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Assessment of Lead in Blood Samples of Children Residing in the Vicinity of Industries

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Abstract

The aim of present study was to determine the lead (Pb) distributions in blood and prevalence of elevated Pb exposure among children, age ranged (5–10 years), residing near industrialized region of Hyderabad city, Pakistan. For comparison, biological samples of children of same age group from non-industrial area were also analyzed. The Pb concentration in blood samples was determined by electrothermal atomic absorption spectrometry, prior to microwave assisted acid digestion. The results showed that significantly higher proportion of children living in the vicinity of industrial area, had blood Pb levels (BLL) in the range of 15.4-35.6 µg/dL, and 8.51-16.7 µg/dL for those of non-industrial area. The blood Pb level was higher in boys of both groups as compared to girls of same age group, but the difference was not significant ($p=0.178$). Negative correlation was observed between BLL and hemoglobin levels ($p<0.001$), while positive correlation was observed between BLL and age.

***Keywords*:** Lead; Industrialized region; Children; Blood; Hemoglobin.

Introduction

Lead (Pb) poisoning have serious and even fatal consequences at any age, but young children are especially vulnerable [1, 2]. In young children, blood Pb levels (BLL) > 10 µg/dL can lower subsequent scores on IQ tests [3, 4]. Nutritional factors and personal habits can influence Pb absorption, increasing risk of intoxication especially in low income family children [5].

Generally, BLL of rural communities can be expected to be significantly lower than those of urban communities, among populations living in the vicinity of certain industrial sources of Pb, such as Pb smelters and mines, a higher incidence of elevated BLL may occur, particularly among young children [6-8]. Lead poisoning is an entirely preventable environmental problem [9]. Despite of differences in the sources of exposure, children continue to present a uniquely vulnerable group [10, 11]. After Pb enters the body by ingestion or inhalation, it has a mean biological half-life in the blood of about 40 days in adult males, and is subsequently stored mainly in calcified tissues [12, 13]. Whole blood has been the primary biological fluid used to assess Pb exposure, both for screening and diagnostic purposes and for biomonitoring in the long term [14]. Lead is constantly exchanged between blood and bone where blood Pb contents correspond to 5% of the total body burden of Pb [15]. An analysis of BLL is, therefore, the first choice for the assessment of internal exposure to Pb in individuals exposed to Pb [16]. Lead circulating in the blood stream is mobile, as compared to that stored in bones. It is

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the mobile Pb, which exerts adverse effects on the body. It has now been generally accepted that under conditions of more or less constant and prolonged exposure, an individual's BLL reflects the quantity of "biologically active" form of Pb in their body. Blood Pb has positive correlation with the symptoms of Pb toxicity [17]. Among the various biopsy materials, serum, scalp hair, urine and other body fluids may be used as bio-indicators for these purposes [18, 19]. Atomic absorption spectrometric methods are frequently used for the specific determination of very low elemental concentrations in biological samples. At present, the mineralization method frequently used for the analysis of biological samples is wet digestion with concentrated acids, using either convective systems or microwave ovens [20]. The main advantage of microwave-assisted samples pretreatment is its requirement of a small amount of mineral acids and a reduction in the production of nitrous vapors.

Over the past several years, Pb poisoning has attracted growing attention in the developed countries. At the same time, however, this problem has not been a subject of concern in most developing countries including Pakistan. Less work has been done on environmental Pb exposures and its impact on growing children in Pakistan, where the labors of industries mostly have low socio-economic status, and they preferred to live in the vicinity of industrial area, to overcome their expenses on transport. With the recent ban of leaded gasoline in Pakistan, emphasis should shift to other sources of exposure in children. Due to lack of anti-emissions equipments, the Pb industries (glass, batteries and ceramics) contaminated its environmental area with lead oxides during the last three decades [21].

In the present study, we aimed to evaluate the Pb exposure for primary school children, not adequately discussed so far. No national figures on Pb-poisoning related effects levels in children living in and around industrial areas in Hyderabad, Pakistan, where many glass industries, re-cycling of Pb from old batteries and other small industries where bangles (traditional jewelry in Asia), made of Pb are currently in operation. For comparison purposes, children of same age group residing in non-industrial area were selected as referents. This study describes the correlation of BLL and environmental exposure among children belongs to two study areas represent different environmental and socioeconomic conditions.

Materials and Methods

Study population

Hyderabad is fourth big city of Pakistan has a tropical temperate weather with average temperature of around 26°C (12°C in winter and 45°C in summer). It is relatively dry with average humidity ranging from 30% to 45% and annual rainfall of 100 mm. These climatic conditions favor children to play outdoor in a rather dusty environment. Aiming to assess children Pb exposure around the industrial area, medical team was set, including epidemiologists and toxicologists from environmental agencies and universities, together with local and regional technical and political health authorities.

A total of 425 children (242 boys and 183 girls) age ranged 5–10 years were selected for study. 245 children were residents of industrial sites, while 180 children belonged to non industrial areas. They all were studying in primary schools present in vicinity of industrial and non industrial areas. A verbal consent was obtained from parents for the child's participation in study. 456 children's mothers accepted to be interviewed, but only 425 mothers allowed blood samples withdrawal from their children. Background and risk factor data were collected through the administration of structured questionnaires to parents of children.

Height and weight measurements were performed on children using a standard bathroom scale according to standard World Health Organization guidelines [22]. Due to restricted resources and high illiteracy rate, questionnaires were administered to a randomly selected subsample of about 50% of the children's parents from industrial area and 80% from nonpolluted areas in local language (Urdu). The information was collected on medical history, frequency of infectious diseases, dietary habits, socio demography, housing conditions, children's behavior (for example, play sites, hand-to mouth activity), environmental and personal hygiene, parental occupational exposure to Pb, work history

of adults, income, and education. The economic status of exposed children, relative to unexposed area was low with poor housing conditions, malnutrition and limited access to basic environmental health services. The IQ of each child was recorded from verbal interview and report cards at their schools. Ethnically all children were Muslims.

Ethical and human subjects issues

The research proposal for this study, including English and Urdu translations of the questionnaire and consent form, was submitted to and approved by the higher education commission of Pakistan.

Sampling

Blood sampling was carried out at primary schools located in both understudy areas. Great care was taken with the washing of the children's hands first with soap and tap water, rinsing with distilled water, then wiping with alcohol. Blood was taken by venepunctures after the application of EMLA (Eutectic Mixture of Local Anesthetics**)** local anaesthetic cream to reduce the pain produced, collected into metal-free safety vacutainer blood collecting tubes (Becton Dickinson, Rutherford, USA) containing >1.5 µg K_2EDTA/mL. These tubes were kept into an ice box and transfer from the survey sites to our laboratory on the same day. We stored 2 mL of blood sample at −20°C to detect Pb content in whole blood, while 3 mL sent for hematological parameters determinations on the same day.

Chemicals and reagents

Ultrapure water obtained form ELGA labwater system (Bucks, UK), was used throughout the work. Concentrated nitric acid 65% and 30% hydrogen peroxide purchased from Merck (Darmstadt, Germany). Standard solutions of Pb were prepared by dilution of certified standard solution (1000 mg/L) Fluka Kamica (Bush, Switzerland). Dilute working standard solutions were prepared immediately prior to their use, by stepwise dilution of the stock standard solution with 0.2 mol/L HNO_3. Stock standard solution of chemical modifiers, $Mg(NO_3)_2$ (5.0 g/L) was prepared from $Mg(NO_3)_2$ (Merck Ltd., Poole, Dorset, UK). All solutions were stored in polyethylene bottles at 4°C. For the accuracy of methodology, the certified reference material (CRM), Clincheck control- lyophilized human whole blood (Recipe, Munich, Germany), was used.

Apparatus

The analysis of Pb was carried out by means of a double beam Perkin-Elmer atomic absorption spectrometer model 700 (Norwalk, CT, USA) equipped with a graphite furnace HGA-400, pyrocoated graphite tube with integrated platform, an autosampler AS-800 and deuterium lamp as background correction system. The hollow cathode lamp of Pb (Perkin) was used as radiation sources at analytical wavelength 283.3 nm. All instrumental conditions were used according the manufacturer's recommendation. A PEL domestic microwave oven (Osaka, Japan), programmable for time and microwave power from 100 to 900W, was used for total digestion of blood samples. Acid washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing solutions.

The electrothermal atomizer condition were: Drying temperature (°C)/ramp/hold (s) (110/15/10), Ashing temperature (°C)/ramp/hold (s) (1200/10/15), Atomization temperature (°C))/ramp/hold (2100/0/5), Cleaning temp. (°C)/ ramp/hold (s) (2300/1/3), using a modifier $Mg(NO_3)_2$ integrated absorbance signals computed by the AA spectrometer were employed throughout.

Microwave assisted acid digestion

Duplicate samples of blood (0.5 mL) of each child (exposed and controls) were directly taken in PTFE vessels. About 2 mL of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) was added to each vessel and kept for 10 min at room temperature, and then the vessels were placed in a covered PTFE container. This was then heated following a one-stage digestion program at 80% of total power (900W). Complete digestion of blood samples required 3 min, after the digestion, the vessels was left to cool and the resulting

solution was evaporated to semidried mass to remove excess acid. About 5 mL of 0.1 mol/L nitric acid was added to the residue and filtered through a Whatman No.42 filter paper and diluted with deionized water up to 10 mL in volumetric flasks. Blank extraction was carried out through the complete procedure. Blanks and standard solutions were prepared in a similar acid matrix.

The validity and efficiency of the microwave assisted digestion method was also checked with conventional wet acid digestion method on same certified reference material (CRM) and real samples as reported elsewhere [23], results are given in Table 1. Analytical results of the certified samples were in agreement with the certified values, confirming the reliability of our methods. The percentage recovery of Pb in CRM sample obtained by conventional digestion was 99.2%. The microwave assisted digestion method was less time-consuming, required less than 10 min to complete the digestion of samples and standards. The mean values for Pb differed >2% from the certified value. The coefficient of variation deviated less than 2%. Non-significant differences ($p>0.05$), were observed when comparing the values obtained by both methods (paired t-test) (Table 1).

Table 1. **Determination of Pb in certified sample (CRM) by Conventional (CDM) and microwave digestion method (MWD) µg/L (n=10)**

Elements	CDM $\bar{x} \mp s$	MWD $\bar{x} \mp s$	t_{value}[a]	% recovery[b]	Certified values
Pb	105±8.2 (7.76)[c]	104±7.3 (6.96)	0.0523	99.2	105 ± 24

Key: [a]Paired t-test between CDM and MWD at df=9, $t_{critical}$ at 95% CL= 2.262

[b] $\%\,\mathrm{Recovery} = \frac{[MDM]}{[CDM]} \times 100$

[c] Values in parenthesis (%) of relative standard deviation

Statistical analysis

All data are expressed as mean and standard deviation (SD). The comparisons of exposed children (EC) and non-exposed children (NEC) of both genders were made using Student's t-test. Multiple regression and Spearman correlation analyses were performed to determine the correlation between BLL, age, and % of hemoglobin. The statistical significance was defined as $p>0.05$. All statistical analyses were performed using the statistical Package for Social Science (SPSS) Version 10.0.

Results and Discussion

Visual examination of the data supported by use of the Kruskal-Wallis one way analysis of variance on ranks and Dunn's multiple comparison indicate the existence of two groups, exposed children and non-exposed children of both gender. In the study population, 143 boys and 102 girls were from industrial areas termed as EC, while 99 boys and 81 girls were selected from non industrial areas termed as NEC. According to hematological testing, >63% EC had less hemoglobin level as compared to NEC (Table 2).

Table 2. **Hematological parameters of children living in industrial and non-industrial areas**

Parameters	Non-industrial	Industrial	Normal Range
Serum ferritin (µg/L)	33.2±1.2	31.2±1.4	< 30
Haemoglobin (mg/dL)	13.3±1.5	11.8±1.2	11.5-16.5
Haematocrit (%)	38.9±2.4	37.5±3.2	35-55
Red blood count RBC (mm^3)	4.6±0.7	4.1±0.6	3.5-5.5
MCV (µm)	82.2±2.6	79.4±2.9	75-100
MCH (pg)	30.2±1.9	28.7±2.6	25-35
MCHC (g/dL)	35.5±1.5	33.4±3.3	31-38
WBC (mm^3)	8.2±0.9	7.6±1.3	3.5-10
Platelets (mm^3)	258.7±33.5	276.6±21.5	100-400

Key: Results are given as $\bar{x} \mp s$

The BLL of non-exposed boys and girls were observed at 95% confidence interval (C.I: 12.1, 13.0) µgd/L, (C.I: 10.2, 10.8) µg/dL respectively. The BLL of exposed boys (C.I: 21.9, 23.1) µg/dL and girls (C.I: 20.6, 21.5) µg/dL were significantly higher ($P>0.001$) than NEC of both gender (Table 3), although age-related increase was found in children of both groups. The BLL among the NEC and EC included in this study are presented in box and whisker plots (Fig. 1).

There were no significant differences of BLL between sexes of both NEC and EC calculated by Kruskal-Wallis Test ($p=0.479–0.481$). Difference and prevalence of elevated BLL in children with age range (5-10 years) of both groups are shown in Fig 2 (a, b), indicating the

correlation between log values of Pb in blood vs. age (years) of EC and NEC. The correlation (r) between the log-transformed data of BLL and age in NEC shows weak correlation (r=0.34), whereas a high correlation was observed between age and BLL of EC (r=0.67), which indicated that the prevalence of elevated BLL increased with age in EC. The correlation of hemoglobin and BLL between NEC and EC of both genders was statistically analyzed by a multiple linear regression equation and Pearson's correlation (Table 4).

Table 3. **Comparison of Pb in whole blood of children living in industrial (EC) and non-industrial area (NEC) (µg/dL)**

Gender	NEC $\bar{x} \mp s$ (Min-Max)	EC $\bar{x} \mp s$ (Min-Max)	p-value
Boys	12.5 ± 2.1 (6.0 – 18.2)	22.5 ± 3.5 (15.4 – 35.5)	0.001
Girls	10.5 ± 1.4 (7.5 – 14.2)	21.0 ± 2.2 (15.5 – 25.6)	0.001

Table 4. **Linear regression and Pearson's coefficient for concentration of hemoglobin (mg/dL) vs. Pb in blood (log values of µg/dL) of NEC and EC**

Population	Boys	Girls
NEC	y = -0.0163x + 2.3137 r = 0.34	y = -0.0215x + 2.2888 r = 0.33
EC	y = -0.0406x + 2.8311 r = 0.71	y = -0.0272x + 2.4621 r = 0.61

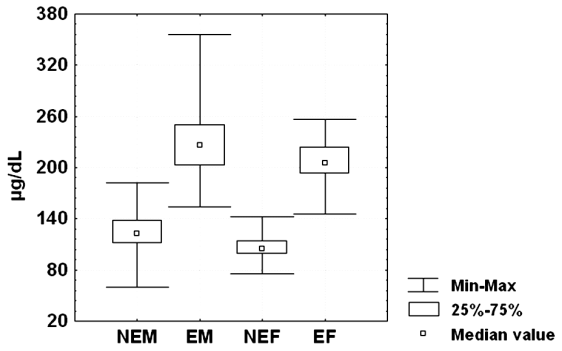

Figure 1. **Determination of Lead in blood samples of industrial and non industrial children of both genders, (NEM; Non-exposed male children, EM; Exposed male children, NEF; Non-exposed female children, EF; Exposed female children).**

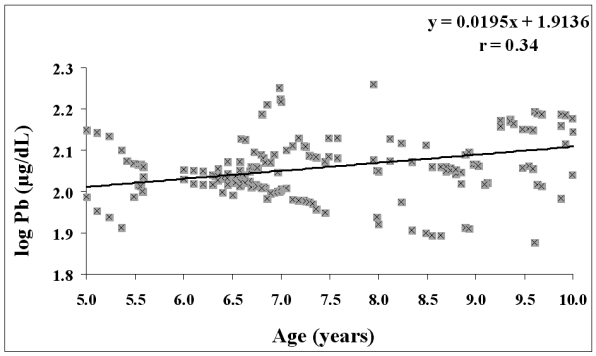

Figure 2a. **Scatter- plot showing correlation between log values of Pb in blood Vs. Age (years) in unexposed children.**

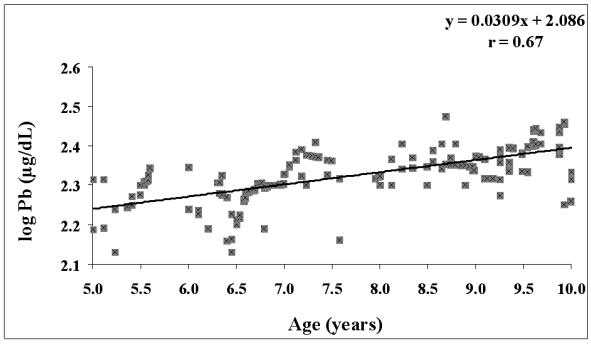

Figure 2b. **Scatter- plot showing correlation between log values of Pb in blood Vs. Age (years) in exposed children.**

In this study, BLL were analyzed in the blood samples of children age ranged 5-10 years of both gender, to assess the Pb burden pollution in children residing in industrial areas and compared with those children residing in unexposed areas. The mean BLL in understudy children living in industrial site area was higher among both boys and girls as compared to those of non-industrial areas. Our results are consistent with previous study [24]. To our knowledge, this is the first report on BLL in children living in Hyderabad city, Pakistan.

In this study, we attempted to assess BLL in children living in Pb contaminated environment. However, not all the potential sources of exposure have been considered, such as exposure to Pb from food or drinking water and smoking habits in the family, which could contribute to children's exposure to Pb at home [7, 25]. It was reported that the BLL of children living near the city centers were over 20 µg/dL [26]. According to our study,

boys tend to have higher BLL than girls. Possible answers lie in the active personality and behavior pattern of boys because they are more likely to play outdoors. We noticed a rise in BLL with age. The average BLL was 30–50% higher in 10 year-old children as compared to 5 year old children (Fig 2). This can be explained by the fact that with the growth of children, the chances of getting exposed to Pb in food, water, dust, and air increased, because elder children were more likely to play outdoor, which placed them at higher risk for Pb poisoning [27]. The findings of elevated BLL associated with age identified school going children as the risk populations for education and intervention of Pb exposure in population of Pakistan living in industrial areas have high prevalence of air pollution.

In our study group, parental occupational exposure to understudy exposed children, because all of the head and elder family members were working in different industries. Socioeconomic status of understudy exposed children's families is mostly belonging to labor class with low income and parental education was almost negligible or up to primary level.

Interesting observations were made in this study regarding the relationship between socioeconomic status and BLL in EC and NEC living in industrial site area and unexposed areas with good living conditions and socioeconomic status. Children from unexposed area and living in financially secure homes had lower BLL than those children, living in industrial areas. Low socioeconomic status may positively influence the concentrations of several toxic elements in children both due to a higher exposure and a low nutritional status influencing the absorption of toxic elements [28]. A positive association between BLL and occupation involving Pb exposure has been reported [29, 30].

Relationship between socioeconomic status and children's BLL have been reported in literature, i.e., Poland [28, 31] and other European countries [7, 32], United States [33], Jamaica [34] and Russia [35]. Workers bringing Pb-rich dust into the home from the workplace, via their clothes, hair, and shoes, may put children engaging in hand-to-mouth activities at an increased risk of Pb exposure [36].

Among NEC, 22% have higher BLL than permissible limit (10 µg/dL); because of high traffic intensity in urban area of Hyderabad, Pakistan which may significantly contributes to the Pb exposure. It was reported in previous studies, the children living in urban areas have higher BLL than children in rural areas [37, 38]. In our country the government controlled schools have no proper funding and basic facilities as the other developed countries have adopted, such as student–teacher ratio, students eligible for a free or discounted lunch. According to school annual report cards, the EC were not good as compared to the NEC, even the EC are not taught Basic English courses, as compared to NEC. The main reason was that the school in industrial areas is running under the auspices of government of Pakistan, where mostly the education standard is very low as compared to private elementary school organized by private agencies. As the expenditure in government schools are very low, so mostly the poor people admit their children to get primary education there. The risk of Pb poisoning is especially high for children in poorer circumstances since actual exposure is more likely, as are iron and calcium deficiencies that result in higher BLL. Subtle effects on IQ loss have been reported for BLL ≥10 µg/dL [3, 39]. The finding of this study suggests another disparity, school underperformance as a function of environmental Pb accompanied by neurotoxity. The results are consistent with previous findings about the trend of elevated Pb on inner city elementary school properties compared with elementary school properties in outer-city locations [40]. Documented effects of environmental Pb exposure in children include impaired neuropsychological (e.g. visuo-spatial memory, executive functioning and attention) and motor functions as well as decreased IQ [41-43]. Several early studies reported that a given blood or tooth Pb level was associated with neurodevelopmental deficits of greater magnitude or persistence among children from the lower socioeconomic strata [44, 45]. In the US and European countries, decrease in BLL were mainly attributed to the elimination of leaded petrol and Pb-soldered food cans. In spite of this, the prevalence of Pb poisoning is still high among

urban low-income populations, due to other sources of exposure [46].

Screening at a young age not only children living in industrial vicinity but also in non-industrial urban areas is very important because early identification of the problem may help to prevent detrimental developmental effects [47, 48]. It is investigated that the greatest Pb susceptibility appears in children who exhibit an increase of BLL between ages 2 and 6 years; in that case BLL at 6 years is more strongly associated with negative cognitive and behavioral outcomes than BLL at early child hood [49]. A comparison of blood lead levels reported for children from various countries in the published literature is given in Table 5. The lead content in the blood samples of exposed children higher than other reported study [50-60], while the level of Pb in non-exposed children are lower than those values, which were carried out in China and Sweden for children on Population and school based, respectively [61, 62].

Table 5. **Comparison of BLL in children from various parts of the world**

Authors	Country	Study Population	Age (years)	n	Mean ± S.dev (µg/dL)
Plusquellec et al., 2010	Canada	Preschool children	4.8 - 6.2	110	5.4 ± 5.0
de Almeida et al., 2010	Brazil	School Children	6 - 8	444	2.4
Nicolescu et al., 2010	Romania	Population Based	8 - 12	37[a]	3.2
				46[b]	5.1
Zahran et al., 2009	USA	School Children	1- 6	117	4.4 ± 1.6
Kim et al., 2009	S. Korea	School Children	8 - 11	261	1.7 ± 0.8
Zhang et al., 2009	China	Population Based	0 - 6	15 727	4.7
				14 737	4.6
				13 584	4.7
Boucher et al., 2009	Canada	Population Based	5	104	5.2
			11	198	2.7
Solon et al., 2008	Philippines	------	0.6 - 6	877	7.1
Zheng et al., 2008	China	Population Based	1 - 7	154	13.2 ± 5.9
Nriagu et al., 2008	Nigeria	------	2 - 9	653	8.9 ± 4.8
Stromberg et al., 2008	Sweden	School Children	7 - 11	1268	18.3
Chiodo et al., 2007	USA	------	7	506	5.0
Lalor et al., 2007	Jamaica	School Children	2 - 6	1081	7.3 ± 1.3
Surkan et al., 2007	USA	------	6 - 10	512	2.3 ± 1.6
Present study	Pakistan	School Children	5 - 10	180[a]	11.6 ± 1.7
				245[b]	21.9 ± 2.94

Key: [a] Non-exposed
[b] Exposed

Conclusion

All under study EC have BLL above the presently accepted limit 10 µg/dL, than NEC, living in non-industrial areas. However prevalence of BLL >10 µg/dL may be a problem in EC especially those from low income homes who are at greater risk of exposure. Future surveillance studies should target children from these communities and involve environmental sampling to validate potential sources of Pb exposure. The finding of higher BLL in Pakistani children, living in industrial site area, which threatens children's growth and development, demanded effective measures for the control of Pb pollution.

Childhood Pb-poisoning has aroused public attention as a public health problem in Pakistan. It is imperative to assess the problem realistically to develop sound and practical

approaches for prevention. Lead poisoning is a health problem in industrial areas of Sindh (Hyderabad and Karachi), and further studies are needed to better define the extent of Pb exposure among children. Limited access to affordable and convenient health care significantly affects the health and well-being of children in poorer communities. Ongoing stringent environmental and personal hygiene control measures are imperative, as is an intensive education program to raise awareness in the community of sources and mechanisms of exposure to Pb.

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