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Relations of blood lead levels to echocardiographic left ventricular structure and function in preschool children

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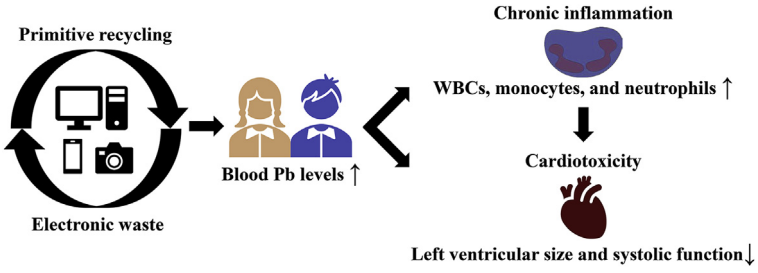
HIGHLIGHTS

- First study on the impact of Pb exposure on child left ventricle.
- Pb cardiotoxicity in child was assessed by using echocardiography.
- Higher blood Pb level was found in e-waste-exposed children.
- Parameters of left ventricle and systolic function were lower in exposed children.
- Pb cardiotoxicity may be linked to chronic low-grade inflammation.

GRAPHICAL ABSTRACT



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ABSTRACT

Lead (Pb) has been proved to exert adverse effect on human cardiovascular system. However, the cardiotoxicity of Pb on children is still unclear. The aim of this study was to evaluate left ventricular (LV) structure and function, by using echocardiographic indices, in order to elucidate the effect of Pb on low-grade inflammation related to left ventricle in healthy preschool children. We recruited a total of 486 preschool children, 310 from Guiyu (e-waste-exposed area) and 176 from Haojiang (reference area). Blood Pb levels, complete blood counts, and LV parameters were evaluated. Associations between blood Pb levels and LV parameters and peripheral leukocyte counts were analyzed using linear regression models. The median blood level of Pb and the counts of white blood cells (WBCs), monocytes, and neutrophils were higher in exposed group. In addition, the exposed group showed smaller left ventricle (including interventricular septum, LV posterior wall, and LV mass index) and impaired LV systolic function (including LV fractional shortening and LV ejection fraction) regardless gender. After adjustment for confounding factors, elevated blood Pb levels were significantly associated with higher counts of WBCs and neutrophils, and lower levels of LV parameters. Furthermore, counts of WBCs, monocytes, and neutrophils were negatively correlated with LV parameters. Taken together, smaller left ventricle and impaired systolic function were found in e-waste-exposed children and associated with chronic low-grade inflammation and elevated blood Pb levels. It indicates that the heart health of e-waste-exposed children is at risk due to the long-term environmental chemical insults.

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1. Introduction

Lead (Pb) is considered as a potent environmental toxic metal, in part due to its non-biodegradable nature, and its toxic effects are well studied. Guiyu has a long history as an e-waste recycling site, resulting in considerable heavy metal contamination, the hazard quotient and hazard index of Pb were the highest in the local environment (Yekeen et al., 2016). Pb exposure can induce numerous adverse effects and disturb physiological functions on the cardiovascular system (Boskabady et al., 2018). Cardiac injury induced by Pb exposure in early stage of life is a great risk factor for heart health in late life (Davuljigari and Gottipolu 2019). Yang et al. (2017) had previously shown that left ventricular (LV) systolic function decreased with blood Pb levels in adults. Moreover, children with cardiac dysfunction, which occurs mostly as a result of LV dysfunction, may be at the risk of malnutrition and failure to thrive (Leitch 2000). Therefore, it is important to identify the impacts of e-waste pollution, particularly Pb pollution on child left ventricle. However, no study used echocardiography to assess LV function in preschool children from e-waste areas. To bridge these knowledge gaps, Pb was selected as a marker of exposure and echocardiography was used to assess the impacts of e-waste pollution on child left ventricle in this study.

Pb exposure has been shown to have a variety of adverse effects on the cardiovascular system. Aberrant intracellular Ca^{2+} homeostasis Yekeen s to systolic dysfunction, diastolic dysfunction, and adverse remodeling (Peana and Domeier 2017). The molecular mechanisms of Pb cardiotoxicity can be explained by the fact that, because of Pb^{2+} exhibits some chemical similarities with Ca^{2+}. Pb^{2+} not only affects cell signaling by replacing Ca^{2+} in protein binding sites, but also alters Ca^{2+} cellular concentration by modulating the activity of ion channels (Ferreira de Mattos et al., 2017; Verstraeten et al., 2008). The cardiotoxicity of Pb is therefore associated with a dysregulation of calcium homeostasis. Another important mechanism underlying Pb toxicity is oxidative stress induction. Previous studies found endothelial cells might endocytose Pb and increase generation of superoxide and hydrogen peroxide, which in turn promotes oxidative stress in endothelial cells (Ni et al., 2004; Vaziri and Ding 2001). The adverse effect on endothelial cells might be associated with an activation of transcriptional factor Nrf2 signaling pathway (Metryka et al., 2018). In the event of endothelium disruption, Pb can reach and accumulate in smooth muscle cells, exacerbating lipid peroxidation and eventually triggering atherosclerosis (Di et al., 2016). These observations suggest that Pb exposure has a direct effect on the cells of the cardiovascular system.

Earlier observations had shown that calcium and reactive oxygen species (ROS) are essential for an appropriate inflammatory response (Grinstein and Klip 1989; Yang et al., 2013). The adverse effect of Pb exposure on calcium homeostasis and oxidative stress suggest that Pb may play an important role in the formation and development of chronic low-grade inflammation. Heo et al. (1998) found Pb induces higher counts of neutrophils, monocytes, and lymphocytes in peripheral blood, and affects the direction of precursor T helper cell differentiation as well as by directly inhibiting T helper cells 1 and stimulating T helper cells 2. Moreover, Pb exposure increases the release of pro-inflammatory cytokines and other mediators, including interleukin (IL) 6, tumor necrosis factor (TNF) α, and C reactive protein (Boskabady et al., 2018; Di Lorenzo et al., 2007; Khan et al., 2008; Zhang et al., 2020). Furthermore, inflammation is also involved in the negative effects of Pb on the cardiovascular system. In vitro studies found Pb exposure increases activity of the cyclooxygenase-2 gene and its promoter in vascular smooth muscle cells via the epidermal growth factor receptor/nuclear factor kappa-B signal transduction pathway (Chang et al., 2011; Chou et al., 2011). The results of a study conducted by Iris Zeller et al. (2010) showed Pb induces an increase in IL-8 production and secretion by vascular endothelial cells. At the same time, IL-8 stimulates smooth muscle cell invasion into the intima and enhances intimal thickening. Feng et al. (2018) investigated how Pb damages the structure of the myocardium, focusing on its effect on the expression of IL-6 and TNF-α in the heart. Collectively, the possible potential mechanism of cardiovascular disease induced by Pb exposure may be mediated by inflammation. Based on these considerations, we hypothesized that: a) E-waste Pb exposure might have adverse effect on the left ventricle in preschool children. b) In addition, we explored if a possible mechanism implicated in heart cardiotoxicity by Pb in children could be through chronic low-grade inflammation. This study aims to determine child blood Pb level and evaluate its effect on peripheral leukocyte counts and LV echocardiographic parameters, to offer a deeper understanding of heart health risk in children with different level Pb exposure.

2. Methods

2.1. Study population

From November to December 2018, we recruited a total of 486 preschool children (2–6 years old) from Guiyu (an e-waste-exposed group, n = 310) and Haojiang (a reference group, n = 176). The two towns, located in Guangdong province, China, have a similar cultural background, socioeconomic status, and ethnicity (Zhang et al., 2019). A questionnaire on personal information, playing and hygiene habits, dietary habits, residential environment, and family status was completed by the children's parents (or guardians). Ethics approval of this research came from the Human Ethics Committee of Shantou University Medical College, China. Before enrollment, informed consent was provided to all participants' guardians for signature.

2.2. Sample collection and physiological parameters

Peripheral venous blood sample was drawn from each child by trained nurses. Blood with anticoagulant (EDTA) was utilized for blood Pb level measurement and peripheral blood cell analysis. Heart rate was measured by a medical doctor after a 15-min rest and showed by beat per minute (bpm). The trained physician gave each child a physical test, including height and weight (Zeng et al., 2017). Body surface area (BSA) and body mass index (BMI) were calculated.

2.3. Blood Pb measurement and routine blood analysis

Blood Pb levels were measured by graphite furnace atomic absorption spectrometry (Jena Zenit 650, Germany) as previously described (Chen et al., 2019). We measured the blood Pb levels in each individual for twice and averaged. The method's accuracy was verified by recoveries between 91.06% and 101.2% from spiked blood samples. The limit of detection of this method was 0.084 μg/dL. Routine blood analysis was performed with an automated Sysmex XT-1800i hematology analyzer (Sysmex Corporation, Kobe, Japan).

2.4. Echocardiographic assessments

Echocardiography examination was performed on all 486 children, using an ultrasound system (SIUI Apogee 1200, China). Examinations were performed according to the guidelines of the American Society of Echocardiography Cardiac (Lang et al., 2005;

Lopez et al., 2010). All participants rested for 10 min before the examination and then underwent echocardiographic studies in the left lateral decubitus position. LV dimensions were measured by using M-mode tracings recorded from a 2-dimensional parasternal long-axis view. Echocardiography provided measurements of LV end-diastolic (LVD) and end-systolic (LVS) diameters, LV end-diastolic volume (EDV), LV end-systolic volume (ESV), LV posterior wall thickness at diastole (LVPW), and interventricular septal thickness at diastole (IVS). LV systolic function was evaluated by the fractional shortening (FS), stroke volume (SV) and ejection fraction (EF). LV mass (LVM) was calculated by Devereux's formula (Devereux et al., 1986). LVM index (LVMI) was calculated using the standard formula: LVMI ($g/m^{2.7}$) = LVM (g)/[height (m)]$^{2.7}$ (Khoury et al., 2009).

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 software (GraphPad, CA) and Statistical Package for the Social Sciences (SPSS), version 23.0 software (IBM Corporation, USA). As appropriate, the Mann-Whitney *U* test, the independent-sample *t*-test, the chi-square test, the Fisher's exact test, and the continuity correction were used to compare the differences between the two groups. Data are presented as the median and interquartile range, or mean and standard deviation, according to distribution characteristics. In addition, linear regression of multivariable-adjusted (adjusted for gender, age, BMI, e-waste contamination within 50 m of residence, residence as workplace, distance of residence from road, family member daily smoking, monthly household income, maternal work associated with e-waste, paternal work associated with e-waste, duration of outdoor play, child contact with e-waste, washing hands before eating, nail biting habit, chewing pencil habit, yearly canned food consumption, yearly vegetable and fruit consumption, yearly iron-rich food consumption, yearly marine product consumption, and yearly salted food consumption) was performed for the associations of blood Pb level with peripheral leukocyte counts and parameters of the left ventricle. The associations of peripheral leukocyte counts with parameters of the left ventricle were also assessed in the same multivariable-adjusted linear regression model. The selection of potential confounders was based on the results of Spearman correlation analysis about the relationship between blood Pb levels, peripheral leukocyte counts, LV parameters and relevant factors (Supplementary Material Table S1-S3). *P*-values < 0.05 were considered statistically significant in a two-tailed test.

3. Results

3.1. Statistical characteristics of the studied populations

The exposed group possessed lower physical development levels than the reference group, for BMI (14.7 ± 1.43 kg/m^2 vs. 15.9 ± 1.35 kg/m^2, $P < 0.001$) and BSA (0.69 ± 0.07 m^2 vs. 0.75 ± 0.09 m^2, $P < 0.001$) (Table 1). The heart rate was higher in the exposed group (102 ± 11.6 bpm vs. 95 ± 12.5 bpm, $P < 0.001$), while the mean age and gender distribution in both groups were similar (both $P > 0.05$). Additionally, children of the two groups lived and played in different surroundings and conditions (such as distance between road and residence, e-waste contamination within 50 m of resident, and daily smoking by family member) (all $P < 0.001$). Moreover, the parents with lower level of education had a lower monthly household income in the exposed group (all $P < 0.001$).

3.2. Blood Pb levels

Once we determined the statistical characteristics of the study subjects, we decided to test the blood Pb levels to see the difference between the two groups (Table 2). Blood Pb level of 5 μg/dL or more is considered a level of concern for children according to the U.S. Center for Disease Control and Prevention (Betts, 2012). The median blood Pb level of the exposed group was 4.51 μg/dL, which was significantly higher than the 3.98 μg/dL for reference children ($P < 0.001$). In addition, the blood Pb levels exceeded the recommended limit of 5 μg/dL in 37.4% (113/302) of the exposed group, but in only 20.6% (36/175) exceeded the threshold of the reference group ($P < 0.001$).

3.3. Peripheral leukocyte counts

To explore the differences of systemic markers of inflammation between the two groups of children, we measured peripheral leukocyte counts. There was higher median absolute value of WBCs in the exposed group (7.49 × 109/L vs. 6.59 × 109/L, $P < 0.001$) (Fig. 1). In addition, neutrophil, monocyte, and lymphocyte counts were significantly higher in the exposed group (median 3.28 × 10^9/L, 0.47 × 10^9/L, and 3.28 × 10^9/L, respectively) compared with the reference group (median 2.96 × 10^9/L, 0.37 × 10^9/L, and 2.86 × 10^9/L, respectively).

3.4. Comparison of echocardiographic LV structure and systolic function

The participants of this study were all healthy children, and no abnormal cardiac structure and systolic dysfunction were found during the echocardiography examination. Regardless of gender, lower median levels of LV geometric patterns were observed in the exposed group than the reference group, for IVS (male: 4.38 mm vs. 5.08 mm, female: 4.09 mm vs. 5.04 mm, both $P < 0.001$), LVPW (male: 5.09 mm vs. 5.76 mm, female: 5.03 mm vs. 5.62 mm, both $P < 0.001$), LVM (male: 33.1 g vs. 42.7 g, female: 30.1 g vs. 34.9 g, both $P < 0.001$), and LVMI (male: 34.3 $g/m^{2.7}$ vs. 29.8 $g/m^{2.7}$, $P < 0.001$, female: 30.3 $g/m^{2.7}$ vs. 27.1 $g/m^{2.7}$, $P < 0.001$) (Table 3). Furthermore, our data show lower median levels of FS (male: 35.0% vs. 37.0%, female: 35.0% vs. 36.0%, both $P < 0.001$) and EF (male: 73.0% vs. 75.0%, female: 72.0% vs. 74.0%, both $P < 0.001$) (Table 3) in the exposed group regardless gender. These observations show that the exposed children had smaller left ventricles and impairment in LV systolic function.

3.5. Relationship between Pb exposure and peripheral leukocyte counts

Spearman correlation analysis had shown positive relationships ($P < 0.01$) between Pb exposure and counts of WBCs ($r_s = 0.140$), neutrophils ($r_s = 0.114$), and monocytes ($r_s = 0.123$) (Table 4). Linear regression of multivariable-adjusted was examined for the association between blood Pb levels and peripheral leukocytes (Table 5). Adjusted regression analysis illustrated that higher blood Pb levels were significantly associated with higher levels of Ln-WBC and Ln-Neutrophil [B (95% CI) = 0.006 (0.001, 0.012), $P = 0.015$, and B (95% CI) = 0.009 (0.000, 0.017), $P = 0.046$].

3.6. Pb exposure and LV structure/systolic function

With the purpose of understanding the association between blood Pb levels and LV structure/systolic function, the Spearman correlation was calculated. We observed negative relationships ($P < 0.05$) between blood Pb levels and LV parameters, including IVS

Table 1
Basic characteristics of the study children.

Characteristics	N	Reference group	N	Exposed group	*P*-value
Age (years, mean ± SD)	176	4.75 ± 1.01	310	4.74 ± 0.84	0.909[a]
2–3 years		42(23.9)		63(20.3)	0.298[b]
4 years		56(31.8)		120(38.7)	
5–6 years		78(44.3)		127(41.0)	
Gender	176		310		0.112[b]
Male		104(59.1)		160(51.6)	
Female		72(40.9)		150(48.4)	
BMI (kg/m^2, mean ± SD)	176	15.9 ± 1.35	304	14.7 ± 1.43	<0.001[a]
BSA (m^2, mean ± SD)	176	0.75 ± 0.09	304	0.69 ± 0.07	<0.001[a]
HR (beats per minute, mean ± SD)	176	95 ± 12.5	308	102 ± 11.6	<0.001[a]
Family member daily smoking	174		309		<0.001[b]
Non-smoking		93(53.4)		87(28.2)	
~2 cigarettes		17(9.8)		54(17.5)	
~10 cigarettes		27(15.5)		83(26.9)	
~20 cigarettes		32(18.4)		62(20.1)	
> 20 cigarettes		5(2.9)		23(7.4)	
E-waste contamination within 50 m of resident	176		308		<0.001[c]
Yes		3(1.7)		73(23.7)	
No		173(98.3)		235(76.3)	
Distance of residence from road (m)	172		310		<0.001[b]
< 10		22(12.8)		119(38.4)	
~50		47(27.3)		84(27.1)	
~100		39(22.7)		57(18.4)	
> 100		64(37.2)		50(16.1)	
Father's educational level	176		310		<0.001[b]
Middle school or lower		28(15.9)		220(70.9)	
Secondary school		27(15.3)		32(10.3)	
High school		25(14.2)		35(11.3)	
College/university		96(54.5)		23(7.4)	
Mother's educational level	176		309		<0.001[b]
Middle school or lower		46(26.1)		225(72.8)	
Secondary school		25(14.2)		27(8.7)	
High school		17(9.7)		20(6.5)	
College/university		88(50.0)		37(12.0)	
Monthly household income (yuan)	171		299		<0.001[b]
< 1500		7(4.1)		5(1.7)	
1500–3000		8(4.7)		28(9.4)	
3000–4500		30(17.5)		73(24.4)	
4500–6000		28(16.4)		89(29.8)	
> 6000		98(57.3)		104(34.8)	

SD, standard deviation; BMI, body mass index; HR, heart rate; BSA, body surface area.
$P < 0.05$ was considered statistically significant.
[a] Analysis by independent-sample *t*-test for differences between two groups.
[b] Analysis by chi-square test for differences between two groups.
[c] Analysis by continuity correction between two groups.

Table 2
Comparison of blood Pb levels in preschool children.

	Reference group (n = 175)	Exposed group (n = 302)	*P*-value
BPb [μg/dL, median (IQR)]	3.98(3.25–4.84)	4.51(3.70–5.67)	<0.001[a]
>5 μg/dL [n (%)]	36(20.6)	113(37.4)	<0.001[b]
≤5 μg/dL [n (%)]	139(79.4)	189(62.6)	

BPb, blood Pb levels; IQR, interquartile range.
$P < 0.05$ were considered statistically significant.
[a] Analysis by the Mann-Whitney *U* test.
[b] Analysis by chi-square test for differences between two groups.

($r_s = -0.149$), LVPW ($r_s = -0.133$), and EF ($r_s = -0.096$) (Table 4). Then, we examined a linear regression model between blood Pb levels and LV parameters (Table 5). Adjusted regression analysis illustrated that higher blood Pb levels were significantly associated with lower levels of Ln-IVS [B (95% CI) = −0.004 (−0.007, −0.001), $P = 0.006$].

3.7. Relationship between peripheral leukocyte counts and LV structure/systolic function

We further explored the correlation between peripheral leukocyte counts and parameters of the left ventricle by Spearman correlation analysis (Table 4). Results indicated that the higher the peripheral leukocyte counts, the lower the LV parameters (all $P < 0.01$). In addition, we examined a linear regression model between peripheral leukocyte counts and LV parameters (Table 6). After adjustment, the results illustrated that higher counts of WBCs, neutrophils, and monocytes were significantly associated with lower levels of Ln-IVS, Ln-LVPW, Ln-LVD, Ln-EDV, Ln-LVM, and Ln-SV.

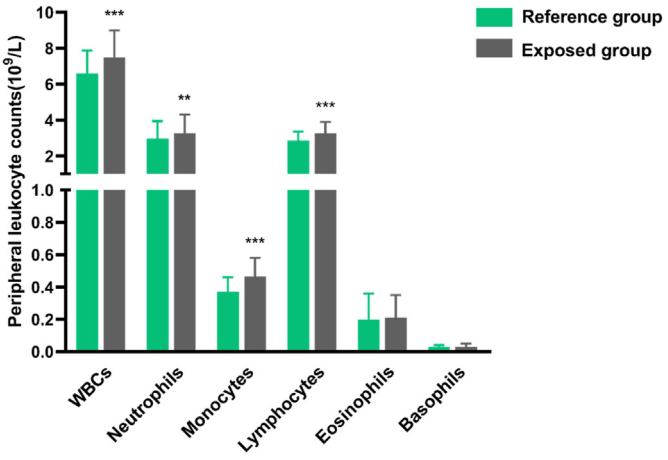

Fig. 1. Peripheral leukocyte counts in preschool children. Counts of WBCs, neutrophils, monocytes, and lymphocytes were higher in exposed group. WBCs, white blood cells. Data are presented as the median (interquartile range), obtained with a Mann-Whitney *U* test. $^{**}P < 0.01$, $^{***}P < 0.001$.

4. Discussion

The major purpose of this cross-sectional study is to investigate the association between Pb exposure and LV structure and systolic function of preschool children. The data showed higher counts of peripheral leukocytes, smaller left ventricle, and impaired LV systolic function in e-waste-exposed children and in association with elevated blood Pb levels. These results favour the possibility that Pb has an adverse effect on child left ventricle by disturbance of inflammatory processes, suggesting that the heart health of children living in e-waste areas is at risk due to the long-term environmental chemical insults.

Pb can be released from primitive recycling and processing of e-waste, threatening the entire community health. Exposure to Pb is usually assessed by biomonitoring its levels in biological specimens. Blood is the preferred matrix for biomonitoring of Pb exposure. It is in continuous contact with the whole organism and in equilibrium with all organs where Pb is stored (Esteban and Castano 2009; Nouioui et al., 2019). Pb could be mobilized from bone back into blood, therefore blood Pb measurements reflect recent exposure and may also represent past exposures (Barbosa et al., 2005). Moreover, it has been reported that the bone-Pb contribution to blood can be 90% or more in exposed children (Gulson et al., 1996). Guiyu, an e-waste recycling area in southeast China, processes more than 1.7 million tons of e-waste every year, and has long history of unregulated e-waste recycling (Leung et al., 2011; Wang et al., 2005). Numerous studies have shown significantly higher Pb concentrations in the sediment, dust, rice and vegetables from e-waste recycling areas (Awasthi et al., 2016; Luo et al., 2011; Zheng et al., 2013). Diet is an important exposure source in children, since they eat more food and drink more water than do adults for each pound of body weight. Moreover, the

Table 3
Comparison of echocardiographic parameters in preschool children.

	Male		*P*-value	Female		*P*-value
	Reference group (n = 104)	Exposed group (n = 160)		Reference group (n = 72)	Exposed group (n = 150)	
IVS [mm, median (IQR)]	5.08(4.89–5.44)	4.38(4.06–4.75)	<0.001	5.04(4.74–5.20)	4.09(3.79–4.57)	<0.001
LVPW [mm, median (IQR)]	5.76(5.36–6.03)	5.09(4.77–5.44)	<0.001	5.62(5.33–5.88)	5.03(4.54–5.41)	<0.001
LVD [mm, median (IQR)]	33.8 (31.7–35.7)	32.6(30.8–34.4)	0.003	31.4 (29.3–33.1)	31.3(29.9–32.6)	0.779
LVS [mm, median (IQR)]	21.3(19.9–22.3)	20.9(19.9–22.1)	0.171	20.0(18.4–21.1)	20.2(19.1–21.4)	0.075
EDV [mL, median (IQR)]	38.6(31.8–45.5)	34.6(29.1–40.5)	0.002	31.0(24.8–36.6)	30.7(26.6–34.7)	0.767
ESV [mL, median (IQR)]	9.7(7.9–11.1)	9.3(7.9–11.1)	0.246	8.0(6.2–9.5)	8.3(7.0–9.9)	0.068
FS [%, median (IQR)]	37.0(35.0–37.0)	35.0(34.0–36.0)	<0.001	36.0(35.0–37.0)	35.0(33.0–36.0)	<0.001
EF [%, median (IQR)]	75.0(73.0–76.0)	73.0(72.0–74.0)	<0.001	74.0(73.0–76.0)	72.0(70.0–74.0)	<0.001
SV [mL, median (IQR)]	28.4(23.2–34.5)	24.9(21.0–29.0)	<0.001	22.9(19.3–27.1)	21.7(19.0–25.5)	0.525
LVM [g, median (IQR)]	42.7(36.5–47.4)	33.1(28.8–38.6)	<0.001	34.9(31.8–40.9)	30.1(26.3–33.2)	<0.001
LVMI [$g/m^{2.7}$, median (IQR)]	34.3(30.3–37.5)	29.8(26.2–33.4)	<0.001	30.3(26.9–33.8)	27.1(24.0–30.4)	<0.001

IVS, interventricular septum; LVPW, left ventricular posterior wall; LVD, left ventricular end-diastolic diameter; LVS, left ventricular end-systolic diameter; EDV, left ventricular end-diastolic volume; EVS, left ventricular end-systolic volume; FS, left ventricular fractional shortening; EF, left ventricular ejection fraction; SV, stroke volume; LVM, left ventricular mass; LVMI, left ventricular mass index. The data of echocardiography are obtained with a Mann-Whitney *U* test. $P < 0.05$ were considered statistically significant.

Table 4
Spearman correlation coefficient between blood Pb levels and peripheral leukocyte counts and LV parameters.

	BPb	WBCs	Neutrophils	Monocytes	IVS	LVPW	LVD	EDV	LVM	FS	EF	SV
BPb	1											
WBCs	0.140**	1										
Neutrophils	0.114**	0.834**	1									
Monocytes	0.123**	0.692**	0.606**	1								
IVS	−0.149**	−0.189**	−0.147**	−0.234**	1							
LVPW	−0.133**	−0.255**	−0.189**	−0.279**	0.629**	1						
LVD	0.013	−0.206**	−0.166**	−0.209**	0.251**	0.309**	1					
EDV	−0.002	−0.216**	−0.169**	−0.223**	0.247**	0.310**	0.986**	1				
LVM	−0.072	−0.264**	−0.204**	−0.294**	0.696**	0.740**	0.778**	0.767**	1			
FS	−0.084	−0.043	−0.003	−0.021	0.197**	0.131**	0.057	0043	0.160**	1		
EF	−0.096*	−0.031	0.008	−0.020	0.207**	0.132**	0.055	0.041	0.163**	0.968**	1	
SV	−0.008	−0.188**	−0.138**	−0.202**	0.281**	0.338**	0.960**	0.952**	0.772**	0.203**	0.202**	1

BPb, blood Pb levels; WBCs, white blood cells; IVS, interventricular septum; LVPW, left ventricular posterior wall; LVD, left ventricular end-diastolic diameter; EDV, left ventricular end-diastolic volume; LVM, left ventricular mass; FS, left ventricular fractional shortening; EF, left ventricular ejection fraction; SV, stroke volume. $P < 0.05$ was considered statistically significant. $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 5
Multiple linear regression analysis for associations between blood Pb levels and peripheral leukocyte counts and LV parameters.

	BPb		
	B(95%CI)	B	*P*-value
Ln-WBC	0.006(0.001, 0.012)	0.110	0.015
Ln-Neutrophil	0.009(0.000, 0.017)	0.092	0.046
Ln-Monocyte	0.006(-0.001, 0.013)	0.080	0.073
Ln-IVS	−0.004(-0.007, −0.001)	−0.114	0.006
Ln-LVPW	−0.001(-0,003, 0.001)	−0.033	0.431
Ln-EF	−0.001(-0.002, 0.001)	−0.046	0.309

Adjusted for gender, age, BMI, e-waste contamination within 50 m of residence, distance of residence from road, residence as workplace, family member daily smoking, monthly household income, paternal work associated with e-waste, maternal work associated with e-waste, child contact with e-waste, duration of outdoor play, washing hands before eating, nail biting habit, chewing pencil habit, yearly canned food consumption, yearly vegetable and fruit consumption, yearly iron-rich food consumption, yearly marine product consumption, and yearly salted food consumption. BPb, blood Pb levels; Ln-WBC, ln-transformed counts of white blood cells; Ln-Neutrophil, ln-transformed counts of neutrophils; Ln-Monocyte, ln-transformed counts of monocytes; Ln-IVS, ln-transformed parameters of the interventricular septum; Ln-LVPW, ln-transformed parameters of the left ventricular posterior wall; Ln-EF, ln-transformed parameters of the left ventricular ejection fraction. B, unstandardized coefficient; CI, confidence interval; β, standardized coefficient. $P < 0.05$ was considered statistically significant.

children interact with the environment in unique ways that can increase exposure to chemicals from play items or dust, and they have no ability to deal with toxic chemicals, thus being more vulnerable (Ackah 2019; Landrigan et al., 1998). In this study, we found elevated blood Pb levels in Guiyu children, which is consistent with our previous studies (Huo et al., 2007; Lu et al., 2018; Xu et al., 2020). The results of Spearman correlation analysis of factors related to blood Pb levels (Supplementary Material Table S1) have suggested that e-waste-exposed children chewing pencil habit (r_s = 0.091), e-waste contamination within 50 m of residence (r_s = 0.127), family member daily smoking (r_s = 0.138), and paternal work associated with e-waste (r_s = 0.097) were positively correlated with blood Pb levels. In summary, results indicate that children from e-waste areas are exposed to higher levels of Pb through multiple routes.

Peripheral leukocytes are a universally available marker of chronic low-grade inflammation. An abnormal count of peripheral leukocytes is usually caused by cancer, an infection, or other conditions that induce systemic inflammation responses. In the human circulation, neutrophils are the most abundant leukocyte type, and have been considered as the key for inflammation. Neutrophils expedite the adhesion of classical monocytes to the endothelium and stimulate macrophage cytokine release by secreting granule proteins (Pfister et al., 2012). Monocytes/macrophages are the main source of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-8 (Metryka et al., 2018). Thus, counts of neutrophils and monocytes/macrophages are an important indicator of low-grade inflammation. In this study, we found higher counts of peripheral leukocytes in e-waste-exposed children. There was a positive correlation between blood Pb levels and the counts of WBCs (r_s = 0.140), neutrophils (r_s = 0.114), and monocytes (r_s = 0.123). The immunotoxicity of Pb has been demonstrated by inhibition of in vitro neutrophil chemotaxis, phagocytosis, and superoxide formation (Governa et al., 1987). It can weaken the formation of neutrophil extracellular traps and impaired migration ability of neutrophils because Pb inhibits the IP3R receptor-mediated release of Ca^{2+}, thereby reducing the host's resistance to various pathogenic microorganisms (Yin et al., 2019). The increased number of blood neutrophils could in part be compensating for their Pb-induced functional impairment (Di Lorenzo et al., 2006). On the

Table 6
Multiple linear regression analysis for associations between peripheral leukocyte counts and LV parameters [B (95%CI)].

	Ln-IVS	Ln-LVPW	Ln-LVD	Ln-EDV	Ln-LVM	Ln-SV
WBCs	−0.007(-0.013, 0.000)*	−0.009(-0.015, −0.004)***	−0.005(-0.009, −0.002)**	−0.018(-0.028, −0.007)**	−0.018(-0.028, −0.008)***	−0.014(-0.025, −0.003)*
Neutrophils	−0.008(-0.016, 0.000)*	−0.009(-0.016, −0.002)**	−0.006(-0.010, −0.001)*	−0.019(-0.033, −0.006)**	−0.019(-0.031, −0.006)**	−0.014(-0.028, 0.000)
Monocytes	−0.106(-0.183, −0.030)**	−0.100(-0.163, −0.037)**	−0.064(-0.108, −0.021)**	−0.220(-0.351, −0.088)**	−0.233(-0.353, −0.113)***	−0.177(-0.310, −0.044)**

Adjusted for gender, age, BMI, e-waste contamination within 50 m of residence, distance of residence from road, residence as workplace, family member daily smoking, monthly household income, paternal work associated with e-waste, maternal work associated with e-waste, child contact with e-waste, duration of outdoor play, washing hands before eating, nail biting habit, chewing pencil habit, yearly canned food consumption, yearly vegetable and fruit consumption, yearly iron-rich food consumption, yearly marine product consumption, and yearly salted food consumption. WBCs, white blood cells; Ln-IVS, ln-transformed parameters of the interventricular septum; Ln-LVPW, ln-transformed parameters of the left ventricular posterior wall; Ln-LVD, ln-transformed parameters of the left ventricular end-diastolic diameter; Ln-EDV, ln-transformed parameters of the left ventricular end-diastolic volume. Ln-LVM, ln-transformed parameters of the left ventricular mass; Ln-SV, ln-transformed parameters of stroke volume. B, unstandardized coefficient; CI, confidence interval; $P < 0.05$ was considered statistically significant. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

other hand, Pb exposure promote ROS production, cell damage and apoptosis, and lead to increases in endogenous waste requiring elimination by neutrophils and monocytes (Zhang et al., 2017). It is therefore understandable that Pb exposure may be associated with increased counts of neutrophils and monocytes in peripheral blood. Our previous studies suggested that Pb-exposed children had higher levels of pro-inflammatory cytokines and counts of $CD4^+$ and $CD8^+$ central memory T cells and lower percentages of natural killer cells (Cao et al., 2018; Lu et al., 2018; Zhang et al., 2016). These findings suggest that Pb exposure was associated with the disruption of host's innate and adaptive immune responses. Under these circumstances, the inflammatory response causes tissues to fail to restore homeostasis, and chronic low-grade inflammation ensues.

Our findings show that associations of blood Pb levels with LV geometric patterns were negative within the range of detection. Blood Pb levels in the exposed group were higher but left ventricle was smaller than these in the reference group. Several studies found that Pb exposure increases levels of creatine kinase isoenzyme-MB and lactate dehydrogenase activity of heart tissue and serum (Davuljigari and Gottipolu 2019; Roshan et al., 2011). This suggests that Pb has a damaging effect on myocardial tissues during chronic exposure. Furthermore, Lu et al. (2018) has found lower systolic blood pressure in e-waste-exposed children, and may be related to decreased peripheral vascular tension. Previous studies have suggested that elevated systolic blood pressure is positively correlated with LVM in adolescents and children (Litwin et al., 2019; Urbina et al., 2019). Blood pressure is considered the major determinant of LV structural alterations, which may be the reason why these results are discrepant compared to the existing research evidence. Chronic Pb exposure has been shown to elevated blood pressure in adults, and studies on Pb exposure and LV structure have focused on adults, Pb exposure therefore has been observed to be associated with LV hypertrophy (Poreba et al., 2010; Yang et al., 2017). Multiple mechanisms have been proposed to explain how Pb intoxication impairs cardiac function. Davuljigari and Gottipolu (2019) demonstrated the adverse effect of early-life Pb exposure to cardiac mitochondrial functions, showing that Pb decreases the activities of mitochondrial superoxide dismutase while increasing malondialdehyde levels. Due to the heart's high density of mitochondria and high energy demand, it is especially vulnerable to Pb-induced mitochondrial dysfunction (Tocchi et al., 2015). A similar result has been found in heart tissue (Markiewicz-Górka et al., 2015). This suggests that Pb induces lipid peroxidation, which plays a significant role in accumulation of lipid hydroperoxides and destruction of the myocardial membrane in heart tissue. In addition, it has been reported increases in histone acetylation and apoptosis induced by Pb of myocardial tissues (Xu et al., 2015). In vitro studies found that extracellular Pb blocked currents through Cav1.2 channels, enhanced their fast inactivation, and diminished their activation, negatively affecting their gating currents (Ferreira de Mattos et al., 2017). Thus, Pb^{2+} reduces cardiac contractility, which is consistent with the findings of our study. Our results show decreased parameters of LV systolic function in the exposed group. Of note, decreased LV systolic function parameters in exposed children did not indicate abnormal systolic function. These observations in the study suggest that e-waste-exposed children may have an increased risk of impairment of their heart structure and function and indicate that Pb exposure is an important risk factor to consider among the cardiovascular diseases in children.

As the data show, our study suggests that low-grade inflammation plays an important role in the cardiotoxicity of Pb. Previous studies have illustrated that Pb exposure causes myocardial injury through inflammation. Roshan et al. (2011) found that exposure to Pb induces significant increases of creatine kinase isoenzyme-MB and hypersensitive C-reactive protein, while brain natriuretic peptide, a biomarker for the diagnosis of congestive heart failure, increases in serum and heart tissue. Additionally, Pb's cardiac injury effect is mediated by pro-inflammatory cytokines, such as TNF-α and IL-6 (Feng et al., 2018). Additionally, histological findings show Pb cardiotoxicity by mononuclear cell infiltration, extensive degeneration, necrosis in cardiac muscle, and disruption in muscle connectivity (Davuljigari and Gottipolu 2019). As it is mentioned above, activated neutrophils release granule proteins that attract monocytes and stimulate macrophages to synthesize cytokines. In this study, we found that elevated blood Pb levels were accompanied by higher counts of WBCs, neutrophils, and monocytes. The counts of WBCs, neutrophils, and monocytes had a negative correlation with LV geometric patterns and systolic function. In our previous studies, we have demonstrated that TNF-α and IL-6 are increased in e-waste-exposed children. Furthermore, there is a positive association between the concentration of IL-6 and blood Pb levels and counts of monocytes (Lu et al., 2018; Zheng et al., 2019). In summary, Pb exposure may perpetuate a low-grade inflammatory milieu, which is not conducive to the heart health in e-waste-exposed children.

There are several limitations that must be acknowledged. First, this is a cross-sectional study and our samples were collected from children in two kindergartens instead of a random population. The results must be interpreted with caution because they show an association, not a causality. Further prospective cohort studies will be required to confirm these associations. Second, participants in the exposed area may be more willing to participate in this study because they may have aware of the adverse health effects of environmental pollution. Although participants were recruited from two towns with homologous background in our study, selection bias still could not be eliminated. Therefore, we have adjusted some potential confounding factors as much as possible in the regression model. Additionally, there was no blood pressure assessment in this study, as our prior studies have evaluated the relationship between Pb exposure and blood pressure in children. In further cohort studies we will explore the association between child blood pressure and left ventricle in the context of Pb exposure. Finally, we only focused on the cardiotoxicity of Pb and the correlations between Pb and LV parameters were weak. Children from e-waste areas are under threat from a variety of hazardous elements. Other chemicals may impact the child cardiovascular system synergistically due to the complex e-waste contamination. This may weaken the effect explained by Pb exposure, and lead to low coefficients. The ongoing study should be continued to explore various risk factors and determine possible mechanisms.

5. Conclusion

On the whole, this is the first study using echocardiography to evaluate the relationship between blood Pb levels and LV structure and systolic function of e-waste-exposed preschool children. We find smaller left ventricle and impaired systolic function among children from the e-waste area, associated with chronic low-grade inflammation and elevated blood Pb levels. The current data support that children in e-waste areas are exposed to cardiotoxic substances, which threaten their heart health. Therefore, there is an urgent need to create safe recycling operations and reduce sources of heavy metal exposure. More than that, additional attention needs to be paid to the heart health in e-waste-exposed children, regular echocardiography examination is necessary.

Contributions of each author

Xijin Xu designed the study, searched the literature, supervised data acquisition, revised the manuscript, and funded the study. Zihan Chen performed echocardiography, statistically analyzed the data, and drafted the manuscript. Xia Huo assisted in the study design, data interpretation, and edited the manuscript. Shaocheng Zhang participated in analyzing the data. Zhiheng Cheng and Yu Huang participated in the questionnaire and sample collections. All authors approved the final version for submission.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.128793.

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