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Original Article

Proposed Serum-Lead Reference Intervals and Clinical Decision Thresholds for an Iranian Population Based on a Seven-Year Retrospective Study

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A B S T R A C T

Introduction: Chronic Lead (Pb) exposure is associated with metabolic, behavioral, and pathological disorders in both pediatric and adult populations. Serum lead levels and toxicity were assessed in patients referred to a clinical laboratory in western Tehran, Iran, over a seven-year period. In addition, lead reference intervals were determined.

Materials & Methods: 1,651 serum Pb (SPb) levels were determined by inductively coupled plasma optical emission spectrometry (ICP-OES), from March 21, 2017, to January 15, 2024. Analysis of variance was used to compare SPb levels across sex and age groups. Reference intervals were calculated according to Clinical and Laboratory Standards Institute guidelines.

Results: Mean ± standard deviation SPb levels were significantly higher in males (9.7 ± 7.6 µg/dL) than in females (6.86 ± 4.07 µg/dL) and children (4.92 ± 4.22 µg/dL; $p < 0.001$). Using a SPb level of >3.5 µg/dL as a screening cutoff based on Centers for Disease Control and Prevention recommendations, 51.1% of children aged ≤5 years screened positive. Older patients had significantly higher SPb levels, with the following age group means (µg/dL): 0-10 years = 5.07 ± 4.58, 11-20 years = 4.89 ± 2.49, 21-30 years = 6.95 ± 4.45, 31-40 years = 7.77 ± 5.84, 41-50 years = 7.75 ± 4.8, and age > 50 = 8.99 ± 6.65.

Conclusion: SPb toxicity should be precisely evaluated in the Iranian population, especially in children. Current reference intervals likely leading to underdiagnosis and an increased risk of chronic sequelae. Population-specific reference intervals are required to improve early identification of Pb toxicity and mitigate its long-term health consequences.

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Introduction

Reference intervals are crucial for the interpretation of clinical laboratory data. These intervals are dependent on numerous factors, including the measurement method patient demographics, and geographical location [1-3]. Several patient-specific variables influence the blood levels of laboratory indices, such as age, sex, lifestyle, comorbid conditions, ethnicity, and family history [4]. Consequently, a central challenge for clinical laboratories is establishing accurate reference intervals or appropriate clinical decision limits [5]. Confounding factors contribute to the variability of these indices, complicating their determination.

Lead (Pb) has no known essential role in human physiology; therefore, elevated blood Lead levels (BLLs) are considered toxic. Clinicians frequently measure BLLs to investigate potential causes of neurologic disorders and anemia in both pediatric and adult patients. Beyond these, Lead toxicity is associated with broader implications, including cardiovascular and hepatic dysfunction, as well as an increased predisposition to cancer. Monitoring BLLs can aid in diagnosing and managing nonspecific clinical signs and symptoms. Notably, Lead exposure has been linked to hypophosphatemia, reduced vitamin D3 levels, and disruption of brain iron, zinc, and copper metabolism [6, 7].

Manufacturers of commercial laboratory assays typically recommend that each clinical laboratory establish its own local reference intervals. Furthermore, ISO 15189 accreditation standards and reference texts stipulate that a laboratory must verify the performance specifications claimed by the manufacturer, particularly in the absence of reference methods or certified reference materials to independently assess accuracy [8, 9]. However, laboratories often utilize different kits, methods, and equipment, which can lead to disparate and non-comparable results for a given sample. This variability stems from differences in analytical sensitivity, specificity, predictive values, and reference intervals, potentially compromising clinical decision-making and hindering harmonization across laboratories [10-12].

In 2012, the Centers for Disease Control and Prevention (CDC) established a blood Lead reference value based on the 97.5th percentile of the National Health and Nutrition Examination Survey (NHANES) data to identify children with elevated exposure levels. This value is periodically updated; for the 2015-2016 and 2017-2018 cycles, it represented the level at which children in the top 2.5% of the distribution would be identified [13].

The chronic effects of prolonged, low-level exposure to Lead are challenging to investigate precisely due to ethical constraints and the limitations of human studies. It is well-established, however, that many toxins exhibit dose-dependent effects, with higher concentrations generally correlating with greater impact. Thus, it is logical to postulate

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that while BLLs below 10 µg/dL may not be overtly toxic for most adults or children over 6 years old, levels exceeding this threshold may be associated with subclinical impairment and an increased risk of pathological effects [14,15]. Clinical signs typically become apparent at BLLs of 80 µg/dL in adults and 45 µg/dL in children, as per CDC guidelines [13, 16-17]. Critical questions remain: do physiological processes begin to show impairment before reaching these overt toxicity thresholds? Do Pb-sensitive enzymes and proteins function optimally at concentrations between 3.5-45 µg/dL in children or 3.5-80 µg/dL in adults with risk factors such as smoking?

Establishing evidence for chronic disorders is complex, considering the clinical sensitivity and specificity of each laboratory method. Most reference intervals are validated for acute conditions, whereas chronic diseases often result from the cumulative effect of long-term predispositions. For example, diabetes is acutely defined by hyperglycemia that can lead to fatal osmotic shifts, while its chronic pathology stems from protein glycation. This glycation triggers inflammatory responses, reactive oxygen species production, and tissue damage, manifesting as nephropathy, retinopathy, and neuropathy.

In summary, redefining toxicity and overload thresholds for metabolites such as Lead is essential for early detection, intervention, prevention of chronic complications, and improvement of quality of life in at-risk populations. The aim of this study was to determine the serum Pb (SPb) reference interval and assess toxicity status in a patient cohort presenting to a private clinical laboratory in western Tehran, Iran. Two key principles must be followed for determining reference intervals: 1) they must have high sensitivity and specificity for early detection and screening at the presymptomatic stage, and 2) they must be effective for monitoring patients and evaluating the effectiveness of treatment.

Therefore, reference intervals should be defined to meet the clinical needs of screening, diagnosis, and monitoring. Reporting population-specific SPb levels is invaluable for healthcare providers, policymakers, physicians, and patients in planning prevention programs and therapeutic interventions. Evaluating these intervals is critical for assessing their applicability in clinical practice. We believe this study will contribute to the revision of Lead toxicity diagnosis and management guidelines.

Materials and Methods

Data acquisition and categorization

We analyzed 1,651 SPb measurements obtained from pediatric and adult patients (male and female) referred to our laboratory between March 21, 2017, and January 15, 2024. Data were extracted retrospectively from the laboratory information system without applying exclusion criteria; only patient age, sex, documented comorbid conditions, and Pb levels were recorded. All patient identifiers were removed to ensure investigator blinding. Patients were referred

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to the clinical laboratory for diagnosis of various diseases, routine examinations, and not exclusively lead poisoning. Doctors order a SPb test to diagnose a possible cause of growth problems in children and to determine the cause of anemia or neurological symptoms in active or passive smokers and drug abusers. SPb levels were quantified using an inductively coupled plasma-optical emission spectrometer (ICP-OES; Agilent Technologies, Inc., USA) at a wavelength of 283.3 nm.

The data were categorized into five groups based on Pb concentration: ≤3.49 µg/dL, 3.5–9.99 µg/dL, 10.0–19.99 µg/dL, 20.0–44.9 µg/dL, and ≥45 µg/dL. The frequency of individuals within each category was calculated to assess the distribution of Pb levels relative to established toxicity thresholds for children (0–5 years and up to 10 years) and adults (males and females) (Table 1).

Reference interval determination

Reference intervals were calculated in accordance with the Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guideline [18] using MedCalc software (Version 22.009). Three statistical methods were employed:

1. The normal distribution method

The normal distribution formula is: $RI=\bar{X}\pm z0.975\times SD$

RI: Reference interval

$\bar{X}$: is the sample mean

z0.975 for 0.95 sample coverage equals 1.96 Therefore, the lower and upper limits obtained from bellow formula:

$Lower\ Limit=\bar{X}-1.96SD$

$Upper\ Limit=\bar{X}+1.96\ SD$

2. The non-parametric percentile method.

The non-parametric percentile lower and upper limits of reference interval are obtained in bellow ways:

$Lower\ Limit= P2.5$

$Upper\ Limit= P97.5$

Where *P2.5* is 2.5th percentile of the population distribution and *P97.5* is 97.5th percentile of the population distribution

3. The robust method

The robust method formula is $RI=\hat{\mu}\pm1.96\hat{\sigma}$

$\hat{\mu}$: mean estimate

$\hat{\sigma}$: standard deviation estimate

Details of the reference intervals determination methods, limitations, sample sizes and other considerations can be found in EP28-A3c guideline [18]. Analyses were performed both including and excluding outliers. The results from each method are reported.

Results

Levene's test indicated a violation of the homogeneity of variances assumption across groups ($p = 0.001$). SPb levels, presented as mean ± standard deviation, were 9.7 ± 7.6 µg/dL in males, 6.86 ± 4.07 µg/dL in females, and 4.92 ± 4.22 µg/dL in children. This difference was statistically significant ($p < 0.001$; 99% CI). The distribution of SPb levels was non-normal, as confirmed by the D'Agostino-Pearson test and visually evident in Figure 1.

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Among the 1,651 SPb measurements, 43 were identified as outliers (range: 19.8–28.0 µg/dL) and 8 were extreme values (range: 29–64 µg/dL). Only two patients had Pb levels of 63 µg/dL and 64 µg/dL; all other samples were below 45.8 µg/dL.

The pediatric cohort consisted of 94 girls and 101 boys. Mean ± SD SPb levels were 4.999 ± 4.179 µg/dL (median: 4 µg/dL; range: 0.5–24.2 µg/dL) for girls and 4.932 ± 4.488 µg/dL (median: 4 µg/dL; range: 0.2–28.0 µg/dL) for boys. The mean SPb level for both sexes was above the 3.5 µg/dL screening threshold.

Using a SPb level of ≤3.5 µg/dL as the reference cutoff, 48.9% of children aged ≤5 years and 43.3% of children aged ≤10 years had values below this level. Consequently, 51.1% of children aged ≤5 years and 56.7% of children aged ≤10 years would have screened positive, necessitating follow-up blood Lead testing within three months according to CDC recommendations [13, 17].

Comorbidities

Of the 195 children, 68 (34.9%) were asymptomatic and referred for routine check-ups, while the remainder presented with signs or symptoms requiring clinical evaluation. Notably, 21 children (10.8%) presented with gastrointestinal pain or other serious health concerns. Other parent- or guardian-reported comorbidities included anorexia, thyroid disorders, anemia, and alopecia; however, these conditions were not precisely documented. Adult patients were also referred to the clinical laboratory for SPb measurement for various reasons. However, lead poisoning is only a suspected cause of health problems and the prescription of this heavy metal measurement is done to confirm the probable diagnosis; this was also true in our study population, especially for adult patients.

Serum Pb levels by age and sex

SPb levels for all participants, stratified by age category and sex, are detailed in Table 2, which includes the results of ANOVA comparisons. SPb levels were significantly lower in younger patients compared to older patients. Concentrations were higher in males than in females, with the pediatric cohort exhibiting the lowest overall levels (Table 2). The distribution of SPb levels, presented as boxplots showing outliers, is illustrated for the seven age groups in Figure 2 and for the three sex groups in Figure 3.

Reference interval estimation

Reference intervals were estimated using three statistical methods to define the upper and lower reference limits. In the pediatric group, the SPb reference interval was estimated to be 0.7–16.9 µg/dL. The intervals were 1.1–16.7 µg/dL for females and 0.7–27.8 µg/dL for males. It is important to note that these estimates were derived from a heterogeneous clinical population and were not established using a pre-selected healthy reference cohort; consequently, no data purification based on health status was performed (Table 3).

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Table 1. Frequency of children, adult females, and adult males categorized by SPb level

Centers for Disease Control and Prevention categorization for children blood Lead screening						
	No recheck	Need recheck within 1 and 3 months		Need recheck within 2 weeks	Need recheck within 48 hours	
Categories	Pb≤ 3.49 µg/dL	Pb= 3.5 to 9.99 µg/dL	Pb= 10 to 19.99 µg/dL	Pb= 20 to 44.9 µg/dL	Pb≥ 45 µg/dL	Total
Children (Up to 5 years)	45(48.91)	35(38.04)	12(13.05)	0(0)	0(0)	95(100)
Children (Up to 10 years)	84(43.3)	93(47.94)	15(7.73)	2(1.03)	0(0)	194(100)
Adult female	220(22.34)	580(58.88)	178(18.07)	7(0.71)	0(0)	985(100)
Adult male	69(14.65)	227(48.2)	139(29.51)	33(7)	3(0.64)	471(100)

Data are presented as number (%)

Note: The cut-off values for children (BLL ≤ 3.49 µg/dL and BLL ≥ 45 µg/dL) are based on the Centers for Disease Control and Prevention criteria for blood Lead screening in children. Values for adult males and females are provided for comparison.

Table 2. Serum Pb levels in the study population, stratified by age and sex

	Years old	Pb Median (Range)	Sig (p<0.05)
All participants	**0-85 (n= 1651)**	6.0 (0.0-64.0); 7.44±5.553	-
Age categories	**A: 0-5 (n=95)**	3.6 (1.0-19.8); 5.02±4.34	(E)(F)(G)
	B: 6-10 (n= 75)	4 (0.20-30.0); 5.07±4.58	(E)(F)(G)
	C: 11-20 (n= 167)	4.9 (1.0-19.0); 4.89±2.49	(D) (E)(F)(G)
	D: 21-30 (n=188)	6.0 (0.20-28.0) 6.95±4.45	(C)(G)
	E: 31-40 (n= 342)	6.95 (0.0-64.0); 7.77±5.84	(A)(B)(C)
	F: 41-50 (n= 327)	7.00 (0.0-29.0); 7.75±4.87	(A)(B)(C)
	G: >50 (n= 457)	8.00 (0.0-63.0); 8.99±6.65	(A)(B)(C) (D)
Sex	**H: Children (n= 194)**	4.0 (0.20-30.0); 4.92±4.22	(I) (J)
	I: Female (n= 985)	6.0 (0.00-32.0); 6.86±4.07	(H) (J)
	J: Male (n= 472)	7.4 (0.0-64.0); 9.69±7.59	(H) (I)

Data are presented as median and mean±SD, SPb= Serum lead

Table 3. Serum Pb reference intervals calculated from raw data using three statistical methods

		Calculated intervals based on three methods of reference interval determination		
	Population	Normal distribution method	Non-parametric percentile (CLSI C28-A3)	Robust method (CLSI C28-A3)
Reference range	Children (with outliers)	0.709 to 15.83	1.00 to 16.86	0.694 to 16.065
	Children (without outliers)*	0.759 to 16.205	1.00 to 16.54	0.746 to 16.612
	Female (with outliers)	1.145 to 16.67	1.100 to 16.00	1.135 to 16.69
	Female (without outliers)**	1.344 to 16.29	1.24 to 15.60	1.347 to 16.36
	Male (with outliers)	0.736 to 27.59	1.00 to 25.00	0.69 to 27.66
	Male (without outliers)***	1.675 to 27.56	2.00 to 24.00	1.66 to 27.78

*Analysis excluded two outliers (0.2 and 30 µg/dL).
**Analysis excluded seven outliers (0, 0.2, 23, 24.3, 26, 27, and 32 µg/dL).
***Analysis excluded seven outliers (0, 0.2, 23, 24.3, 26, 27, and 32 µg/dL).

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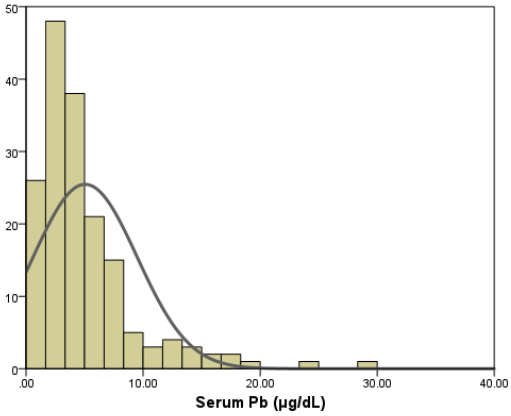


Fig. 1. Distribution of serum Pb levels in the pediatric study population (ages 1–10 years). The distribution was non-normal.

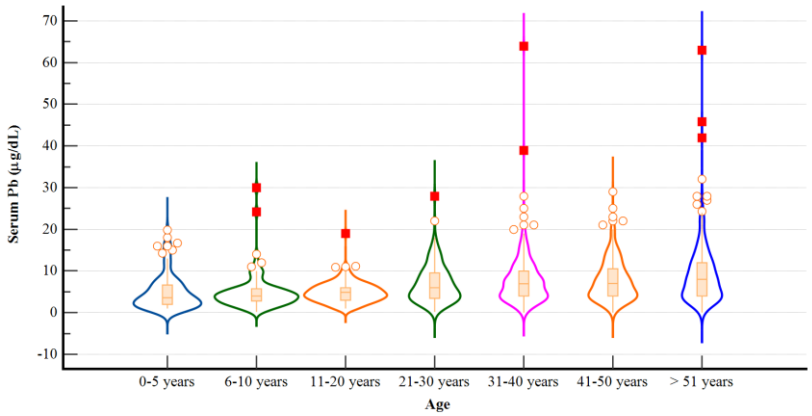


Fig. 2. Violin and box plots comparing serum Pb levels across seven age categories. Violin plots illustrate the data distribution density; box plots show the median, quartiles, and mean (± SD). Outliers are indicated by circles (near) and cubes (far).

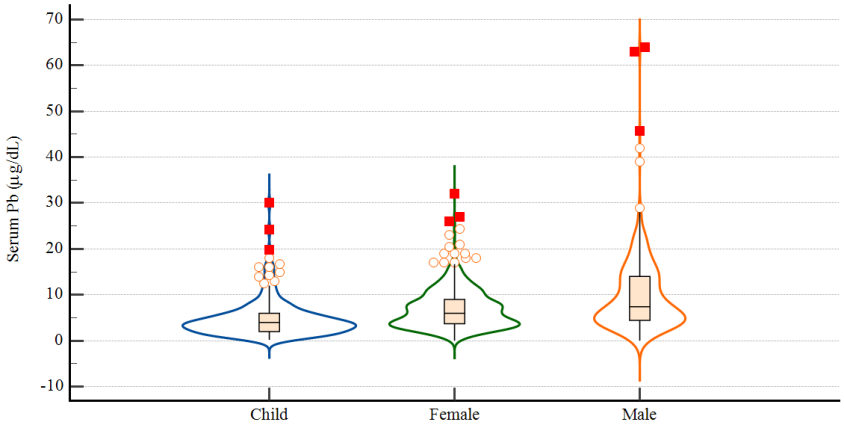


Fig. 3. Violin and box plots comparing serum Pb levels among children, adult females, and adult males. Violin plots illustrate the data distribution density; box plots show the median, quartiles, and mean (± SD). Outliers are indicated by circles (near) and cube (far).

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Discussion

Importance of determining reference intervals for SPb

The in-house establishment of reference intervals presents a significant challenge for clinical laboratories. This is compounded by the fact that available resources—including online references, textbooks, and commercial kit inserts—often propose significantly different limits and cutoffs. Laboratories frequently rely on the reference intervals provided in kit inserts [19, 20]. However, for SPb measurement, where a commercial kit is not employed, laboratories must instead consult reference textbooks or other certifiable resources [18, 21, 22]. Consequently, specialists often source reference intervals from textbooks or online searches to annotate patient reports. While websites, fact sheets, and research articles can be valuable sources, any intervals derived from research articles must be critically appraised and validated using an evidence-based laboratory medicine framework before clinical implementation [23, 24].

A primary obstacle is that conducting a formal reference interval study is resource-intensive, requiring significant time, funding, and ethical approval for patient recruitment, and is susceptible to selection bias. Conversely, the analysis of raw, blinded data without predefined health-based inclusion and exclusion criteria—as performed in this study—also introduces confounding factors that may distort the estimated intervals. In the following section, we provide a brief overview of blood Pb reference intervals cited in selected authoritative resources and literature.

Several reputable online resources provide reference intervals for tests, such as Pb, that are validated by in-house methods. These include Medscape, Lab Tests Online-UK, and the Mayo Clinic. For example:

- Medscape states a normal blood Pb level is <10 µg/dL for children and <25 µg/dL for adults.
- The Mayo Clinic indicates that a level ≥5 µg/dL in children is potentially unsafe and warrants monitoring, while levels ≥45 µg/dL generally require treatment.
- Lab Tests Online-UK defers to the CDC guidelines for the screening and management of childhood Pb exposure.

Authoritative textbooks are considered primary references in the field. According to the 7th edition of Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, the reference interval for whole blood Pb is <25 µg/dL for both children and adults, with levels >99 µg/dL considered toxic; for a 24-hour urine collection, the reference interval is <80 µg/dL [8]. In contrast, the 24th edition of Henry's Clinical Diagnosis and Management by Laboratory Methods reports a normal whole blood Pb value of <50 µg/dL [25].

Importance of monitoring BLLs

Routine assessment of BLLs in the general population is crucial for informing public health policies and prevention strategies. This is particularly critical in developing countries,

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where rapid population growth and urbanization are major priorities. The use of inaccurate or insufficiently sensitive cutoff values for elevated BLLs can result in missed or delayed diagnoses, thereby impeding timely intervention and follow-up. Therefore, the aim of this study was to conduct a comprehensive evaluation of blood Lead status within the described population.

Our findings provide a relevant context for comparison with other studies. For instance, Deng et al. evaluated the relationship between BLLs and cognitive function in a cohort of older US adults (mean age: 69.6 years), reporting a mean plasma Lead level of 1.8 ± 1.6 µg/dL. Although their study did not identify a direct association between concurrent BLLs and cognitive function, the authors emphasized that cumulative Pb exposure earlier in life may contribute to cognitive decline in later years [26].

In a study of pediatric populations, Firouzkouhi Moghaddam et al. [27] investigated the relationship between SPb levels and attention-deficit/hyperactivity disorder (ADHD). They reported that children with ADHD had significantly higher SPb levels than healthy controls (6.73 ± 2.40 µg/dL vs. 3.03 ± 1.31 µg/dL, respectively) [27]. Although their study was conducted in southeastern Iran and ours in the capital, Tehran, the elevated Pb levels observed in both the case and control groups in their report suggest that elevated BLLs may be a widespread public health issue across various regions of Iran. This widespread exposure could potentially contribute to a range of neurodevelopmental and other health problems.

A systematic review of pediatric BLLs in Iran reported concentrations ranging from 0.65 to 57.1 µg/dL across different regions [28]. Expanding on this, Mahmoudi and colleagues [29] conducted a comprehensive review of studies evaluating BLLs in various Iranian demographic and clinical groups. A concerning finding was that the majority of the studied populations exhibited elevated BLLs, with some cases reaching overtly toxic concentrations. Unfortunately, the available data lack information on the subsequent clinical decision-making and follow-up management by physicians following a diagnosis of elevated BLL. As patient management is tailored to clinical status, signs, and symptoms, the specific interventions undertaken in these cases remain unclear.

If the reference intervals provided on laboratory reports serve as the primary guide for clinical decisions, then the accuracy and clinical applicability of these intervals are of paramount importance. However, as noted previously, there is significant discrepancy in the reported reference intervals for BLLs among different resources and textbooks. Furthermore, many established intervals are based primarily on the acute effects of Lead toxicity, potentially overlooking the insidious health consequences of chronic, low-level Pb accumulation.

Heidari and colleagues have emphasized that Pb is a potent neurotoxin with a propensity to accumulate and persist in brain tissue more so than in other organs [30]. Specifically, Pb has

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a half-life of approximately 35 days in blood but can persist in the brain for up to 2 years. During short-term exposure, Pb is distributed to various organs, including the liver, kidneys, lungs, brain, spleen, muscles, and heart. After several weeks, it is predominantly redistributed and stored in the bones and teeth [31-33]. Within the brain, Pb accumulation can disrupt blood-brain barrier integrity, impair synaptogenesis and myelination, and interfere with catecholamine metabolism. Furthermore, Pb accumulation in tissues can increase oxidative stress, induce neuronal apoptosis, and damage subcellular structures such as the endoplasmic reticulum and mitochondria. Pb also interferes with signaling molecules, essential metal homeostasis, epigenetic regulation, and hemoglobin synthesis [26].

Critically, the pathological manifestations of Pb subcellular effects may not be immediately apparent through acute clinical signs. Therefore, to mitigate the metabolic and genetic consequences of Pb accumulation, early identification of Pb body burden through the redefinition of clinically appropriate blood Pb reference intervals is crucial.

The present study found that the measured SPb levels exceeded the current CDC reference value for the pediatric population. This finding is consistent with previous reports from Iran [27, 28]. However, reference intervals for BLLs in Iran require further rigorous investigation, calculation, and definition for use in clinical laboratories. Discrepancies in established reference intervals complicate the diagnosis, treatment, and monitoring of Pb toxicity. This challenge is likely applicable to many developing countries, particularly in the Middle East, and globally.

To facilitate early diagnosis and identify individuals at higher risk of Pb accumulation, we propose a reference interval for blood Pb of < 3.5 µg/dL for children and < 5 µg/dL for adults, with clinical signs and symptoms considered concurrently. Levels above these thresholds should be considered indicative of Pb exposure, warranting reassessment within three months for children and a thorough investigation into the exposure source. For adults, we propose that a BLL above 15 µg/dL may serve as a more sensitive indicator of potential toxicity than the commonly cited thresholds of 25–100 µg/dL used to define poisoning risk. This proposed adult value aligns more closely with the reference interval calculated for the female cohort in this study using the non-parametric percentile method (CLSI EP28-A3c).

Based on our findings, we recommend the following clinical decision thresholds:

- **Children:** Normal: ≤ 3.49 µg/dL; **Action:** Reassess within 3 months: 3.5–9.99 µg/dL; **Action:** Identify exposure source if ≥ 10 µg/dL.
- **Adults:** Normal: ≤ 4.99 µg/dL; **Action:** Reassess within 3 months: 5.0–14.99 µg/dL; **Action:** Identify exposure source if ≥ 15 µg/dL.

These proposed thresholds should be validated and periodically revised based on geographical region.

Our recommendation to lower the pediatric reference value is supported by Okoye et al. (2023), who concluded that a value of 3.5

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μg/dL provides a more sensitive criterion for healthcare providers to diagnose and prevent Pb toxicity in children [34]. In the current study, 10.8% of children for whom a SPb test was ordered presented with gastrointestinal complaints. Other frequently reported conditions included anorexia, thyroid dysfunction, anemia, and alopecia. These manifestations may be associated with deficiencies in essential trace elements and vitamins or with overload of toxic elements like Pb; however, establishing a direct causal relationship in clinical studies remains challenging. Further investigation using animal models and *in vitro* systems is needed to elucidate the acute pathogenic mechanisms of Pb toxicity. Elucidating the chronic effects of Pb poisoning remains a complex and demanding area of research, even in controlled experimental models.

Conclusion

Analysis of a seven-year dataset from a private laboratory revealed notably elevated mean SPb levels in the studied population. Discrepancies in established Pb reference intervals, combined with insufficient recognition of the chronic effects of Pb poisoning in clinical practice, contribute to diagnostic inaccuracy and challenges in clinical decision-making. A critical area for further investigation is the elucidation of comorbidities associated with Pb exposure. Obtaining robust evidence for these associations is necessary to refine reference intervals for BLLs accurately. We posit that regional reports on Pb exposure from across the globe are essential to develop a comprehensive understanding of its toxicity. The compilation and analysis of such geographically diverse data will facilitate the establishment of robust, evidence-based reference intervals and guide effective global strategies for the prevention, diagnosis, and management of Pb-related health issues.

Ethical Considerations

The relevant ethics committee approved the study protocol and the informed consent procedures.

Funding Statement

Not applicable.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgment

To ensure the linguistic quality of the manuscript, the text was reviewed using an AI-based editing service (https://chat.deepseek.com/). The tool was prompted to "edit these sentences as a native English language scientist, for publication in a medical journal."

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Authors' Contributions

SR.S-J and T.S contribute in data gatherings, blinding and purification. N.S and A.A contributed in data analysis and preparation of the manuscript.

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