models to analysis the relationships between the factors and BPb in children from reference area, however, the result showed no significant for the regression model (data not shown).

Table 1
Demographic characteristics of the study population.

Demographic variables	Reference group (n = 152)	Exposed group (n = 332)	Statistics	p-Value
Age (years) (mean ± SD)	4.2 ± 1.1	4.4 ± 0.9	t = 2.102	0.037
Gender [n (%)]				
Male	95 (62.5)	173 (52.1)	χ^2 = 4.556	0.033
Female	57 (37.5)	159 (47.9)		
Blood Pb level (μg/dL) [median (IQR)]	4.5 (3.4, 5.6)	6.5 (5.1, 8.1)	Z = −9.649	0.000
≥5 μg/dL [n (%)]	59 (38.8)	253 (76.2)	χ^2 = 63.626	0.000
<5 μg/dL [n (%)]	93 (61.2)	79 (23.8)		
Erythrocyte Pb level (μg/dL) [median (IQR)]	11.9 (9.2, 15.1)	17.0 (13.0, 21.4)	Z = −8.998	0.000
Sucking/biting toys [n (%)]				
None	112 (74.2)	214 (67.3)	χ^2 = 5.369	0.147
Occasionally	36 (23.8)	101 (31.8)		
Often	3 (2.0)	2 (0.6)		
Always	0 (0.0)	1 (0.3)		
Child contact with e-waste [n (%)]				
No contact with e-waste	139 (92.1)	207 (64.2)	χ^2 = 41.670	0.000
Occasionally taken to the e-waste recycling site	6 (4.0)	86 (26.7)		
Frequently taken to the e-waste recycling site	6 (4.0)	29 (9.0)		
Paternal education levels [n (%)]				
Illiterate	0 (0.0)	1 (0.3)	χ^2 = 149.101	0.000
Primary school	0 (0.0)	21 (6.4)		
Middle school	21 (13.9)	197 (60.4)		
Vocational school	26 (17.2)	35 (10.7)		
High school	27 (17.9)	38 (11.7)		
College/university	77 (51.0)	34 (10.4)		
Monthly household income (yuan) [n (%)]				
<1500	1 (0.7)	12 (4.4)	χ^2 = 33.012	0.000
1500–3000	13 (8.9)	48 (17.7)		
3000–4500	27 (18.5)	69 (25.5)		
4500–6000	24 (16.4)	65 (24.0)		
>6000	81 (55.5)	77 (28.4)		

Values of $p < 0.05$ were considered statistically significant. IQR: interquartile range.

3.3. Hematological parameter distributions in children

We stratified by gender and analyzed the differences of erythrocyte parameters and monocyte counts between the exposed and reference groups (Fig. 1 and Table S1). Although all hematological parameters were within the normal range, the RBC counts of the girls were significantly higher in the exposed group as compared to the reference group ($4.77 \pm 0.35 \times 10^{12}$/L vs. $4.62 \pm 0.27 \times 10^{12}$/L, $p < 0.01$). Furthermore, the total HCT was also found to be higher in children from the e-waste-exposed area than from the reference area (38.2 ± 2.2% vs. 37.5 ± 2.2%, $p < 0.01$). However, the MCH of e-waste-exposed girls was significantly lower compared with reference girls (27.5 ± 1.5 pg vs. 28.1 ± 1.4 pg, $p < 0.05$). Correspondingly, the MCHC was also lower both in the boys and girls living in the e-waste-exposed area as compared to the children from the reference area (343 ± 8.2 g/L vs. 346 ± 9.8 g/L, $p < 0.05$ and 342 ± 7.9 g/L vs. 345 ± 7.7 g/L, $p < 0.01$, respectively). At the same time, we also found that the total monocyte counts were significantly increased in the exposed group when compared with reference group ($0.54 \pm 0.21 \times 10^9$/L vs. $0.49 \pm 0.17 \times 10^9$/L, $p < 0.05$). There were no significant differences in MCV, HGB and RDW between the two groups (all $p > 0.05$).

Table 2
Multiple stepwise regression analysis for factors related to the ln-transformed Pb concentrations in blood from e-waste recycling area.

Model	Change in ln(BPb)	
	β	B (95% CI)
Sucking/biting toys	0.139	0.104 (0.012, 0.196)*
Child contact with e-waste	0.211	0.087 (0.036, 0.137)**
Monthly household income	−0.191	−0.062 (−0.103, −0.021)**
Paternal education level	−0.160	−0.054 (−0.098, −0.011)*

Data was adjusted by gender and age. Ln(BPb): ln-transform of blood Pb levels; β: standardized coefficients; B: unstandardized coefficients; CI: confidence interval.
** Significant at $p < 0.01$.
* Significant at $p < 0.05$.

3.4. Effect of Pb exposure on erythrocyte parameters

To explore the association between BPb or EPb and erythrocyte parameters, we examined the relationships between Pb concentrations in blood or erythrocytes and RBC, HCT, MCV, HGB, MCH, MCHC and RDW from the e-waste recycling and reference areas (Table 3). In children from the e-waste recycling area, the BPb negatively correlated with the HCT (r_s = −0.168, p = 0.002), MCV (r_s = −0.204, $p < 0.001$), HGB (r_s = −0.158, p = 0.004) and MCH (r_s = −0.178, p = 0.001), but positively correlated with RDW (r_s = 0.132, p = 0.016). At the same time, EPb also negatively correlated with the HCT (r_s = −0.295, $p < 0.001$), MCV (r_s = −0.237, $p < 0.001$), HGB (r_s = −0.279, $p < 0.001$) and MCH (r_s = −0.211, $p < 0.001$), but positively correlated with RDW (r_s = 0.162, p = 0.003). However, no significant relationships were observed in the reference area (Table 3). Next, we divided e-waste-exposed children into high Pb- and low Pb-exposed groups, using the blood threshold concentration of 5 μg/dL Pb according to the criteria of United States CDC (Betts, 2012). Multiple linear regression analysis was performed to explain the associations between BPb or EPb and erythrocyte indices after being adjusted for the confounders of gender and age (Table 4). In the high Pb-exposed group, we found negative associations between BPb and HCT, MCV, HGB, MCH or MCHC (B = −0.001, B = −0.333, B = −0.628, B = −0.140 and B = −0.392,respectively). The EPb was also negatively related to the HCT, MCV, HGB, MCH and MCHC (B = −0.001, B = −0.138, B = −0.333, B = −0.057 and B = −0.153, respectively), but only the EPb was positively associated with the RDW (B = 0.011) (Table 4). Nevertheless, the regression models indicated that the associations between BPb or EPb and erythrocyte parameters were not significant in the low Pb-exposed group (data not shown).

3.5. Effect of Pb exposure on erythrocyte CR1 expression

In order to explore the association between Pb exposure and CR1, we examined the relationships between BPb or EPb and erythrocyte CR1 level in the e-waste-exposed and reference areas. Through our Pearson's correlation analyses, we noticed that these correlations did not reach statistical significance when CR1 was assayed by flow cytometry (Fig. S1). However, we found that the surface expression of CR1 on the erythrocyte membrane displayed significant differences between the two groups. Regardless of gender, a lower level of CR1 expression was observed among children from the e-waste-exposed area (median: 6257.515; range: 3046.550 to 20,931.610) compared to reference area (median: 8162.840; range: 3926.100 to 20,751.650) ($p < 0.01$) (Fig. 2). Furthermore, for all preschool children from the e-waste-exposed and reference areas, both BPb and EPb were negatively related to the cell surface erythrocyte CR1 levels (r_s = −0.155 and r_s = −0.125, respectively, all $p < 0.05$) (Table S2). Considering the sample size and confounding factors, such as socioeconomic status, we further used the separate regression models to analyze the association of child BPb or EPb and erythrocyte CR1 expression both in the e-waste-exposed and reference areas after adjusting for age, gender, maternal and paternal education levels and monthly household income (Table 6). The expression of erythrocyte CR1 decreased with the BPb and EPb when they were categorized in quartiles (p value for the trends were all <0.05). Compared to low BPb and EPb (quartile 1), high BPb and EPb (quartile 4) were associated with lower erythrocyte CR1 levels (β_{Q4} = −0.161,

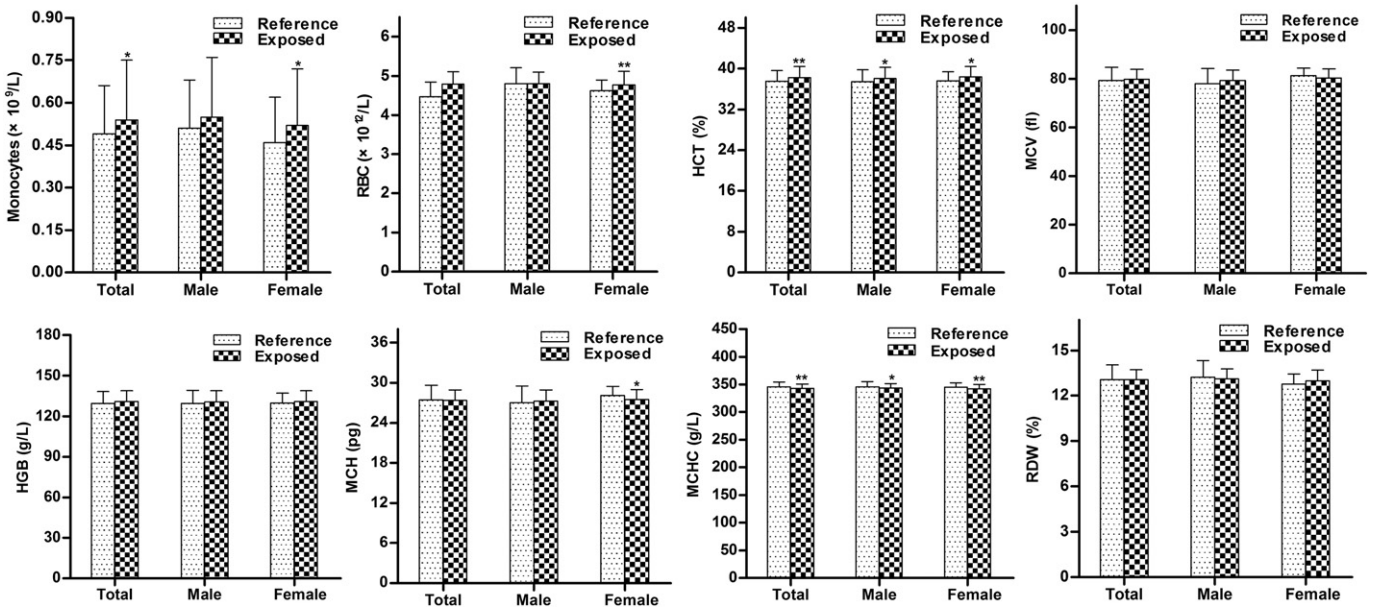


Fig. 1. Hematological profile stratified by gender between e-waste-exposed and reference groups. RBC: red blood cell; HCT: hematocrit; MCV: mean corpuscular volume; HGB: hemoglobin; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width. Exposed group, $n = 332$; reference group, $n = 152$. Results are presented as mean ± SD; data analysis by the independent-sample t-test. ** Significant at $p < 0.01$. * Significant at $p < 0.05$.

95%CI: -0.319, −0.008; $\beta_{Q4} = -0.186$, 95%CI: -0.350, −0.030, respectively).

3.6. Changes in erythrocyte CR1 immune adherence in children

The percentage of children exceeding the mean level of E-C3bRR in all subjects was higher in the exposed group when compared with the reference group (55.4% vs. 32.4%, $p < 0.05$). However, there was no significant difference in E-ICRR between the two groups ($p > 0.05$) (Table 5). In addition, we did not find any associations between Pb exposure and E-C3bRR or E-ICRR in preschool children ($p > 0.05$) (Table S2).

4. Discussion

In the current study, we investigated the changes in erythrocyte parameters, CR1 level and the ability of CR1 to function in an immune adherence assay, and explored the association of erythrocyte changes with environmental Pb exposure in preschool children from Guiyu, a typical e-waste processing area. The most common biological indicator of measuring Pb exposure is the BPb. We found that children living in Guiyu possess a high BPb compared to the reference area, with more than half of the children from exposed group exceeding the BPb limit value of 5 μg/dL, consistent with our previous research (Guo et al., 2014; Huo et al., 2007; Yang et al., 2013; Zeng et al., 2016c). Informal recycling of e-waste and the resulting heavy metal pollution has become a serious burden on the ecosystem in Guiyu. For instance, our previous research revealed soil Pb levels that were 2.32 times, whereas the ratios for dust samples were 4.10 times higher than the reference area (Yekeen et al., 2016). We have found Pb levels of 18.74 mg/kg in Guiyu roadside plants (Alabi et al., 2012), and in preliminary studies, we have also found that the Pb concentrations in $PM_{2.5}$ was 160 ng m^{-3} from Guiyu which is also higher when compared to other Asian cities (Zheng et al., 2016). In addition, the concentration of Pb in the surface water of Guiyu exceeds the Grade I standard when compared to China's Sea Water Quality Standards, and Pb concentration in sediment of the riverine system is high within Guiyu (Guo et al., 2009). Consequently, informal processing of e-waste results in release of Pb which not only pollutes the factory vicinity, but the community as a whole. Considering the reason for elevated BPb in children, we analyzed many relevant factors, including their playing habits, health and diet, as well as residence

Table 3
Pearson's correlation coefficients among individual blood and erythrocyte Pb concentrations, and erythrocyte related parameters from e-waste recycling and reference areas in China.

Variables	E-waste recycling area				Reference area			
	Blood Pb level		Erythrocyte Pb level		Blood Pb level		Erythrocyte Pb level	
	r_s	p	r_s	p	r_s	p	r_s	p
RBC	0.030	0.592	−0.046	0.402	−0.019	0.816	−0.069	0.396
HCT	−0.168	0.002	−0.295	0.000	0.030	0.718	−0.135	0.098
MCV	−0.204	0.000	−0.237	0.000	0.041	0.613	−0.024	0.772
HGB	−0.158	0.004	−0.279	0.000	0.031	0.706	−0.121	0.136
MCH	−0.178	0.001	−0.211	0.000	0.037	0.653	−0.031	0.709
MCHC	−0.011	0.847	−0.027	0.629	0.008	0.924	−0.036	0.656
RDW	0.132	0.016	0.162	0.003	0.018	0.826	0.086	0.292

Values of $p < 0.05$ were considered statistically significant. r_s: Pearson's correlation coefficient; RBC: red blood cell; HCT: hematocrit; MCV: mean corpuscular volume; HGB: hemoglobin; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width.

Table 4
Multiple linear regression analysis of the associations between blood or erythrocyte Pb levels and erythrocyte-related parameters in the high Pb-exposed group from e-waste recycling area.

Variables	High Pb-exposed group[a]	
	Blood Pb level B (95% CI)	Erythrocyte Pb level B (95% CI)
HCT	−0.001 (−0.002, 0.001)**	−0.001 (−0.001, −0.001)***
MCV	−0.333 (−0.504, −0.162)***	−0.138 (−0.198, −0.079)***
HGB	−0.628 (−0.953, −0.304)***	−0.333 (−0.443, −0.222)***
MCH	−0.140 (−0.206, −0.074)***	−0.057 (−0.080, −0.034)***
MCHC	−0.392 (−0.716, −0.069)*	−0.153 (−0.267, −0.039)**
RDW	0.027 (−0.001, 0.056)	0.011 (0.001, 0.021)*

Data was adjusted by gender and age. B: unstandardized coefficients; CI: confidence interval; RBC: red blood cell; HCT: hematocrit; MCV: mean corpuscular volume; HGB: hemoglobin; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width.

[a] Blood Pb level ≥ 5 μg/dL.

* Significant at $p < 0.05$.

** Significant at $p < 0.01$.

*** Significant at $p < 0.001$.

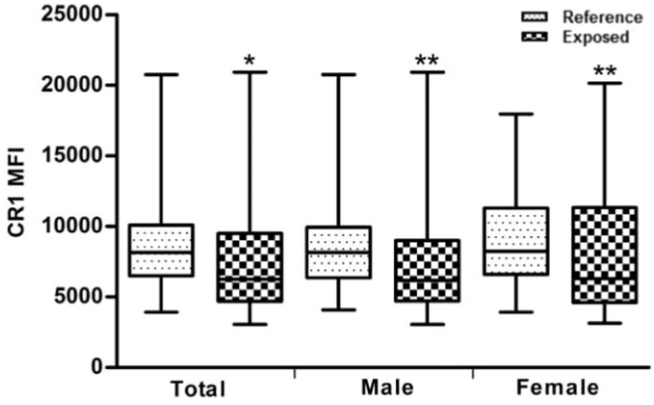


Fig. 2. Surface membrane CR1 levels of erythrocytes from e-waste-exposed and reference groups. Exposed group, $n = 130$; reference group, $n = 133$. Results are presented as medians (minimum, maximum); data analysis by the Mann-Whitney U test. **Significant at $p < 0.01$. *Significant at $p < 0.05$.

Table 6
Change in erythrocyte CR1 expression with increases in BPb and EPb for all preschool children after being adjusted for confounders.

	CR1 expression			
	n (%)	β	B (95% CI)	*p*-Value
BPb quartiles (μg/dL)				
Q1 (<3.78)	66 (25.1)		0	
Q2 (3.78–5.22)	66 (25.1)	−0.074	−0.076 (−0.231, 0.079)	0.338
Q3 (5.22–7.00)	66 (25.1)	−0.042	−0.043 (−0.198, 0.112)	0.587
Q4 (>7.00)	65 (24.7)	−0.161	−0.163 (−0.319, −0.008)	0.040
p for trend				0.006
EPb quartiles (μg/dL)				
Q1 (<9.99)	65 (24.7)		0	
Q2 (9.99–14.0)	66 (25.1)	−0.110	−0.113 (−0.274, 0.047)	0.166
Q3 (14.0–18.6)	66 (25.1)	−0.092	−0.093 (−0.251, 0.064)	0.246
Q4 (>18.6)	66 (25.1)	−0.186	−0.190 (−0.350, −0.030)	0.020
p for trend				0.004

Data was adjusted by age, gender, paternal and maternal education level and monthly household income. Values of $p < 0.05$ were considered statistically significant. β: standardized coefficients; B: unstandardized coefficients; CI: confidence interval.

environment. We observed that sucking/biting toys and child contact with e-waste are related to the elevated BPb. However, monthly household income and paternal education level are negatively associated with BPb. In conclusion, the bad habits of children promote Pb accumulation, but paternal education and household income may convey information about the patterns of potential Pb exposure, as well as health care for children living in the e-waste recycling area.

Previous reports demonstrate that acute Pb exposure can decrease hemoglobin synthesis, increase hemolysis arising from membrane fragility, and increase erythrocyte destruction in the spleen (Goyer and Cherian, 1995; Mrugesh et al., 2011; Palacios et al., 2012). In the present data, the RBC counts of e-waste-exposed girls are higher than the girls from the reference group, and the HCT in the exposed group is also higher than the reference group for both girls and boys. Even though the values of erythrocyte parameters are in the normal reference range for Guiyu children who live in a long-term low Pb exposure environment, their MCH and MCHC are lower than the reference children. Jacob et al. (2000) found that increasing BPb by 10.0 μg/dL is associated with a small increase in the number of RBC, and in girls will reduce the MCV and MCH. The stimulating effect of Pb on erythrocyte formation observed is somewhat stronger in girls (Jacob et al., 2000), similar to our results. According to an occupational Pb exposure report, HGB, HCT, MCHC, MCH and MCV are significantly lower in petrol station attendants (BPb = 15.11 μg/dL), compared to reference people.

Kim et al. (2002) and Liu et al. (2015) demonstrated the value of EPb or HCT in the adjustment of BPb for hematotoxicity studies of Pb toxicity. In view of the hematotoxicity at the different Pb exposure levels, it appears that, EPb and BPb are inversely related to the HCT, MCV, HGB and MCH, and positively related to the RDW in e-waste recycling area. However, we did not find these relationships in children from the reference area. A possible reason is that both the BPb and EPb of children from e-waste-exposed areas are higher than reference area and the mean BPb of these children are high, above 5 μg/dL. In support of this, multiple linear regression analysis finds that the BPb and EPb are inversely associated with HCT, MCV, HGB, MCH and MCHC only in the high Pb-exposed group. We did not find any associations in the low Pb-exposed group. In conclusion, these results indicate that the changes in these erythrocyte parameters are closely associated with high Pb exposure, especially when the BPb concentration is above 5 μg/dL. Therefore, children who have a BPb above 5 μg/dL should be closely monitored for Pb toxicity. In addition, the level of Pb in blood is a highly reliable biological marker of recent exposure to Pb (Betts, 2012). In order to evaluate the Pb toxicity to erythrocytes, combination of BPb and EPb is more accurate indicator. Furthermore, Dobrakowski et al. (2016) noticed that short-term occupational exposure to Pb results in a decreased HGB within the normal ranges, but does not affect RBC counts, which is similar to our results in preschool children. One possible reason is that the present Pb exposure level does not influence the erythrocyte lifespan in e-waste-exposed children, but long-term Pb exposure is responsible for the levels of erythrocyte membrane molecular and hemoglobin. Prior research has shown that the changes in hemoglobin concentrations are likely caused by Pb-induced inhibition of δ-aminolevulinic acid dehydratase or pyrimidine 5′-nucleotidase activity, which is associated with anemia (Jang et al., 2011; Kim et al., 2002). The hemoglobin inside erythrocytes could be important effectors of innate immunity responsible for killing microbial invaders (Liepke et al., 2003). Therefore, long-term Pb exposure might not only affect heme synthesis, but also be a detriment to the antimicrobial activity of hemoglobin in preschool children. Moreover, association analysis shows that changes in EPb, due to Pb exposure associate negatively with changes in MCV, but positively with changes in RDW. Thus, our findings show that long-term Pb exposure may decrease MCV and cause anisocytosis in preschool children.

Table 5
Immune adherence of erythrocyte CR1 in children between e-waste-exposed and reference groups.

Variables	Reference group	Exposed group	Statistics	*p*-Value
E-C3bRR				
≥37.67%[a]	11 (32.4)	72 (55.4)	$\chi^2 = 5.719$	0.017
<37.67%	23 (67.6)	58 (44.6)		
E-ICRR				
≥42.98%[b]	11 (32.4)	64 (49.2)	$\chi^2 = 3.039$	0.079
<42.98%	23 (67.6)	66 (50.8)		

Values of $p < 0.05$ were considered statistically significant. E-C3bRR: erythrocyte C3b receptor rosette rate; E-ICRR: erythrocyte immune complex rosette rate.
[a] Mean value of E-C3bRR in all subjects.
[b] Mean value of E-ICRR in all subjects.

Erythrocyte immunity represents the first line of defense in innate immunity, and erythrocyte membranes express the complement regulatory protein CR1 that regulates activation of the complement system and provides essential protection against self-damage (Ruiz-Argüelles and Llorente, 2007). Animal studies examined the associations between chemical pollutants exposure and erythrocyte CR1 expression and function, but no previous studies have examined the relationship between Pb exposure and erythrocyte CR1 levels in a population. Because 90% of CR1 exists in erythrocytes, and the number of erythrocytes is higher than that of leucocytes in blood, the amount of circulating immune complexes associated with erythrocytes is 500- to 1000-times greater than with leucocytes. Therefore, immune complexes in circulation are predominantly removed by erythrocytes (Siegel et al., 1981). In humans, the CR1 level of erythrocytes also varies with age, with levels being high at birth, and subsequently decreasing to low levels between

6 and 24 months of age, a period at which children are most susceptible to infection (Odhiambo et al., 2015; Waitumbi and Stoute, 2004). The present study shows that children from Guiyu have higher BPb or EPb, but lower erythrocyte CR1 levels than the reference children. Barros et al. (2009) demonstrated that complement regulatory proteins play an important role in protecting erythrocyte destruction through the activation of complement. Individuals with low erythrocyte CR1 expression are ill-equipped to clear immune complexes and prevent immune complex-mediated stimulation of macrophages (Odera et al., 2011). Studies confirm that Pb could change the physicochemical state of proteins and lipids in erythrocyte and lymphocyte membranes (Apostoli et al., 1988; Mrugesh et al., 2011; Slobozhanina et al., 2005). We observed that total BPb and EPb are associated with lower erythrocyte CR1 level, with 4th quartile of exposure (BPb > 7.00 μg/dL, EPb > 18.6 μg/dL) showing the strongest associations in all preschool children. Interestingly, we did not find a correlation trend between BPb or EPb and erythrocyte CR1 with statistical significance in e-waste-exposed and reference areas. The possible reason could be that only when the Pb exposure level is higher, the correlation become manifest. In addition, sample size would also have an effect on the statistical efficiency. Although we did not find a direct relationship between Pb exposure and erythrocyte CR1 expression in children from the e-waste recycling area, the Pb exposure still correlated with a potential risk for CR1 expression in analyses of the total population, and the surface erythrocyte CR1 level is also significantly decreased in e-waste exposed children. CR1 plays critical roles in the immune response of B and T lymphocytes (Erdei et al., 2009), and individuals afflicted by some of these diseases, including systemic lupus erythematosus and severe malaria-associated anemia, develop low erythrocyte CR1 levels as a result of infection (Boaz et al., 2008; Ross et al., 1985). The loss of CR1 observed coincides with a rise in IgG- and IgM-containing immune complexes, and might present an attempt to clear these proinflammatory complexes from the circulation (de Oliveira et al., 2014). To summarize, our results indicate that reduced erythrocyte CR1 levels are directly linked with elevated EPb and BPb, suggesting that Pb could confer a potential risk to erythrocyte immunity for preschool children.

Immune adherence of erythrocytes is reflected by the E-C3bRR and E-ICRR. Decreased activity of CR1 would decrease the erythrocyte-mediated removal of immune complexes and result in a decline in E-C3bRR. On the contrary, an increase in immune complexes can result in the E-ICRR elevated (Birmingham and Hebert, 2001; Oudin et al., 2000). The results in the present study show that the E-C3bRR is higher in the exposed group than the reference group, in the absence of a difference in E-ICRR. We also notice that the monocyte counts are elevated in Guiyu children, which might benefit clearance due to erythrocyte CR1 binding with immune complexes, which are then delivered to the liver and spleen for phagocytosis by macrophages. Therefore, for the exposed children, circulating immune complexes taken up by erythrocytes are removed completely and vacancies of erythrocyte CR1 are not occupied by these immune complexes. Interestingly, animal studies found the levels of E-C3bRR decreased and E-ICRR increased with the rise of aluminum and nickel chloride exposure, and suggested that the immune function of erythrocytes is suppressed by exposure to these metals (Li et al., 2014; Zhu et al., 2011). Erythrocyte immune adherence depends on many factors, including the number of erythrocytes, circulating immune complexes, monocytes and CR1 levels (Kullo et al., 2011). In the present data, the erythrocyte CR1 immune adherence activity is not significantly associated with the BPb or EPb in preschool children. The possible reason is that the erythrocyte counts have no obvious change and the number of monocytes is increased in the exposed children, which is not enough to influence the clearance of immune complexes from the body.

This analysis has limitations associated with the cross-sectional design of the study. First, the child subjects might have been affected by other heavy metals and organic pollutants due to the multiple contaminants present in the e-waste area. However, we choose to investigate the effects of Pb on erythrocytes because of the ability of erythrocytes to concentrate Pb. Pb is a ubiquitous environmental contaminant, especially in Guiyu, and hinders erythrocyte and leukocyte function (García-Lestón et al., 2011; Kim and Lee, 2013; Kossowska et al., 2010). A second limitation is that the sample size is not sufficiently large to better analyze the relationships between the Pb exposure and erythrocyte CR1 expression or immune function. A third limitation is that more indicators of erythrocyte immune function need to be confirmed that could supply us with more information. In our future work, we will measure additional indicators related to erythrocyte immune function and other potential chemical pollutants from a larger population.

5. Conclusions

E-waste exposed children have higher BPb and EPb, and lower erythrocyte parameters and CR1 levels. Our findings suggest that elevated EPb and BPb are related to the disadvantageous changes in HCT, MCV, HGB, MCH, and MCHC in preschool children from the e-waste recycling area. The combination of BPb and EPb values is more accurate for evaluating the Pb toxicity to erythrocyte. Furthermore, in the high Pb-exposed group, the Pb toxicity to erythrocytes is more significant compared to the low Pb-exposed group in children from the e-waste recycling area. Although we did not find a direct relationship between Pb exposure and erythrocyte CR1 expression in children from our e-waste recycling area, the Pb exposure still existed as potential risk factor for depressing CR1 levels in local children, as judged by our analyses of the total population, and erythrocyte CR1 levels also being significantly decreased in e-waste-exposed children. We conclude that long-term Pb exposure may cause adverse effects on blood morphology, hemoglobin biosynthesis and CR1 levels, which might pose a non-negligible threat to erythrocyte immunity in preschool children, especially those children living in an e-waste recycling area. More indicators related to erythrocyte immunity should be measured to elucidate the toxicological effects of Pb. Preventive measures are therefore essential to reduce environmental Pb exposure, to lower the body burden and to control and avoid further detrimental health outcomes.

Conflict of interest

The authors declare they have no competing financial interests.

Acknowledgements

The work was supported by the National Natural Science Foundation of China (21577084), Guangdong Science and Technology Planning Project (2013B051000078) and Education Department of Guangdong Government under the Top-tier University Development Scheme for Research and Control of Infectious Diseases (2015082). We would like to thank Dr. Stanley L. Lin his constructive comments and English language editing. We are grateful to all the recruited children and their guardians for participating in this project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.03.080.

References

Aguilar-Dorado, I.C., Hernández, G., Quintanar-Escorza, M.A., Maldonado-Vega, M., Rosas-Flores, M., Calderón-Salinas, J.V., 2014. Eryptosis in lead-exposed workers. Toxicol. Appl. Pharmacol. 281, 195–202.

Alabi, O.A., Bakare, A.A., Xu, X., Li, B., Zhang, Y., Huo, X., 2012. Comparative evaluation of environmental contamination and DNA damage induced by electronic-waste in Nigera and China. Sci. Total Environ. 423, 62–72.

Apostoli, P., Romeo, L., De Matteis, M.C., Menegazzi, M., Faggionato, G., Vettore, L., 1988. Effects of lead on red blood cell membrane proteins. Int. Arch. Occup. Environ. Health 61, 71–75.

Awandare, G.A., Spadafora, C., Moch, J.K., Dutta, S., Haynes, J.D., Stoute, J.A., 2011. Plasmodium falciparum field isolates use complement receptor 1 (CR1) as a receptor for invasion of erythrocytes. Mol. Biochem. Parasitol. 177, 57–60.

Barros, M.M., Yamamoto, M., Figueiredo, M.S., Cancado, R., Kimura, E.Y., Langhi, D.M.J., et al., 2009. Expression levels of CD47, CD35, CD55, and CD59 on red blood cells and signal-regulatory protein-alpha,beta on monocytes from patients with warm autoimmune hemolytic anemia. Transfusion 49, 154–160.

Beck, Z., Brown, B.K., Matyas, G.R., Polonis, V.R., Rao, M., Alving, C.R., 2011. Infection of human peripheral blood mononuclear cells by erythrocyte-bound HIV-1: effects of antibodies and complement. Virology 412, 441–447.

Betts, K.S., 2012. CDC updates guidelines for children's lead exposure. Environ. Health Perspect. 120, a268.

Birmingham, D.J., Hebert, L.A., 2001. CR1 and CR1-like: the primate immune adherence receptors. Immunol. Rev. 180, 100–111.

Boaz, O.O., Collins, O.O., Walter, O.O., Christine, A., Dominic, W.M., José, A.S., 2008. Reduced immune complex binding capacity and increased complement susceptibility of redcells from children with severe malaria-associated anemia. Mol. Med. 14 (3–4), 89–97.

Breivik, K., Arrmitage, J.M., Wania, F., Jones, K.C., 2014. Tracking the gloal generation and exports of e-waste. Do existing estimates add up? Environ. Sci. Technol. 48, 8735–8743.

Brouwers, N., Cauwenberghe, C.V., Engelborghs, S., Lambert, J.C., Bettens, K., Bastard, N.L., et al., 2012. Alzheimer risk associated with a copy number variation in the complement receptor 1 increasing C3b/C4b binding sites. Mol. Psychiatry 17, 223–233.

Cao, J., Xu, X., Hylkema, M.N., Zeng, E.Y., Sly, P.D., Suk, W.A., et al., 2016. Early-life exposure to widespread environmental toxicants and health risk: a focus on the immune and respiratory systems. Ann. Glob. Health 82, 119–131.

Coelho, S.D., Pastorinho, R., Itai, T., Isobe, T., Kunisue, T., Nogueira, A.J., et al., 2016. Lead in duplicate diet samples from an academic community. Sci. Total Environ. 573, 603–607.

Dai, Y., Wang, Q.W., He, S., Zhang, Z., Gao, C., 2015. Correlation of ECR1 A3650G polymorphism with neonatal respiratory distress syndrome. Genet. Test. Mol. Biomarkers 19, 18–23.

Dobrakowski, M., Boroń, M., Czuba, Z.P., Birkner, E., Chwalba, A., Hudziec, E., et al., 2016. Blood morphology and the levels of selected cytokines related to hematopoiesis in occupational short-term exposure to lead. Toxicol. Appl. Pharmacol. 305, 111–117.

Erdei, A., Isaák, A., Török, K., Sándo, N., Kremlitzka, M., Prechl, J., et al., 2009. Expression and role of CR1 and CR2 on B and T lymphocytes under physiological and autoimmune conditions. Mol. Immunol. 46, 2767–2773.

Fearon, D.T., 1980. Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte, and monocyte. J. Exp. Med. 152, 20–30.

Fyfe, A., Holme, E.R., Mckay, I.C., Zoma, A., Hunter, J., Lucie, N.P., et al., 1987. The relative roles of genetic and environmental factors in the regulation of erythrocyte C3b receptor (ECR1) numbers in normal individuals. Clin. Exp. Immunol. 70, 231–237.

García-Lestón, J., Roma-Torres, J., Vilares, M., Rui, P., Cunha, L.M., Prista, J., et al., 2011. Biomonitoring of a population of portuguese workers exposed to lead. Mutat. Res. 721, 81–88.

Goyer, R.A., Cherian, M.G., 1995. Toxicology of metals. Handb. Exp. Pharmacol. 115.

Guo, Y., Huang, C., Zhang, H., Dong, Q., 2009. Heavy metal contamination from electronic waste recycling at Guiyu Southeastern China. J. Environ. Qual. 38, 1617–1626.

Guo, Y., Huo, X., Li, Y., Wu, K., Liu, J., Huang, J., et al., 2010. Monitoring of lead, cadmium, chromium and nickel in placenta from an e-waste recycling town in China. Sci. Total Environ. 408, 3113–3117.

Guo, P., Xu, X., Huang, B., Sun, D., Zhang, J., Chen, X., et al., 2014. Blood lead levels and associated factors among children in Guiyu of China: a population-based study. PLoS One 9, e105470.

Heacock, M., Kelly, C.B., Asante, K.A., Birnbaum, L.S., Bergman, A.L., Bruné, M.N., et al., 2016. E-waste and harm to vulnerable populations: a growing global problem. Environ. Health Perspect. 124, 550–555.

Huo, X., Peng, L., Xu, X., Zheng, L., Qiu, B., Qi, Z., et al., 2007. Elevated blood lead levels of children in Guiyu, an electronic waste recycling town in China. Environ. Health Perspect. 115, 1113–1117.

Jacob, B., Ritz, B., Heinrich, J., Hoelscher, B., Wichmann, H., 2000. The effect of low-level blood lead on hematologic parameters in children. Environ. Res. 82, 150–159.

Jang, W.H., Lim, K.M., Kim, K., Noh, J.Y., Kang, S., Chang, Y.K., et al., 2011. Low level of lead can induce phosphatidylserine exposure and erythrophagocytosis: a new mechanism underlying lead-associated anemia. Toxicol. Sci. 122, 177–184.

Java, A., Liszewski, M.K., Hourcade, D.E., Zhang, F., Atkinson, J.P., 2015. Role of complement receptor 1 (CR1; CD35) on epithelial cells: a model for understanding complement-mediated damage in the kidney. Mol. Immunol. 67, 584–595.

Kasten-Jolly, J., Yong, H., Lawrence, D.A., 2010. Impact of developmental lead exposure on splenic factors. Toxicol. Appl. Pharmacol. 247, 105–115.

Kathleen, C., 2010. Lead poisoning in children. Am. Fam. Physician 81, 751–757.

Katyal, M., Tiwari, S.C., Kumar, A., Dinda, A.K., Arora, V., Kumar, R., et al., 2004. Association of complement receptor 1 (CR1, CD35, C3b/C4b receptor) density polymorphism with glomerulonephritis in Indian subjects. Mol. Immunol. 8, 1325–1332.

Kim, Y., Lee, B.K., 2013. Increased erythrocyte lead levels correlate with decreased hemoglobin levels in the Korean general population: analysis of 2008–2010 Korean national health and nutrition examination survey data. Int. Arch. Occup. Environ. Health 86, 741–748.

Kim, Y., Lee, H., Lee, C.R., Park, D.U., Yang, J.S., Park, I.J., et al., 2002. Evaluation of lead exposure in workers at secondary lead smelters in South Korea: with focus on activity of erythrocyte pyrimidine 5′-nucleotidase (P5N). Sci. Total Environ. 286, 181–189.

Kossowska, B., Dudka, I., Bugla-Płoskońska, G., Szymańska-Chabowska, A., Doroszkiewicz, W., Gancarz, R., et al., 2010. Proteomic analysis of serum of workers occupationally exposed to arsenic, cadmium, and lead for biomarker research: a preliminary study. Sci. Total Environ. 408, 5317–5324.

Kullo, I., Ding, K., Shameer, K., Mccarty, C., Jarvik, G., Denny, J., et al., 2011. Complement receptor 1 gene variants are associated with erythrocyte sedimentation rate. Am. J. Hum. Genet. 89, 131–138.

Lapin, Z.J., Höppener, C., Gelbard, H.A., Novotny, L., 2012. Near-field quantification of complement receptor 1 (CR1/CD35) protein clustering in human erythrocytes. J. NeuroImmune Pharmacol. 7, 539–543.

Li, J., Wu, B., Cui, H., Peng, X., Fang, J., Zuo, Z., et al., 2014. Effects of nickel chloride on the erythrocytes and erythrocyte immune adherence function in broilers. Biol. Trace Elem. Res. 161, 173–179.

Liepke, C., Baxmann, S., Heine, C., Breithaupt, N., Ständker, L., Forssmann, W.G., 2003. Human hemoglobin-derived peptides exhibit antimicrobial activity: a class of host defense peptides. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 791, 345–356.

Lin, Y., Xu, X., Dai, Y., Zhang, Y., Li, W., Huo, X., 2016. Considerable decrease of antibody titers against measles, mumps, and rubella in preschool children from an e-waste recycling area. Sci. Total Environ. 573, 760–766.

Liu, J., Xu, X., Wu, K., Piao, Z., Huang, J., Guo, Y., et al., 2011. Association between lead exposure from electronic waste recycling and child temperament alterations. Neurotoxicology 32, 458–464.

Liu, C., Huo, X., Lin, P., Zhang, Y., Li, W., Xu, X., 2015. Association between blood erythrocyte lead concentrations and hemoglobin levels in preschool children. Environ. Sci. Pollut. Res. Int. 22, 9233–9240.

Marzocchi-Machado, C.M., Alves, C.M., Azzolini, A.E., Polizello, A.C., Carvalho, I.F., Lucisano-Valim, Y.M., 2005. CR1 on erythrocytes of Brazilian systemic lupus erythematosus patients: the influence of disease activity on expression and ability of this receptor to bind immune complexes opsonized with complement from normal human serum. J. Autoimmun. 25, 289–297.

Mrugesh, T., Dipa, L., Manishika, G., 2011. Effect of lead on human erythrocytes: an in vitro study. Acta Pol. Pharm. 68, 653–656.

Odera, M., Otieno, W., Adhiambo, C., Stoute, J.A., 2011. Dual role of erythrocyte complement receptor type 1 in immune complex-mediated macrophage stimulation: implications for the pathogenesis of plasmodium falciparum malaria. Clin. Exp. Immunol. 166, 201–207.

Odhiambo, C.O., Otieno, W., Adhiambo, C., Odera, M.M., Stoute, J.A., 2015. Increased deposition of C3b on red cells with low CR1 and CD55 in a malaria-endemic region of western Kenya: implications for the development of severe anemia. Proc. Natl. Acad. Sci. 6, 2715–2719.

de Oliveira, R.B., Wang, J.P., Ram, S., Gazzinelli, R.T., Finberg, R.W., Golenbock, D.T., 2014. Increased survival in B-cell-deficient mice during experimental cerebral malaria suggests a role for circulating immune complexes. MBio 5, e00949-14.

Oudin, S., Libyh, M.T., Goossens, D., Dervillez, X., Philbert, F., Réveil, B., et al., 2000. A soluble recombinant multimeric anti-Rh(D) single-chain Fv/CR1 molecule restores the immune complex binding ability of CR1-deficient erythrocytes. J. Immunol. 164, 1505–1513.

Palacios, J., Roman, D., Cifuentes, F., 2012. Exposure to low level of arsenic and lead in drinking water from Antofagasta city induces gender differences in glucose homeostasis in rats. Biol. Trace Elem. Res. 148, 224–231.

Pham, B.N., Kisserli, A., Donvito, B., Duret, V., Reveil, B., Tabary, T., et al., 2010. Analysis of complement receptor type 1 expression on red blood cells in negative phenotypes of the knops blood group system, according to CR1 gene allotype polymorphisms. Transfusion 50, 1435–1443.

Ross, G.D., Yount, W.J., Walport, M.J., Winfield, J.B., Parker, C.J., Fuller, C.R., et al., 1985. Disease-associated loss of erythrocyte complement receptors (CR1, C3b receptors) in patients with systemic lupus erythematosus and other diseases involving autoantibodies and/or complement activation. J. Immunol. 135, 2005–2015.

Ruiz-Argüelles, A., Llorente, L., 2007. The role of complement regulatory proteins (CD55 and CD59) in the pathogenesis of autoimmune hemocytopenias. Autoimmun. Rev. 6, 155–161.

Senbagavalli, P., Geetha, S.T., Karunakaran, K., Rekha, V.V.B., Venkatesan, P., Ramanathan, V.D., 2008. Reduced erythrocyte CR1 levels in patients with pulmonary tuberculosis is an acquired phenomenon. Clin. Immunol. 128, 109–115.

Siegel, I., Liu, T.L., Gleicher, N., 1981. The red-cell immune system. Lancet 2, 556–559.

deSilva, P.E., 1981. Determination of lead in plasma and studies on its relationship to lead in erythrocytes. Br. J. Ind. Med. 38, 209–217.

Slobozhanina, E.I., Kozlova, N.M., Lukyanenko, L.M., Oleksiuk, O.B., Gabbianelli, R., Fedeli, D., et al., 2005. Lead-induced changes in human erythrocytes and lymphocytes. J. Appl. Toxicol. 25, 109–114.

Tedder, T.F., Fearon, D.T., Gartland, G.L., Cooper, M.D., 1983. Expression of C3b receptors on human be cells and myelomonocytic cells but not natural killer cells. J. Immunol. 130, 1668–1673.

Waitumbi, J.N., Stoute, J.A., 2004. Age-related changes in red blood cell complement regulatory proteins and susceptibility to severe malaria. J. Infect. Dis. 190, 1183–1191.

Wang, F.S., Chu, F.L., Jin, L., Li, Y.G., Zhang, Z., Xu, D., et al., 2005. Acquired but reversible loss of erythrocyte complement receptor 1 (CR1, CD35) and its longitudinal alteration in patients with severe acute respiratory syndrome. Clin. Exp. Immunol. 139, 112–119.

Xu, X., Chen, X., Jian, Z., Pi, G., Fu, T., Dai, Y., et al., 2015. Decreased blood hepatitis B surface antibody levels linked to e-waste lead exposure in preschool children. J. Hazard. Mater. 298, 122–128.

Xu, L., Ge, J., Huo, X., Zhang, Y., Lau, A.T., Xu, X., 2016. Differential proteomic expression of human placenta and fetal development following e-waste lead and cadmium exposure in utero. Sci. Total Environ. 550, 1163–1170.

Yang, H., Huo, X., Yekeen, T.A., Zheng, Q., Zheng, M., Xu, X., 2013. Effects of lead and cadmium exposure from electronic waste on child physical growth. Environ. Sci. Pollut. Res. 20, 4441–4447.

Yekeen, T.A., Xu, X., Zhang, Y., Wu, Y., Kim, S.S., Reponen, T., et al., 2016. Assessment of health risk of trace metal pollution in surface soil and road dust from e-waste recycling area in China. Environ. Sci. Pollut. Res. 23, 17511–17524.

Zeng, X., Gong, R., Chen, W.Q., Li, J., 2016a. Uncovering the recycling potential of "new" WEEE in China. Environ. Sci. Technol. 50, 1347–1358.

Zeng, X., Xu, X., Boezen, H.M., Huo, X., 2016b. Children with health impairments by heavy metals in an e-waste recycling area. Chemosphere 148, 408–415.

Zeng, X., Xu, X., Zheng, X., Reponen, T., Chen, A., Huo, X., 2016c. Heavy metals in $PM_{2.5}$ and in blood, and children's respiratory symptoms and asthma from an e-waste recycling area. Environ. Pollut. 210, 346–353.

Zhang, Y., Huo, X., Cao, J., Yang, T., Xu, L., Xu, X., 2016. Elevated lead levels and adverse effects on natural killer cells in children from an electronic waste recycling area. Environ. Pollut. 213, 143–150.

Zhao, Y.Z., Jia, J., Li, Y.B., Guo, C.X., Zhou, X.Q., Sun, Z.W., 2014. Effects of endosulfan on the immune function of erythrocytes, and potential protection by testosterone propionate. J. Toxicol. Sci. 39, 701–710.

Zheng, X., Xu, X., Yakeen, T.A., Zhang, Y., Chen, A., Kim, S., et al., 2016. Ambient air heavy metals in $PM_{2.5}$ and potential human health risk assessment in an informal electronic-waste recycling site of China. Aerosol Air Qual. Res. 16, 388–397.

Zhu, Y., Zhao, H., Li, X., Zhang, L., Hu, C., Shao, B., et al., 2011. Effects of subchronic aluminum exposure on the immune function of erythrocytes in rats. Biol. Trace Elem. Res. 143, 1576–1580.

Zipfel, P.F., Skerka, C., 2009. Complement regulators and inhibitory proteins. Nat. Rev. Immunol. 9, 729–740.