Research Article

Serum S100B, NSE, and GRIA1 levels as neurological biomarkers in lead exposure

Esra FIRAT OĞUZ[1],*, Fatma Meriç YILMAZ[2], Engin TUTKUN[3], Ömer Hınç YILMAZ[3], Müjgan ERCAN[4], Sevilay SEZER[1]

[1]Department of Clinical Biochemistry, Ankara Numune Education and Research Hospital, Ankara, Turkey
[2]Department of Biochemistry, Faculty of Medicine, Yıldırım Beyazıt University, Ankara, Turkey
[3]Ankara Occupational Diseases Hospital, Ankara, Turkey
[4]Aydın Public Health Laboratory, Aydın, Turkey

Background/aim: The central nervous system is one of the major targets in lead exposure. Biomarkers for the diagnosis and follow-up of lead exposure have not been identified. In this study, serum S100B, neuron-specific enolase (NSE), and glutamate receptor 1 (GRIA1) levels were determined as possible biomarkers for lead neurotoxicity.

Material and methods: Twenty-five subjects with chronic lead exposure and 25 controls were included in the study. NSE and S100B were measured by electrochemiluminescence immunoassay with a Cobas E601 analyzer. GRIA1 levels were measured with an ELISA kit using a quantitative sandwich enzyme immunoassay technique.

Results: GRIA1 levels were significantly higher in the lead exposure group than in the control group. No significant differences for NSE, S100B, ALT, AST, or creatinine in sera were found between lead exposure and control groups.

Conclusion: Subjects with chronic lead exposure are found to have increased glutamate receptor levels and do not seem to have glial or neuronal damage, which can be demonstrated with the elevation of NSE and S100B levels. GRIA1 levels might be used as a biomarker for the neurotoxicity of lead.

Key words: Lead exposure, neuron-specific enolase, GRIA1, S100B, neurotoxicity

1. Introduction

Lead (Pb) is a widespread heavy metal that can persist in water, plants, and soil in the environment naturally in an inert form (1). Human exposure can be associated with the use of lead-containing ceramic dishware, food cans, and paints or, more commonly, it may be occupational in individuals working in industrial areas like automobile batteries, paints, ceramics, plastics, and cosmetics (2,3).

The central nervous system (CNS) is a major target in lead exposure. Lead is known to be a risk factor for a variety of neurologic and psychiatric disorders. Lead-induced brain injury appears to affect cognitive functions in the frontal cortex, motor ability in the cerebellum, and memory in the hippocampus (3,4). Several mechanisms are offered for lead-induced neurotoxicity, including cholinergic dysfunction, glutamate receptor changes, increased oxidative stress, and impaired antioxidant defense system in the brain (5). Lead acts like calcium in the CNS and enters cells from calcium channels. Lead exposure causes an elevation in intracellular calcium as the result of high uptake to astrocytes. Lead also activates protein kinase C

(PKC) like calcium and PKC activation leads to calcium release from intracellular stores. This causes secretion of excitatory neurotransmitters, phospholipase activation, and oxidative stress in the CNS (6).

S100B is a biomarker with low molecular weight (9–13 kDa) and belongs to the calcium-binding protein family. It is found in glial cells of the CNS, especially in astrocytes. Neuron-specific enolase (NSE) is a glycolytic enzyme found in neuronal cytoplasm and known as a biomarker of brain damage with S100B. After neuronal damage, their elevated levels are found in blood and cerebrospinal fluid according to the increased permeability of the blood–brain barrier (BBB) (7,8). Glutamate is one of the major neurotransmitters in brain and affects *N*-methyl-D-aspartate (NMDA) receptor activation, which is mediated by calcium. Glutamate receptors are divided into two groups: the fast-acting ligand-gated ionotropic channels and the slower-acting metabotropic receptors. The ionotropic receptors are cation-specific ion channels. These receptors are subdivided into three groups: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA),

kainate, and NMDA receptor channels (9). Glutamate receptors are important for synaptic transmission during learning and memory. AMPA receptors were demonstrated to mediate most excitatory responses in the brain (10). Some studies revealed that ionotropic NMDA receptors have important roles in lead-induced neurotoxicity (11–13).

Glutamate receptor 1 (GRIA1) is one of the ionotropic excitatory receptors in mammalian brains and is important in neural communication, learning, and memory (14). GRIA1 is responsible for glutamate-mediated excitation of neural cells and is important in memory formation and learning. GRIA1 levels were investigated in acute mercury intoxication and were suggested to be a potential neurological biomarker in mercury-induced neurotoxicity (15).

In this study, we aimed to study NSE, S100B, and GRIA1 levels in subjects with chronic lead exposure and determine whether these parameters could be used as biomarkers of lead neurotoxicity.

2. Materials and methods

2.1. Patients
The exposure group consisted of workers in the battery industry (n = 15) and smelters (n = 10), who were admitted to the Ankara Occupational Diseases Hospital for annual health examinations. The mean lead-related work duration was 7.6 years for battery workers and 5.4 years for smelters. Twenty-five healthy adults admitted to the hospital for routine check-ups, without a history of occupational Pb exposure and with normal physical examination and no chronic illness, were included in the study as the control group. Subjects with Pb exposure were divided into two groups according to their neurological examination results. This study was approved by the local ethics committee of Ankara Keçiören Training and Research Hospital (date: 22.02.2012 / no.: B.10.4.ISM.4.06.68.49)

2.2. Blood samples and measurement
Venous blood samples were collected in vacutainer tubes and centrifuged at $1300 \times g$ for 10 min. Sera were separated and stored at –20 °C until analysis.

GRIA1 was measured with an ELISA kit (Cusabio Biotech Co., P.R. China; catalog number: CBS-EL009898HU) using a quantitative sandwich enzyme immunoassay technique. Detection range of the assay was 23.44–1500 pg/mL. Intra- and interassay precision were <8% and <10%, respectively.

NSE (catalog number: REF 12133113 122) and S100B (catalog number: REF 03175243 190) were measured by electrochemiluminescence immunoassay in a Cobas E601 analyzer (serial number: 2115-01; Roche, Germany). Detection range of the NSE assay was 0.050–370 ng/mL. Detection range of the S100B assay was 0.005–39 µg/L.

Repeatability of the S100B assay was 1.0%, 1.8%, and 0.7% at concentrations of 0.09 µg/L, 0.26 µg/L, and 2.25 µg/L, respectively. Reproducibility of the S100B assay was 3.1%, 2.5%, and 2.9% at concentrations of 0.09 µg/L, 0.26 µg/L, and 2.24 µg/L, respectively. Repeatability of the NSE assay was 1.6%, 0.8%, and 0.7% at concentrations of 0.90 ng/mL, 11.9 ng/mL, and 95.1 ng/mL. Reproducibility of the NSE assay was 2.2%, 3.1%, and 3.8% at concentrations of 0.87 ng/mL, 11.4 ng/mL, and 87.3 ng/mL.

Whole-blood lead determination was performed using EDTA tubes in the toxicology laboratory of the Ankara Occupational Diseases Hospital using Varian AA 240Z (Agilent Technologies, Inc., Santa Clara, CA, USA) atomic absorption spectrophotometry (serial number: AA0904M070). Standard solutions were prepared by dilution of certified standard solutions (High-Purity Standards, Charleston, SC, USA). Two-level quality control materials were used (Seronorm; Sero AS, Billingstad, Norway). The lead calibration curve ranged from 0 to 100 µg/dL. Limits of detection and quantification were 0.02 and 0.1 µg/dL, respectively. The relative standard deviation of measurements was 4.2%.

2.3. Statistical analysis
The findings of this study were analyzed with SPSS 18 (SPSS Inc., Chicago, IL, USA). The conformity of continuous variables to normal distribution was tested with the Kolmogorov–Smirnov test. The descriptive statistics of continuous variables were expressed as median (min–max). The presence of a statistically significant difference between the groups in terms of continuous variables was examined with the Mann–Whitney U test for nonparametric variables. The presence of a correlation between the groups was examined with Spearman's rho tests. $P < 0.05$ was considered the threshold of statistical significance for all tests. The area under the curve (AUC), specificity, and sensitivity values were calculated with receiver operating characteristic (ROC) analysis.

3. Results
Subjects in the lead exposure group were 25–62 years of age (median: 38) and those of the control group were 18–73 years of age (median: 37). In the lead exposure group, the median blood lead level was 34.2 µg/dL with a minimum value of 1.24 and a maximum value of 73.7 µg/dL. Mean exposure time was 6.21 ± 6.396 years. The GRIA1 level was significantly higher in the lead exposure group than in the control group (P = 0.011). No significant difference was determined between the lead exposure and the control group for NSE, S100B, ALT, AST, or creatinine levels (P > 0.05) (Table 1).

Subjects who had lead exposure were divided into two groups according to their neurological examinations. Fourteen of the subjects had neurological signs in their

examination. Twelve of these 14 subjects had pain and numbness of the hands and others had pain and numbness of the feet. Eleven of the subjects had no neurological signs in their examination. There was no significant difference in the levels of GRIA1, NSE, or S100B between these two groups (P > 0.05) (Table 2).

There were no correlations between lead exposure and GRIA1, NSE, S100B, ALT, AST, and creatinine levels (Table 3).

ROC analysis was performed for GRIA1. The AUC for GRIA1 was 0.709. Sensitivity and specificity for GRIA1 levels to detect lead-related neurotoxicity were found as 70% and 56%, respectively (cut-off: 39.1 pg/mL) (Figure).

4. Discussion
The CNS is a major target in lead exposure. Lead is known to be a risk factor for a variety of neurologic and psychiatric disorders.

In the present study, we studied the levels of neurological biomarkers S100B, NSE, and GRIA1 in lead exposure and control groups. We found that GRIA1 levels were significantly higher in the lead exposure group.

However, according to ROC analysis, the sensitivity and specificity values for GRIA1 levels to detect lead-induced neurotoxicity did not indicate that it is a good biomarker.

GRIA1 is one of the excitatory receptors in mammalian brains and is responsible for glutamate-mediated excitation of neural cells. It is important in memory formation and learning (14). Overactivation of glutamate receptors AMPA and NMDA is involved in the process of cell damage by increasing the intracellular Ca^{2+} concentration in neurons and activating the generation of free radicals, proteases, and phospholipases (16). These receptors are thought to be vulnerable to effects of Pb as Pb mimics calcium and activates PKC, this activation disturbing the signaling mechanisms (17–19). Studies indicate that Pb is accumulated in brain regions, especially in the hippocampus and cerebellum (20–22). In the hippocampus Pb affects Ca^{2+}-dependent cellular processes. Studies suggested the NMDA receptor as a target in lead-induced neurotoxicity (11,23). In the present study, we think that the finding of higher GRIA1 levels in the exposure group support the literature findings and GRIA1 levels may be thought to be a potential biomarker in lead neurotoxicity.

Table 1. Comparison of serum GRIA1, NSE, and S100B and routine chemical parameters of the lead exposure and control groups

	Lead exposure group (n = 25)	Controls (n = 25)	P
GRIA1 (pg/mL)	39.9 (33.5–64.5)	36.2 (32.0–51.2)	0.011*
NSE (ng/mL)	14.8 (10.6–35.4)	15.6 (4.9–30.0)	0.516
S100B (µg/L)	0.058 (0.026–0.160)	0.049 (0.027–0.135)	0.607
ALT (IU/L)	16 (3–91)	27 (11–52)	0.109
AST (IU/L)	18 (11–39)	21 (12–42)	0.662
Creatinine (mg/dL)	0.8 (0.6–1.3)	0.95 (0.77–1.25)	0.180

GRIA1, NSE, S100B, creatinine, ALT, and AST are expressed as median (min–max).
GRIA1: Glutamate receptor 1, NSE: neuron-specific enolase, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Table 2. Comparison of groups with and without neurological signs.

	Neurological signs (+) (n = 14)	Neurological signs (–) (n = 11)	P
GRIA1 (pg/mL)	41.9 (33.5–54.6)	39.4 (35.8–64.5)	0.584
NSE (ng/mL)	15.0 (10.6–35.4)	14.9 (11.0–22.0)	0.661
S100B (µg/L)	0.051 (0.026–0.160)	0.058 (0.042–0.079)	0.380
Lead (µg/dL)	36.3 (1.2–73.0)	29.4 (13.4–47.2)	0.228

GRIA1, NSE, S100B, and lead are expressed as median (min–max).
GRIA1: Glutamate receptor 1, NSE: neuron-specific enolase.

Table 3. Correlation between blood lead levels and other parameters.

Lead	r	P
GRIA1 (pg/mL)	−0.382	0.600
NSE (ng/mL)	0.107	0.611
S100B (µg/L)	−0.98	0.640
ALT (IU/L)	−0.074	0.726
AST (IU/L)	0.208	0.318
Creatinine (mg/dL)	−0.384	0.058

GRIA1: Glutamate receptor 1, NSE: neuron-specific enolase, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Figure. ROC curve for GRIA1.

However, there was no significant difference in the levels of GRIA1 between the lead-exposed groups with and without peripheral neurological signs and this result might be related to GRIA1 levels indicating Pb exposure in the CNS but not in the peripheral nervous system, as GRIA1 levels are known to have important roles in the CNS (13). Basu et al. also studied NMDA receptor levels in mink exposed to mercury, another heavy metal, and found decreased levels as a result of impaired glutamate homeostasis (24). In another study GRIA1 levels were investigated in cases of mercury exposure and were found to be higher than in the control group. High levels of GRIA1 were related to improvement of the neurological symptoms of exposed patients and it was suggested as a potential neurological biomarker in mercury-induced neurotoxicity (15).

On the other hand, no difference was found in terms of S100B and NSE levels between groups. Lead can cross the BBB by acting like calcium and accumulates in astroglial cells, disturbs immature astroglial cells, and blocks the formation of myelin sheath (2). During toxic conditions, glial cells undergo some changes like reactive gliosis. After glial reactions one of the most affected markers is S100B. S100B has two roles according to its levels. At nanomolar concentrations it acts as a neuroprotective factor and at micromolar concentrations it may act as a proinflammatory cytokine and promotes neuroinflammatory reactions and neuronal dysfunction (7). In our study we did not find a significant difference in the levels of S100B between lead-exposed workers and the control group. Marchi et al. measured S100B levels in patients with iatrogenic BBB disruption by intraarterial mannitol infusion and found that low levels of S100B were associated with BBB opening without CNS damage. High S100B release indicates synthesis and glial damage (25). Pleines et al. studied patients with traumatic brain injury (TBI) and found very low S100B levels in normal individuals without brain injury (22). We thought that
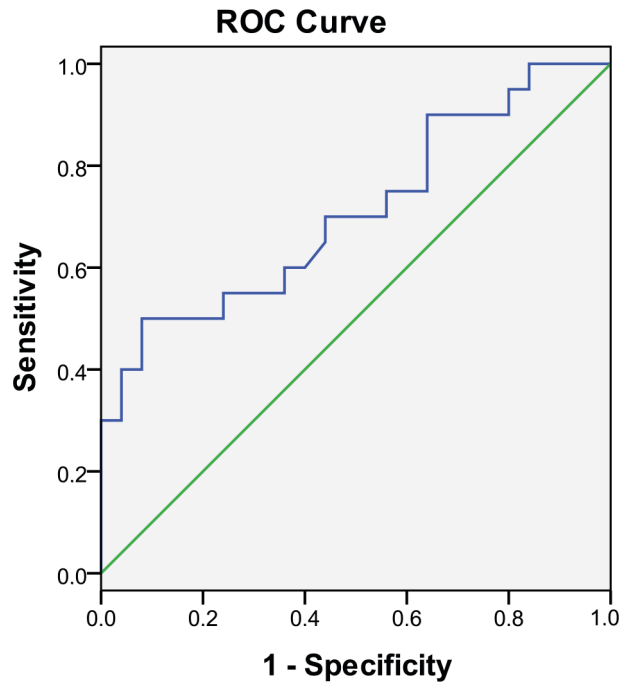
in our lead-exposed group there was not glial damage in the brain for S100B to act like a neuroprotective factor and we therefore observed low levels of S100B. Zheng et al. studied Pb-exposed workers and found that high blood Pb concentrations were associated with significantly lower sensory and motor conduction velocities in the median, ulnar, and peroneal nerves (20). In neurological examinations we saw peripheral neurological signs like pain and numbness of the hands or feet in 10 participants, but there was no significant difference between the two groups. Bilińska et al. electrophysiologically recorded the subclinical damage in the peripheral nervous system in their study with asymptomatic workers chronically exposed to inorganic Pb (9,21). In our study 9 of 14 participants who had positive neurological examinations and complaints had electromyography as well and all of them were found to be in normal ranges. S100B is usually found in glial cells of the CNS in the largest amounts and it increases with glial damage or activation. Therefore, a reason for S100B to be nonsignificant might be that all participants had peripheral neurological damage.

NSE is a glycolytic enzyme found in the cytoplasm of neurons and neuroendocrine cells and shows functional damage of neurons. Because of the high molecular weight of NSE, it cannot pass through membranes. It is released after cell destruction and increased concentrations can be found in peripheral blood or liquor (7,26). Yardan et al. studied S100B and NSE in patients with CO poisoning

and found no elevation in levels of these parameters in patients without loss of consciousness but significant elevation in unconscious patients (26). Gradisek et al. studied NSE and S100B in patients with TBI and found no differences in concentrations of these biomarkers between the group of survivors and patients who died because of non-TBI causes (27). We did not find any significant difference between lead exposure and control groups in terms of NSE levels. In another study it was indicated that protective features of the CNS prevent molecules from passing through the peripheral circulation. Therefore, it

is recommended to evaluate behavioral and molecular measurements with the results of biomarkers of exposure to neurotoxicants (28).

In conclusion, the results of our study showed that subjects with chronic lead exposure are found to have increased glutamate receptor levels and do not seem to have glial or neuronal damage, which can be demonstrated with the elevation of NSE and S100B levels. GRIA1 levels might be used as a biomarker for the neurotoxicity of lead. Further studies are needed to identify the mechanisms involved in the neurotoxicity of lead.

References

1. Gillis BS, Arbieva Z, Gavin IM. Analysis of lead toxicity in human cells. BMC Genomics 2012; 13: 344.

2. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates. Interdiscip Toxicol 2012; 5: 47-58.

3. Verstraeten SV, Aimo L, Oteiza PI. Aluminium and lead: molecular mechanisms of brain toxicity. Arch Toxicol 2008; 82: 789-802.

4. Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic effects and biomarkers of lead exposure: a review. Rev Environ Health 2009; 24: 15-45.

5. Shukla PK, Khanna VK, Khan MY, Srimal RC. Protective effect of curcumin against lead neurotoxicity in rat. Hum Exp Toxicol 2003; 22: 653-658.

6. Raunio S, Tahti H. Glutamate and calcium uptake in astrocytes after acute lead exposure. Chemosphere 2001; 44: 355-359.

7. Giacoppo S, Bramanti P, Barresi M, Celi D, Foti Cuzzola V, Palella E, Marino S. Predictive biomarkers of recovery in traumatic brain injury. Neurocrit Care 2012; 16: 470-477.

8. van Munster BC, Korse CM, de Rooij SE, Bonfrer JM, Zwinderman AH, Korevaar JC. Markers of cerebral damage during delirium in elderly patients with hip fracture. BMC Neurol 2009; 9: 21.

9. Santiago AR, Gaspar JM, Baptista FI, Cristovao AJ, Santos PF, Kamphuis W, Ambrosio AF. Diabetes changes the levels of ionotropic glutamate receptors in the rat retina. Mol Vis 2009; 15: 1620-1630.

10. Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. Pharmacol Rev 1999; 51: 7-61.

11. Nihei MK, Guilarte TR. Molecular changes in glutamatergic synapses induced by Pb^{2+}: association with deficits of LTP and spatial learning. Neurotoxicology 2001; 22: 635-643.

12. Bressler J, Kim KA, Chakraborti T, Goldstein G. Molecular mechanisms of lead neurotoxicity. Neurochem Res 1999; 24: 595-600.

13. Xu J, Yan HC, Yang B, Tong LS, Zou YX, Tian Y. Effects of lead exposure on hippocampal metabotropic glutamate receptor subtype 3 and 7 in developmental rats. Journal of Negative Results in Biomedicine. 2009; 8: 1-8.

14. Petroff OA. GABA and glutamate in the human brain. Neuroscientist 2002; 8: 562-573.

15. Yilmaz FM, Yilmaz H, Tutkun E, Uysal S, Carman KB, Dilber C, Ercan M. Serum biochemical markers of central nerve system damage in children with acute elemental mercury intoxication. Clin Toxicol (Phila) 2014; 52: 32-38.

16. Schreiber SS, Baudry M. Selective neuronal vulnerability in the hippocampus--a role for gene expression? Trends Neurosci 1995; 18: 446-451.

17. Nava-Ruiz C, Mendez-Armenta M, Rios C. Lead neurotoxicity: effects on brain nitric oxide synthase. J Mol Histol 2012; 43: 553-563.

18. Garza A, Vega R, Soto E. Cellular mechanisms of lead neurotoxicity. Med Sci Monit 2006; 12: 57-65.

19. Kim HC, Jang TW, Chae HJ, Choi WJ, Ha MN, Ye BJ, Kim BG, Jeon MJ, Kim SY, Hong YS. Evaluation and management of lead exposure. Annals of Occupational and Environmental Medicine 2015; 27: 30.

20. Villeda-Hernandez J, Barroso-Moguel R, Mendez-Armenta M, Nava-Ruiz C, Huerta-Romero R, Rios C. Enhanced brain regional lipid peroxidation in developing rats exposed to low level lead acetate. Brain Res Bull 2001; 55: 247-251.

21. Garcia-Arenas G, Ramirez-Amaya V, Balderas I, Sandoval J, Escobar ML, Rios C, Bermudez-Rattoni F. Cognitive deficits in adult rats by lead intoxication are related with regional specific inhibition of cNOS. Behav Brain Res 2004; 149: 49-59.

22. Nava-Ruiz C, Alcaraz-Zubeldia M, Mendez-Armenta M, Vergara P, Diaz-Ruiz A, Rios C. Nitric oxide synthase immunolocalization and expression in the rat hippocampus after sub-acute lead acetate exposure in rats. Exp Toxicol Pathol 2010; 62: 311-316.

23. Nihei MK, Desmond NL, McGlothan JL, Kuhlmann AC, Guilarte TR. N-methyl-D-aspartate receptor subunit changes are associated with lead-induced deficits of long-term potentiation and spatial learning. Neuroscience 2000; 99: 233-242.

24. Basu N, Scheuhammer AM, Rouvinen-Watt K, Grochowina N, Evans RD, O'Brien M, Chan HM. Decreased N-methyl-D-aspartic acid (NMDA) receptor levels are associated with mercury exposure in wild and captive mink. Neurotoxicology 2007; 28: 587-593.

25. Marchi N, Rasmussen P, Kapural M, Fazio V, Kight K, Mayberg MR, Kanner A, Ayumar B, Albensi B, Cavaglia M et al. Peripheral markers of brain damage and blood-brain barrier dysfunction. Restor Neurol Neurosci 2003; 21: 109-121.

26. Yardan T, Cevik Y, Donderici O, Kavalci C, Yilmaz FM, Yilmaz G, Vural K, Yuzbasioglu Y, Günaydın YK, Sezer AA. Elevated serum S100B protein and neuron-specific enolase levels in carbon monoxide poisoning. Am J Emerg Med 2009; 27: 838-842.

27. Gradisek P, Osredkar J, Korsic M, Kremzar B. Multiple indicators model of long-term mortality in traumatic brain injury. Brain Inj 2012; 26: 1472-1481.

28. Evans HL. Markers of neurotoxicity: from behavior to autoantibodies against brain proteins. Clin Chem 1995; 41: 1874-1881.