Erythrocyte Acetylcholinesterase as a Biomarker of Environmental Lead Exposure

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V. B. Patel et al. (eds.), *Biomarkers in Toxicology*, Biomarkers in Disease: Methods, Discoveries and Applications, https://doi.org/10.1007/978-3-030-87225-0_4-1

Abstract

Lead is a prime, ubiquitous, environmental toxicant and multi-organ poison. One of the most recognized deleterious effects of lead exposure is neurotoxicity, which may at least in part arise from perturbation in cholinergic neurotransmission with possible impact on acetylcholinesterase activity. Although primarily located at the neuromuscular junction and cholinergic brain synapses, this pivotal enzyme is also present in peripheral cells such as human erythrocytes. Erythrocyte acetylcholinesterase, which correlates positively with brain acetylcholinesterase and represents neurotoxic targets in brain, is a conventional biomarker for the neurotoxic effects of pesticide exposure. However, recent reports have unveiled the sensitivity of this extra-neural enzyme to other environmental contaminants particularly lead, bringing to attention its relevance in the assessment of environmental lead exposure and lead-induced neurotoxicity. This chapter summarizes the evidence of the remarkable diversity of erythrocyte acetylcholinesterase as a biomarker of environmental lead exposure and lead-induced alterations in human cholinergic system as well as the possible factors surrounding its applicability in this regard.

Keywords

Cholinergic neurotransmission · Environmental lead exposure · Erythrocyte acetylcholinesterase · Environmental contaminant · Lead · Lead-induced neurotoxicity · Multiorgan poison · Neurotoxic effects · Toxicant · Toxic metal

Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
BLL	Blood lead level
CNS	Central nervous system
LIN	Lead-induced neurotoxicity
Pb	Lead

Introduction

Lead (Pb) is a prime toxic environmental contaminant and multi-organ poison that has been known since antiquity (Anetor et al. 2016; Nwobi et al. 2021). Lead occurs naturally in ores with other metals but released into the environment during mining or processing of these ores (Hsu and Sabatini 2019). This heavy metal, although toxic, has some unique physico-chemical characteristics that guarantee its applicability in various products such as protection equipment, electrical equipment, paint, petrol, cosmetics, jewellery, and toys, among others (WHO 2021). The various anthropogenic activities, the uncontrolled involvement of lead in products as well as the unavailability of natural breakdown mechanisms for this toxic metal, have culminated to its continuous widespread environmental contamination as well as concomitant increased human exposure and attendant health risks. Although environmental lead exposure is a significant health concern of global magnitude, it is more common in fast industrializing countries with large chemical burden and developing countries that have weak or unimplemented environmental regulations and inadequate product content control and policies (Obeng-Gyasi 2019).

Environmental lead exposure from different sources occurs through routes such as ingestion, inhalation as well as dermatological contact with lead-contaminated products (Al Osman et al. 2019). However, following absorption of lead into the bloodstream, it is distributed to almost all major organs' systems where it induces different types of toxic effects of which the neurotoxic effect is considered to be of major concern and the most deleterious (Anetor et al. 2002; Anetor et al. 2008; Nwobi et al. 2019a). Yet, reliable and sensitive methods for predicting and assessing neurotoxicity remain a challenge to neuroscientists and toxicologists.

Lead-induced neurotoxicity (LIN) may manifest as perturbation of cholinergic neurotransmission that may present as alteration in the activity of acetylcholinesterase (AChE): a key enzyme that hydrolyses the neurotransmitter acetylcholine to ensure nerve impulse intermittency (Ortega et al. 2021). Although this enzyme is primarily located at the neuromuscular junction and cholinergic brain synapses, it can also be found at peripheral cells such as the human erythrocytes (Nwobi et al. 2019a; Felsztyna et al. 2020). Several reports have shown that erythrocyte AChE has several similar characteristics with neuronal AChE, correlates positively with brain AChE, and reflects neurochemical targets in the brain (Nehru and Sidhu 2001; Lionetto et al. 2013; Gupta et al. 2015; Nwobi et al. 2019a).

Although erythrocyte AChE has long been known as a biomarker for the neurotoxic effects of pesticide exposure (Assis et al. 2018), emerging reports over the past few years have shown that this enzyme is also sensitive to other environmental contaminants such as the toxic metals (Frasco et al. 2005; Phyu and Tangpong 2014; Fu et al. 2018). In line with this, the relevance of erythrocyte AChE in the assessment of human environmental lead exposure and LIN has continued to gain attention over the years (Ademuyiwa et al. 2007; Khan et al. 2009; Gupta et al. 2015; Nwobi et al. 2019a). This chapter summarizes the available evidence of the remarkable diversity of the involvement of erythrocyte acetylcholinesterase as a biomarker of environmental lead exposure and lead-induced alterations in the human cholinergic system.

Chemistry, Forms, and Properties of Lead

Lead is a naturally occurring element with the chemical symbol Pb, derived from its Latin name, *plumbum*. Lead has an atomic number of 82 and a relatively high atomic weight of 207.2, making it a heavy metal. It has four stable isotopes: ^{208}Pb (51 to 53%), ^{207}Pb (20.5 to 23%), ^{206}Pb (23.5 to 27%), and ^{204}Pb (1.35 to 1.5%) (ATSDR 2020). Lead is a member of group 14 or p-block, subgroup IVA, and period 6 of the periodic table of elements. The 82 electrons in a lead atom have the shell structure of 2.8. 18.32. 18.4 and the ground state electron configuration of [Xe] 4f14 5d10 6 s2 6p2. As such, the valence shell of the lead atom in the ground state has two "s" and two "p" electrons. Notably, the two "s" electrons are resistant to ionization and are thus sometimes referred to as inert pair, making lead to be considered to have a stable oxidation state or valency of +2 (ATSDR 2020). Lead has a bluish-white color when freshly cut, develops a dull grayish color when exposed to air, and has a shiny chrome-silver luster, when it is melted into a liquid. It has a density of 11.34 g/cm^3, low melting point of 327.46° C, and boiling point of 1,750 °C to 1,755 °C (ATSDR 2020).

Lead constitutes 0.002% of the Earth's crust and exists naturally in ore with other metals and not in the elemental state (Hsu and Sabatini 2019). The main lead ore is galena (lead sulfide) (WHO 2010). Others include anglesite (lead sulfate), cerussite (lead carbonate), mimetite (lead chloroarsenate), and pyromorphite (lead chlorophosphate) (WHO 2010). Lead can also occur in other forms such as organic lead as found in tetra-ethyl lead used as additive in petrol (Galadima et al. 2012), or inorganic lead as occurs in old paint, soil, dust, and various consumer products.

Lead has a range of unique properties, which make it invaluable in various domestic and industrial applications. These include low melting point, high resistance to corrosion and fire, ability to absorb radiation, and sounds and other vibrations as well as being soft, malleable, and a relatively poor conductor of electricity (WHO 2021).

Sources and Routes of Environmental Lead Exposure

Human exposure to environmental lead was low prior to the industrial revolution. However, in recent years, it has risen because of various human activities such as industrialization, large-scale mining, uncontrolled applications of lead to products, and the continuous use of such lead products, all of which are coupled with a lack of environmental regulation and product content control and policies. This makes environmental lead exposure a problem of serious concern.

Several potential sources of lead exposure exist. However, the various sources and their relative importance may differ both within and between countries and regions (Obeng-Gyasi 2019; WHO 2021). Nevertheless, some of the major sources of lead include leaded paint, lead emissions from industries, leaded water pipes and fittings, lead-glazed food vessels, lead-containing traditional folk medicine and cosmetics (WHO 2021) (Fig. 1).

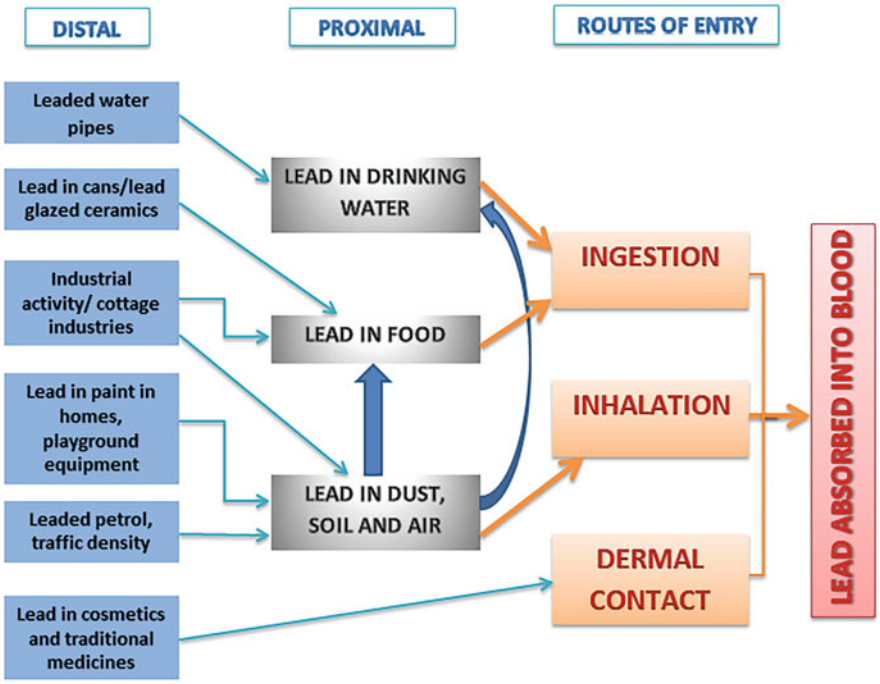

Fig. 1 Major sources and routes of environmental lead exposure. (Adapted from WHO 2021)

Although the recognized routes of lead exposure include ingestion, inhalation, and dermatological contact, ingestion and inhalation have been reported to be the most significant routes (Fig. 1) (WHO 2021). Numerous cases of oral lead poisoning emanate from regularly ingesting small amounts of lead-contaminated substances such as dust or dirt, flakes of lead paint, food, water, and traditional remedies, among others. Young children, however, are more vulnerable to oral lead poisoning than adults are because they spend so much time in one place, play on the ground, have frequent hand-to-mouth contact, and eat objects that may contain or be contaminated with lead (WHO 2010).

Inhaling lead fumes or particles work is a common occupational route of exposure, but it can also happen at home if there is lead-contaminated airborne dust Exposure through inhalation of lead-contaminated air, dust or fumes is quite common in occupational settings but may also occur within the domestic environment (Pelclová et al. 2016). Dermal exposure can occur in the workplace or through the use of lead-containing cosmetics, but it is usually regarded as a minor and limited method of lead uptake (WHO 2021).

Toxicokinetics of Lead

Absorption, Distribution, and Elimination of Lead

The absorption of lead from the gastrointestinal tract is affected by several factors such as age, nutritional status, genetic factors, and the type of lead involved (WHO 2021). The absorption rate of ingested lead in adults is low (3–10%), compared with children, who have a higher rate (40–50%) (WHO 2021). Lead absorption can also be increased by fasting as well as nutritional deficiencies of important minerals such as calcium, zinc, and iron (Kordas et al. 2018; Rădulescu and Lundgren 2019).

Absorption of particulate lead by inhalation is dependent on particle size, concentration, and ventilation rate and is worthy to note that children may have higher exposure because of their increased air intake per unit of body weight compared to adults (WHO 2010). It has been reported that while small particles of lead (<1 μm) are deposited in the lower respiratory tract, from where they are almost entirely absorbed, larger particles (1–10 μm) are deposited in the upper airways, transferred by mucociliary transport to the esophagus and swallowed (WHO 2021). Dermal absorption is dependent on the skin's integrity and the lead's physicochemical qualities; it is minimal for inorganic lead and much greater for organic lead compounds (ATSDR 2020).

As soon as absorbed, lead gets to its first receptacle – blood, where about 99% is bound to erythrocytes, it is distributed to soft tissues such as the kidney, heart, and brain as well as the calcified tissues such as the bone – which serves as the major repository of lead (Fig. 2). Blood and soft tissues represent the active pool and bone the storage pool (ATSDR 2020). The blood lead level (BLL) represents recent exposure to lead from exogenous sources. It could also reflect lead redistributed from skeletal stores if there had been previous exposure to lead. This may be particularly important in pregnancy, when stored lead is released because of bone turnover (Osorio-Yáñez et al. 2021). About two-third of inorganic lead is excreted in the urine and the other one-third secreted in bile, into the intestine, and then excreted through the feces (Charkiewicz and Backstrand 2020). Lead is eliminated in two phases after exposure: The first (elimination from blood and soft tissues) takes about 20–30 days, and the second (slow elimination from the blood) involves excretion from the bones (Charkiewicz and Backstrand 2020). In general, the lead elimination half-life from blood and soft tissues is approximately 30 days while that of bone is 10–20 years; as a result, lead can be eliminated at an exceedingly slow rate, facilitating its accumulation in the body with more propensity to toxicity.

Toxicodynamics of Lead

Lead does not have any physiological function but induces a variety of toxicities ranging from subclinical to clinical toxicities with overt signs and symptoms. No safe threshold for lead exposure or blood lead level has been reported thus far, implying that the ideal blood lead level is zero although achieving this limit is rarely

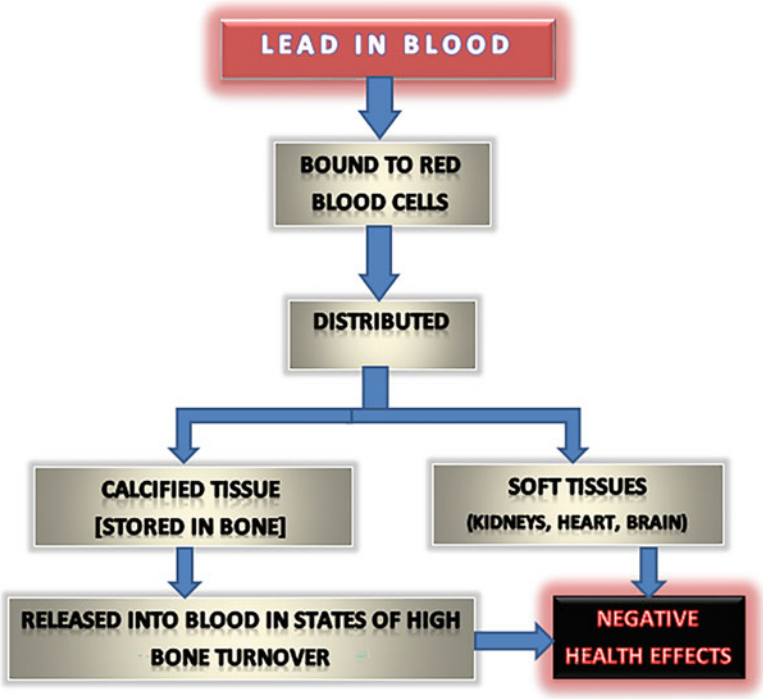

Fig. 2 Distribution of lead. (Adapted from WHO 2021)

feasible (Vorvolakos et al. 2016; ATSDR 2020; Ruckart et al. 2021). This may explain why no reference range for blood lead exists, but acceptable level (ACCLPP 2012). Lead has the ability to affect nearly all biochemical components, organs, and systems in the body. The principal systems affected may include among others the cardiovascular, hematological, renal, reproductive, skeletal, and neurological systems (Table 1).

Lead and Hematological Toxicity

One of the most well-studied systems on which lead has profound deleterious effect is the hemopoietic system, particularly its effects on the hem biosynthethic pathway – a pathway that has served as a surrogate for the diagnosis of lead poisoning (Patharkar et al. 2019). Three important enzymes in this pathway are downregulated by lead. The enzymes are aminolevulinic acid synthetase which catalyzes the formation of δ-aminolevulinic acid, δ-aminolevulinic acid dehydratase which catalyzes the formation of porphobilinogen from δ-aminolevulinic acid, and ferrochelatase which catalyzes the insertion of iron into protoporphyrin to form hem. Out of these 3 enzymes, δ-aminolevulinic acid dehydratase has been reported to be

Table 1 Lead toxicity and effects

Lead toxicity	Effects	Selected references
Lead and hematological toxicity	Inhibition of hem biosynthetic pathway key enzymes; aminolevulinic acid dehydratase, aminolevulinic acid synthetase, and ferrochelatase Decrease in life span of circulating erythrocytes through cell membrane fragility Anemia	Patharkar et al. (2019) Qader et al. (2021) WHO (2021)
Lead and renal toxicity	Hyperuricemia, gout, decreased renal clearance, tubular reabsorption, and glomerular filtration rate Increased risk of nephropathy and renal failure	Jung et al. (2019) Nakhaee et al. (2019)
Lead and cardiovascular disease	Hypertension Coronary heart disease, stroke, and peripheral arterial disease	Gambelunghe et al. (2016) Chowdhury et al. (2018), Obeng-Gyasi et al. (2018)
Lead and reproductive toxicity	Infertility in men and women Reduced sperm count Reduced fetal growth, decreased birth weight, preeclampsia, preterm birth and spontaneous abortion, and teratogenic effects	Ma et al. (2019) Wu et al. (2012) Dutta et al. (2021) WHO (2021)
Lead and bone metabolism	Alteration of essential bone minerals such as calcium and zinc Reduction of bone mineral content and the mechanical characteristics of long bones Alteration in biomarkers of bone turnover	Nwobi et al. (2019) Qi et al. (2020) Olchowik et al. (2014), Rodríguez and Mandalunis (2018) Nwobi et al. (2021)
Lead and neurotoxicity	Decreased cognition, intelligence quotient and behavior scores, alterations in attention, changes in visual-motor, reasoning skills, and impaired reading ability in children Increased rates of malaise, amnesia, headache, fatigue, lethargy, irritability, dizziness, and weakness in occupationally exposed adults Reduction in nerve conduction velocity, sensory and motor neuropathies	Crump et al. (2013) ATSDR (2020), Reuben et al. (2020) WHO (2021)

the most sensitive to lead (Qader et al. 2021). Lead also shortens the life span of circulating erythrocytes by making cell membranes more fragile (WHO 2021). The combined effects of lead on erythrocyte cell membranes as well as inhibition of enzymes involved in hem-biosynthesis may lead to anemia (WHO 2021).

Lead and Renal Toxicity

Lead induces proximal tubular injury in the kidneys, which manifests as proximal tubule nuclear inclusion bodies that may lead to tubulo-interstitial disease and fibrosis. Hyperuricemia and gout are also common findings associated with chronic lead toxicity (Jung et al. 2019). This may emanate from isolated proximal tubular

defects leading to increased tubular reabsorption and decreased secretion of uric acid. Proximal tubular defects may also lead to decreased renal clearance, tubular reabsorption, and glomerular filtration rate (Jung et al. 2019). These may predispose to increased risk of nephropathy and related renal failure (Nakhaee et al. 2019).

Lead and Cardiovascular Disease

In addition to hypertension (Gambelunghe et al. 2016), epidemiological reports have linked lead exposure with various cardiovascular-related clinical outcomes such as cardiovascular disease mortality, coronary heart disease, stroke, and peripheral arterial disease (Chowdhury et al. 2018; Obeng-Gyasi et al. 2018).

Lead and Reproductive Toxicity

Lead causes reproductive malfunction and infertility in men and women (Ma et al. 2019). Reduced sperm count in male partners of infertile couples has been associated to increased lead levels in seminal fluid (Wu et al. 2012). Maternal exposure, even to low lead levels, has been linked with reduced fetal growth, decreased birth weight, preeclampsia, preterm birth, and spontaneous abortion as well as teratogenic effects (Dutta et al. 2021).

Lead and Bone Metabolism

Lead exposure alters the metabolism of important essential minerals involved in skeletal metabolism such as calcium and zinc. Lead and calcium share similar metabolic characteristics because of their comparable biochemical nature as divalent cations (Godwin 2001). However, compared to calcium, lead has larger ionic radius, higher electronegativity and uneven charge distribution in the electron cloud, thereby, allowing it to bind to protein-binding sites with greater affinity than calcium and impairing physiological functions such as bone mineralization (Godwin 2001; Nwobi et al. 2019b). In the same vain, zinc, another divalent cation, which has stimulatory effect on osteoblastic bone formation and mineralization, could also be altered by lead (Qi et al. 2020). The perturbation in the metabolism of these essential bone minerals may manifest as reduction in bone mineral content and the mechanical characteristics of long bones (Olchowik et al. 2014; Rodríguez and Mandalunis 2018). Furthermore, it could cause a disruption in bone turnover by generating an imbalance in the dual processes of bone formation and resorption (Nwobi et al. 2021).

Lead and Neurotoxicity

When compared to other organ systems, the nervous system is the most susceptible and the major target for lead toxicity (Fang et al. 2021). Chronic lead exposure can induce subtle alterations in neurological function in children and adults (Vlasak et al. 2019). Young children, on the other hand, are more vulnerable due to their high rate of lead absorption and higher penetration of lead through the blood-brain barrier, which is the most sensitive to damage (Nwobi et al. 2019a; WHO 2021). Children may develop neurological and cognitive sequelae such as decreased cognition, intelligence quotient and behavior scores, alterations in attention, changes in visual-motor and reasoning skills and impaired reading ability, and antisocial behavior, which may persist into adulthood (Crump et al. 2013; ATSDR 2020).

Increased rates of malaise, amnesia, headache, fatigue, lethargy, irritability, dizziness, and weakness have been reported in occupationally exposed adults, which may become more obvious and life-threatening in old age (ATSDR 2020; Reuben et al. 2020). These workers have also been reported to have reduced nerve conduction velocity, sensory and motor neuropathies, and wrist drop and/or foot drop (WHO 2021).

Cholinergic System

The cholinergic system plays a pivotal role in several CNS functions including cognition (Manzo et al. 1995). The dysfunction of this system has been reported to be responsible for the behavioral disturbances and learning and memory deficits in humans and animals (Nehru and Sidhu 2001). Several studies have suggested that the alteration in cholinergic neurotransmission involving the cholinesterases may be responsible for the neurotoxic effects of lead (Bressler and Goldstein 1991; Goldstein 1992; Anetor et al. 2002). This implies that the disrupted biochemical intracellular communication may occur before the well-known, overt clinical signs of LIN.

Cholinesterases are enzymes that hydrolyze serine and have a high affinity for choline esters. Varieties of cholinesterase enzymes with different properties exist in the animal tissues; however, the two main types are acetylcholinesterase (AChE, EC 3.1.1.7), also known as true cholinesterase, and butyrylcholinesterase (BChE, EC 3.1.1.8), also known as pseudocholinesterase (Hajjawi 2012; Rosenberry et al. 2017). Although the three-dimensional structures of AChE and BChE are strikingly similar, they differ in the amino acids that line a deep, narrow gorge at the bottom of which a catalytic site is located. In human BChE, there is replacement of 6 of the 14 aromatic amino acids that line AChE's gorge with aliphatic amino acids. As a result, BChE has a larger acyl pocket than AChE but lacks the peripheral site found in AChE (Hajjawi 2012; Rosenberry et al. 2017).

The main and most known function of AChE is to hydrolyze acetylcholine, a major neurotransmitter and neuromodulator, into choline and acetic acid, thereby, modifying nerve impulses involved in neural communication (Huerta-Ocampo et al.

2021). Butyrylcholinesterase, on the other hand, hydrolyzes butyrylcholine more efficiently than acetylcholine and is commonly engaged in exogenous chemical detoxification and bioactivation (Hajjawi 2012).

Acetylcholinesterase

In its basic working state, AChE is a dimmer amphipathic protein with a molecular weight of 160 K and a single inter-subunit disulfide bond (Rosenberry and Soggin 1984). Acetylcholinesterase is a fast-acting, uncompetitive enzyme with a turnover rate of 10^3 to 10^4 s^{-1} and a catalytic rate around the diffusion limit (Quinn 1987). Acetylcholinesterase hydrolyzes acetylcholine, a key neurotransmitter, thereby, ensuring nerve impulse intermittency. Apart from being primarily found in neuromuscular junctions and cholinergic brain synapses, AChE is also found in peripheral tissues such as the human erythrocytes, where it is firmly attached (Felsztyna et al. 2020).

X-ray crystallographic research of acetylcholinesterase revealed that catalysis occurs in a 20-Å deep active site gorge comprising a catalytic triad of serine, histidine, and glutamate residues located at the base of the active site, denoted as the cholinergic site, acylation, or A-site. Acetylcholinesterase has another distinct region near the rim of the gorge or enzyme surface, called the peripheral site or P-site (Soreq and Seidman 2001; Johnson et al. 2005; Rosenberry et al. 2017) (Fig. 3).

The P-site is lined with aromatic residues that are pivotal in the binding and orientation of aromatic and/or cationic substrates as they move from the P-site to the A-site (Rosenberry et al. 2017). The P-site has been reported to serve as an intermediate binding site for cationic ligands such as lead, which, thereafter, proceed to the acylation site (Johnson et al. 2005; Rosenberry et al. 2017). Binding to P-site can result in allosteric activity change of the acylation step, which inadvertently affects the whole rate of enzyme reaction. The activity of AChE reduces at high substrate levels, which by corollary may imply high lead level (Mallender et al. 2000; Rosenberry et al. 2017). This phenomenon known as substrate inhibition has been suggested to occur through steric blockade of product release caused by the binding of an additional substrate molecule to the P-site (Szegletes et al. 1998; Rosenberry et al. 2017). This implies that the binding of lead to the P-site of the enzyme molecule may result in the creation of a complex whose stability may give rise to varying degrees of alteration of enzyme-effective function with consequent possible conformational change of the enzyme (Auf der Heide 2007) (Fig. 3).

Extraction and Purification of Erythrocyte Acetylcholinesterase

A mature human erythrocyte also known as red blood cell is a non-nucleated, biconcave, disc-shaped cell filled with hemoglobin that transports oxygen and carbon dioxide between the lungs and tissues (Ciaccio et al. 2021). Acetylcholinesterase is a glycosylphosphatidylinositol-anchored protein that is firmly connected to

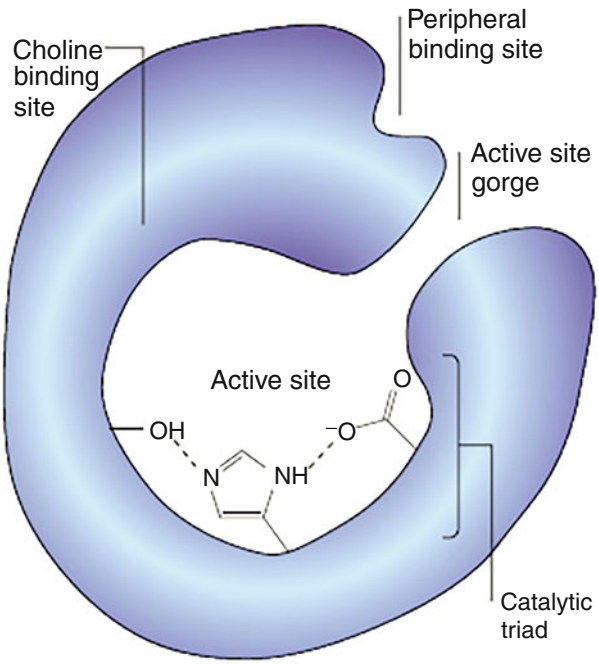

Fig. 3 Structural features of the acetylcholinesterase enzyme. The cholinergic site is at the bottom of a deep, narrow gorge while the peripheral binding site is near the rim of the gorge or enzyme surface. The amino acids serine 203, histidine 447, and glutamate 334 form a catalytic triad in human acetylcholinesterase. (Reproduced with permission from Soreq and Seidman (2001))

the outer membrane of the human erythrocyte by hydrophobic and hydrostatic forces (Felsztyna et al. 2020). The catalytic efficiency of erythrocyte AChE is determined by its amphipathic extraction and purification medium such as detergents like Triton X-100 and sodium chloride (0.14 M) (Gupta et al. 2015). The role of sodium chloride is to break the electrostatic interactions with membrane proteins that protect some of the enzymes from detergent action (Gupta et al. 2015). This allows the detergent to attack the membrane more efficiently and solubilize more membrane-bound enzymes.

The biochemical assay of acetylcholine involves the hydrolysis of acetylthiocholine to form sulfhydryl groups which react with Ellman reagent (5,5-′-dithiobis(2-nitrobenzoic acid)) to generate a yellow color detected at the wavelength of 405 nm, whose intensity is proportional to choline and by implication, AChE activity (Ellman et al. 1961). A clinical assay involves the hydrolysis of acetylcholine by AChE to form choline and acetic acid; the change in pH due to the liberation of acetic acid is measured using metanitrophenol - an indicator, whose intensity of yellow color in alkaline solution decreases as acetic acid concentration is increased (Hajjawi 2012).

Erythrocyte Acetylcholinesterase in Biological Monitoring of Environmental Lead Exposure

Environmental lead exposure in association with its health risks, such as LIN, is an issue of serious concern. Hence, there is need for a continuous, accessible, reliable and sensitive monitoring of lead exposure particularly in the developing countries where exposure to lead recently appears more intense (Attina and Trasande 2013; Obeng-Gyasi 2019; WHO 2021). The lack of both human CNS cells or tissues and sensitive neurochemical indicators as well as ethical and practical considerations are the principal reasons the majority of the human research on LIN in the past relied greatly on neurobehavioral tests or electrophysiological measurements (Seppalainen 1988; Anger 1990; Manzo et al. 1996). Although these methods may be highly valuable in the clinical setting as useful metrics in detecting/diagnosing behavioral changes and neurological disorders, they are unable to identify specific nervous system components affected by the neurotoxicant (Ademuyiwa et al. 2007). However, these limitations have been overcome through the use of direct biochemical methods involving promising peripheral alternatives that are not only present in more easily and ethically obtainable body fluids such as blood, but also exhibit biochemical signals of neurotransmission similar to those involved as neurotoxic targets in the CNS (Manzo et al. 1995; Manzo et al. 1996; Ademuyiwa et al. 2007). Thus, the extraneuronal enzyme – erythrocyte AChE, which is a prime candidate in this case – may be considered to be very useful in assessing exposure and response of the CNS when functional damage may not yet be apparent – silent neurotoxicity (Nwobi et al. 2019a). Several reports have shown that erythrocyte AChE exhibits similar characteristics with those of neuronal enzyme, correlates positively with brain AChE, and reflects neurochemical targets in the brain (Nehru and Sidhu 2001; Ademuyiwa et al. 2007; Lionetto et al. 2013; Gupta et al. 2015).

Blood Lead Level Versus Erythrocyte Acetylcholinesterase in Lead-Induced Neurotoxicity

Although many sample matrices such as blood, bone, tooth, hair, nail, saliva, and urine have been studied, the most used biomarker of lead exposure is the blood lead level (BLL) (Sommar et al. 2014). However, BLL has been considered insufficiently sensitive owing to some limitations such as poor response to changes at increased lead exposure, as well as exhibition of large inter-individual difference in health response to a particular lead level. Blood lead level also has a short half-life of approximately 30 days; hence, it primarily reflects current exposures and inadequately assesses long-term risk such as LIN, which has a more insidious impact (Nwobi et al. 2019a). As a result, the early detection of LIN appears to be a significant concern of global magnitude and a critical area of research, thus necessitating a continuing search for its accessible and inexpensive biomarkers. Interestingly, erythrocyte AChE is promising in surmounting these limitations because it has

a longer half-life of 2–3 months and its activity appears to be altered in LIN (Majidi et al. 2018).

Reports from animal models, humans, and in vitro investigations exist on the use of AChE as a surrogate biomarker for lead exposure (Ademuyiwa et al. 2007; Ani et al. 2007; Reddy et al. 2007; Khan et al. 2009; Gupta et al. 2015; Nwobi et al. 2019a). Lead has been reported to directly affect AChE activity in different brain regions such as the hippocampus, cerebellum, cortex, and midbrain, resulting in alteration in motor coordination activity and perturbation in cognitive behavior (Reddy et al. 2003; Ani et al. 2007; Reddy et al. 2007). A remarkable observation by these investigators is that the perturbation of AChE activity by lead persists even after the cessation of the stimulus exposure, underscoring the strength of the biological association between AChE and lead.

In an in vitro study by Gupta et al. 2015, the effect of various doses of lead on human erythrocyte AChE activity showed strong inhibition of the enzyme in a time-dependent, uncompetitive manner (Gupta et al. 2015). This report also underscores the neurological far-reaching implication of lead exposure on AChE activity and biomarker of effect potential.

Ademuyiwa et al. 2007, reported lead inhibition on the activity of erythrocyte AChE in adult artisans occupationally exposed to lead in Abeokuta, Nigeria. The investigators suggested that erythrocyte AChE could be employed as a putative biomarker for lead exposure, specifically lead-induced cholinergic system alterations in individuals living in a lead-contaminated environment (Ademuyiwa et al. 2007). In the same vein, Khan et al. 2009, investigated erythrocyte and plasma AChE activity in adult painters in Lucknow city, India, and reported that lead exposure, assessed by increased BLL, disrupted cholinergic function by remarkably inhibiting the erythrocyte AChE activity in the workers (Khan et al. 2009).

Nwobi et al. 2019a, investigated the potential role of BLL, erythrocyte AChE activity, and intelligence quotient in the early identification of LIN in apparently healthy children that had elevated BLL, an indication of environmental lead exposure based on ACCLPP 2012. However, their findings showed increased erythrocyte AChE activity which associated positively with BLL (Fig. 4) and negatively with intelligence quotient (Nwobi et al. 2019a). Thus, suggesting that erythrocyte AChE may be useful for the early identification of childhood lead exposure and LIN.

The Possible Mechanisms Surrounding the Interference of Lead with Erythrocyte Acetylcholine Activity

The mechanisms surrounding the interference of lead with erythrocyte AChE activity so far appear to be incompletely elucidated, and no one unifying molecular explanation has been proposed to explain it. As a result, various research groups have proposed or speculated on plausible molecular pathways. Some investigators have associated the altered erythrocyte AChE activity in lead exposure to the effect of free radicals generated by lead (Tsakiris et al. 2000; Khan et al. 2009). Although some authors have linked the altered AChE activity to the effect of binding of lead to

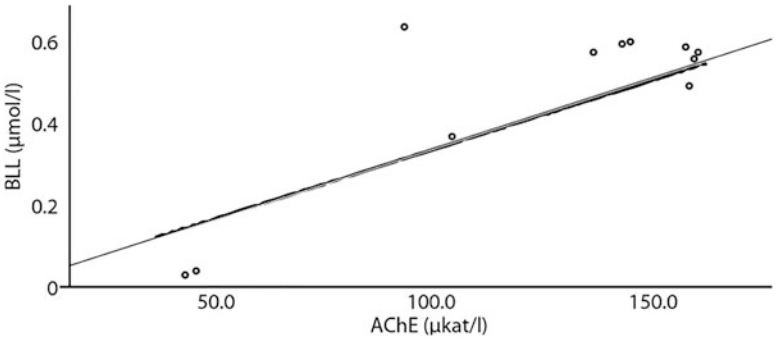

Fig. 4 Scatter plot showing linear relationship between blood lead level (BLL) and erythrocyte acetylcholinesterase (AChE) activity in children in Ibadan, Nigeria. (Reproduced from Nwobi et al. (2019a))

the thiol group in the enzyme (Phyu and Tangpong 2014), others have argued that erythrocyte AChE does not contain free thiol groups in its structure to which lead could bind (Rosenberry 1975). However, most recently, the alteration in erythrocyte AChE activity has been linked to the displacement of nutritionally essential metals such as calcium, from their protein-binding sites by lead (Anetor et al. 2002; Sharma et al. 2015; Nwobi et al. 2019).

The structure of AChE may shed more light on the AChE-lead interaction. It has been reported that the tertiary structure of the EF-hand regions of AChE corresponds closely to the well-characterized EF-hand motifs of calcium-binding proteins such as calmodulin, suggesting that divalent cations may be critical to the folding, maintenance of structure, function, and activity of AChE (Tsigelny et al. 2000). Other divalent metals such as lead can also bind to EF-hand motifs of calcium-binding proteins (McPhalen et al. 1991). Lead, a toxic metal, at low concentration has been reported to increase the activity of calcium-binding protein, which on the other hand is inhibited at higher concentration of lead (Kasten-Jolly and Lawrence 2018).

This could explain at least in part, the lead-induced increase in erythrocyte AChE activity in school children reported by Nwobi et al. (2019), which appeared not to be in line with the findings of other researchers who worked on occupationally exposed adults but reported lead-induced inhibition of AChE activity (Ademuyiwa et al. 2007; Khan et al. 2009). This explanation seems plausible for two reasons. First, Nwobi et al. (2019) recruited children that were not occupationally exposed to lead compared to other researchers that employed occupationally exposed adult workers. Second, the mean BLL in the children recruited by Nwobi et al. (2019) was quite small, 0.4 µmol/L (8.3 µg/dL), compared to the mean BLL of occupationally exposed workers, 35 µg/dL and 21 µg/dL, reported by Ademuyiwa et al. (2007) and Khan et al. (2009), respectively. Reports have shown that the activity of AChE reduces at high substrate levels, which by corollary may imply high lead level (Mallender et al. 2000; Rosenberry et al. 2017). This may further explain why the erythrocyte AChE, which was exposed to low lead level compared to the

occupationally exposed adults, positively correlated with the BLL in Nwobi et al. 2019a. However, these reports underscore the need for more population-based studies to compare the effect of low and high level of lead exposure on erythrocyte AChE in children and adults.

Application of Erythrocyte Acetylcholinesterase to Prognosis

The application of erythrocyte AChE as a biomarker of environmental lead exposure may imply higher correlation with brain AChE activity and longer time of interaction with lead, permitting reliable detection of lead for a longer period after exposure. Hence, erythrocyte AChE could be considered an important promising index to determine the degree of environmental lead exposure and LIN as well as a monitoring alternative to BLL, a conventional biomarker of lead exposure. However, so far, it is not yet completely clear to what extent the data from erythrocyte AChE activity may be superior to the information obtained from the measurements of BLL regarding their usefulness as putative biomarkers of environmental lead exposure due to the following reasons:

1. There is inconsistent result on the erythrocyte AChE activity on exposure to low and high lead exposure, necessitating further research to identify low concentration cut-off at which lead activates the enzyme activity and high concentration cut-off at which the lead inhibits the enzyme activity.
2. Erythrocyte AChE also shows sensitivity to other environmental contaminants other than lead (Frasco et al. 2005; Phyu and Tangpong 2014; Fu et al. 2018), implying that the combinations of different environmental contaminants may exert additive or synergistic inhibitory effect on erythrocyte AChE activity. As a result, the changes in the activity of this enzyme may be interpreted as an integrative measurement of the overall neurotoxic risk posed by the load of all the bioavailable environmental contaminants rather than just lead.
3. Erythrocyte AChE activity may also be altered by some health conditions such as pregnancy, anemia, bleeding, and reticulocytosis, which may confound the evaluation and interpretation of enzyme activity (Auf der Heide 2007).

Clearly, it is pertinent to consider or resolve the foregoing confounding issues before erythrocyte AChE can be accepted as an alternative biomarker that is superior to the use of BLL for the assessment of environmental lead exposure.

Application of Erythrocyte Acetylcholinesterase to Other Conditions

Erythrocyte AChE plays diverse significant roles in health and diseases (Saldanha 2017). This enzyme aids in the preservation of erythrocyte shape and size as well as serves as a marker of membrane integrity (Gupta et al. 2015). It is also involved as

the Yt antigen of the Cartwright blood group and has been linked to the expression of hemoglobin (Bartels et al. 1993).

The activity of erythrocyte AChE declines as erythrocytes age, and it likewise reduces with age in people as oxidative stress rises in response to increasing age (Prall et al. 1998; Saldanha 2017). Erythrocyte AChE enzyme activity increases in healthy females compared to males (Hilário et al. 2003; Saldanha 2017). These gender-related changes in the enzyme's activity and the fluidity of the membrane hydrophobic area under the impact of adrenaline may explain, at least in part, the differences in responses, attitudes, and behaviors under stress circumstances between men and women (Hilário et al. 2003; Saldanha 2017). These differences may hold some useful promise in the management of diseases and medication reactions, at least at the cellular level.

Increased erythrocyte AChE activity has been linked with Parkinson's disease, essential hypertension, glaucoma, retinal vasculitis, amyotrophic lateral sclerosis, and Hirschsprung's disease, implying that this enzyme is significantly involved in inflammation (Silva-Herdade and Saldanha 2013; Saldanha 2017). Decreased erythrocyte AChE activity has been reported in patients with type 1 diabetes as well as individuals with paroxysmal nocturnal hemoglobinuria, which put them at risk of complement system lysis (Suhail and Rizvi 1989; Ueda et al. 1990). Decreased erythrocyte AChE activity has also been observed in farmers that are exposed to pesticides. This could result from the direct harmful effects of the pesticides on the integrity of the erythrocyte membrane (Lozano-Paniagua et al. 2016). It is also noteworthy that there is the need to rule out these confounders while considering erythrocyte AChE as a promising and reliable biomarker of environmental lead exposure or LIN.

Mini-Dictionary of Terms

- Acetylcholine: A major neurotransmitter in the cholinergic system.
- Acetylcholinesterase: A cholinergic enzyme (also known as true cholinesterase) that rapidly hydrolyzes acetylcholine into acetic acid and choline, thereby terminating its action and ensuring the intermittence of nerve impulses.
- Blood lead level: The most commonly used biomarker of lead exposure that represents absorbed doses of lead.
- Central nervous system: The part of the nervous system that consists of the brain and spinal cord as well as coordinates the activity of the entire nervous system.
- Erythrocyte: A red blood cell, which in humans, is a biconcave, nonnucleated disc that contains the pigment hemoglobin, which imparts the red color to blood as well as transports oxygen and carbon dioxide to and from the tissues.
- Lead: A heavy metal that is a prime ubiquitous environmental and occupational toxicant as well as a multiorgan poison.
- Lead-induced neurotoxicity: The alteration of the normal activity of the central nervous system or damage to it, because of exposure to lead.

Key Facts of Environmental Lead Exposure

- Lead is a prime ubiquitous environmental contaminant as well as a multiorgan poison.
- Major routes of lead exposure include ingestion, inhalation, and dermatological contact.
- Blood lead level is the most commonly used biomarker of lead exposure.
- The contemporary population's body lead burden is 500–1000 times more than that of their preindustrial forebears (WHO 2010).
- Lead exposure is responsible for 900,000 deaths, loss of 21.7 million years of healthy life, 62.5% of developmental intellectual disability of unclear etiology, 8.2% of hypertensive heart disease, 7.2% of the ischemic heart disease, and 5.65% of stroke in the world (IHME 2019).
- The global cost of lead exposure in children is projected to be $977 billion per year or 1.20% of global GDP (Attina and Trasande 2013).

Key Facts of Erythrocyte Acetylcholinesterase as a Biomarker of Environmental Lead Exposure

- Lead-induced neurotoxicity is the most deleterious effect of lead exposure and could manifest as alteration in cholinergic neurotransmission.
- Erythrocyte acetylcholinesterase is an extra neuronal enzyme that is similar to the brain acetylcholinesterase and represents neurochemical targets in the brain.
- The structure of this enzyme shows an acylation site for binding of acetylcholine and a peripheral site where lead could bind.
- The applicability of erythrocyte acetylcholinesterase activity as a biomarker of environmental lead exposure should be independently confirmed by the use of blood lead level or other molecular approaches.

Summary Points

- Lead is a prime, toxic, ubiquitous environmental contaminant and multiorgan poison that poses significant health challenge, particularly in developing countries, which also bear the brunt of the associated health and socioeconomic implications.
- One of the most recognized deleterious effects of lead exposure is neurotoxicity particularly in the central nervous system.
- Blood lead level is the commonly used marker of lead exposure but has been considered insufficiently sensitive owing to some limitations such as poor response to changes at increased lead exposure, exhibition of large inter-individual difference in health response to a particular lead level, and possession of a short half-life of approximately 30 days, which make it primarily reflect

current exposures and inadequately assesses long-term risk such as led-induced neurotoxicity, which has a more insidious impact.

- However, the extraneural enzyme – erythrocyte acetylcholinesterase – appears to be an important tool to determine the degree of environmental lead exposure and lead-induced neurotoxicity because it implies higher correlation with brain acetylcholinesterase activity, represents neurochemical targets in brain, and has longer time of interaction with lead, which allows reliable detection of lead for a longer period after exposure, unlike blood lead level.
- It is note-worthy that the applicability of erythrocyte acetylcholinesterase in this regard appears to have some confounding factors, which may interfere with the evaluation and interpretation of enzyme activity; it shows sensitivity to other environmental contaminants apart from lead, its activity may differ on exposure to low and high lead exposure, and it may be altered by some health conditions.
- It is pertinent to consider and resolve the above limitations before accepting erythrocyte acetylcholinesterase as a sufficiently reliable, specific, and sensitive biomarker for environmental lead exposure and lead-induced neurotoxicity.
- Hence, at the moment, although erythrocyte acetylcholinesterase appears to hold some promise as a useful biomarker of environmental lead exposure and lead-induced neurotoxicity, its activity should be verified independently using blood lead levels or other molecular approaches.

Cross-References

► Biomarkers of Lead Exposure: Platforms and Analysis

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