Evaluation Nitrotyrosine and Nitric Oxide Levels in the Blood of Acute Mercury Intoxication

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Abstract

Background: Several mechanisms shown in present data for identify the mercuric chloride's biologocal toxicity, and oxidative stress is one of those mechanisms. Some studies showed mercury have some effects on human body. Such as; bind to sulfhidril groups which on proteins, expose the reactive oxygen molecules and lead to formation of free oxygen radicals. Superoxide radical can lead to peroxidation of lipid membrans, changes in activities of proteins and antioxidant enzymes and gene transcription. The nitrotirozyne formed by conjoint of peroxinitrit and tirozyne residues of proteins and it can be used as a marker for evaluate potential cytotoxic effects of NO (Nitric Oxide). Although animal experiments carried out to date, there was no human series about mercury poisoning and effects of this toxicity on nitrotirozyne and NO in the literature as we evaluated in our study. With this purpose, we planned the identify nitrotirozyne and NO levels in serums of children who exposured to mercury.

Methods: 65 children who exposure mercury in school's laboratory from Gaziantep and Kahramanmaraş were included in the study. Those children had> 10 µg/l blood-mercury levels and >15 µg/l urine- mercury levels. 23 children who refer out pediatric clinic for another reasons enrolled in the study as control group.

Results: NO and nitrotirozyne levels found higher in mercury exposed children compared the control group.

Conclusıon: Markers of oxidative stress such as NO and nitrotirozyne increase at the acute mercury intoxication.

Introduction

Mercury is an element that can be found in air, water and soil; metallic (elemental) mercury is found in nature in the form of inorganic salts and organic compounds, and any form of it can have toxic effects.[1]

Free radicals are constantly formed in organisms and regularly eliminated by the antioxidant defense system. Oxidative stress occurs when the oxidative balance shifts towards free radicals due to insufficient antioxidant defense mechanism or increased free radical formation.[2,3]

Studies have shown that various metals such as mercury, lead, and cadmium cause cellular damage by forming free radicals and initiating oxidative stress in many tissues.[4,5]

The number of studies investigating the effects of acute mercury intoxication on the oxidative stress biomarkers NO and nitrotyrosine is very few and its effect on humans is not fully understood. Therefore, this study was conducted to investigate the effect of mercury on oxidative stress biomarkers in humans.

Materials And Methods

In this study, a total of 65 cases were evaluated as the patient group, which includes 42 girls and 23 boys. These cases were diagnosed with acute mercury poisoning and admitted to Kahramanmaraş Sütçü İmam University Faculty of Medicine, Pediatric Neurology Outpatient Clinic, and Gaziantep Pediatrics Hospital Pediatric Neurology Polyclinic between 20 February 2012 and 3 March 2012. In the control group, those who applied to the Pediatrics Outpatient Clinic with various complaints but did not have a history of intoxication and neurological findings. Blood samples taken from a total of 23 healthy volunteers (17 girls and 6 boys) due to different diagnoses were included in the study. In order to determine the blood and urine mercury levels, samples were taken from the cases who applied with complaints such as nausea, headache and skin rash and were known to have contact with mercury. Then because of the suspicion of mercury exposure, 24-hour urine and blood samples were collected into mercuryfree hypodermic tubes in compliance with previously described procedures. In addition, blood samples were also taken to investigate the relationship of acute mercury poisoning with blood Nitric Oxide and Nitrotyrosine levels at the same time. After the blood samples were centrifuged, their serums were collected and stored at -20°C. Before performing the level determination in the laboratory, blood samples kept at -20 C were allowed to dissolve at + 4 Cº. Nitric Oxide and Nitrotyrosine levels were studied in Kahramanmaraş Sütçü İmam University Faculty of Medicine Biochemistry Research Laboratory. During admission, blood spots were used to analyze serum mercury levels. The blood samples were centrifuged and the resultant serum samples were stored at − 20°C. Inductively- coupled mass spectrometry (ICP-MS) was used to analyze mercury levels in blood at Refik Saydam Hygiene Center National Poison Solidarity Center (Ankara, Turkey). Nitric oxide (NO) determination in serum was determined by spectrophotometric measurement of the color formed as a result of the reaction with nitrite sulfanilamide produced by Griess reaction and modified cadmium reaction and N-naphthylenediamine (NNDA) diazotization at 545 nm. Results were defined in μmol/L. 3-NT levels in serum samples were measured by double sandwich ELISA method. Patients with blood mercury levels above 10 μg/l and/or urine mercury levels above 15 μg/l were included in the study.

Statistical analysis

Statistical analyses were performed using SPSS20.0 software. Descriptive statistics were given as mean ± standard deviation (SD). The χ2-test, Wilcoxon test, and Student's t-test or Mann-Whitney U-test were used for comparison of categorical data between groups and for the comparison of continuous data, respectively. The confidence interval was set at 95% and statistical significance at $p \leq 0.05$. The independent samples t-test was used to compare the differences between the groups.

Results

Gender distribution of the people included in our study as follows; in the patient group with mercury poisoning, 42 (64.6%) case were girls and 23 (35.4%) cases were boys. While in the control group, 17 cases (73.9%) were female and 6 cases (26.1%) were male. There was no statistically significant difference between the two groups in terms of gender ($p = 0.415$)

Spearman correlation test was applied because the data did not fit the normal distribution. A statistically significant correlation was found between NO and Nitrotyrosine levels (p = < 0,01, r = 0,605). No correlation was found among others (NO and mercury p = 0,153, Nitrotyrosine and mercury p = 0,819) (Table 1).

When the mean blood NO levels in the patient and control groups were compared; The NO level in the patient group was 12,8420 ± 3.5968 (µmol/liter), while it was 5.6452 ± 2,3446 (µmol/liter) in the control group (Table 1). The difference between NO levels between the two groups was statistically significant (p = < 0.001).

When the mean blood Nitrotyrosine levels in the patient and control groups were compared; patient group was 1004.9 ± 129.7 nmol/lt, while in the control group was 707.1 ± 167.2 nmol/lt (Table 2). The difference between the two groups was statistically significant (p < 0.001).

In the patient group, the mean blood mercury level was 225,022 ± 543,1 µg/l in female patients and 122,139 ± 310,13 µg/l in male patients. However, no significant difference was found between the genders (p = 0,22). In the same group, the mean blood mercury level was 186,13 ± 414,06 µg/l in the children aged between 7 and 12 years and 191,03 ± 531,97 µg/l in the children aged between 13 and 19 years. No significant difference was found between the two age groups (p = 0,96). Similarly, mean NO level was 12,36 ± 3,20 (µmol/lt) in the 7–12 year age group and 13,30 ± 3,93(µmol/lt) in the 13–19 year age group. No significant difference was found between the two groups (p = 0.29) and mean Nitrotyrosine level was 976,65 ± 119,11 (nmol/lt) in the 7–12 year age group and 1032,42 ± 135,50 (nmol/lt) in the 13–19 year age group. No significant difference was found between the two groups (p = 0.83).

Discussion

Toxic metals such as mercury, aluminum and lead, initiate oxidative stress by forming reactive oxygen products (ROS). Many studies in adults have shown that mercury can contribute to the formation of free radicals and cause cellular damage by initiating oxidative stress in many tissues.[6] It has been suggested that mercury impairs cellular antioxidant defense mechanisms, especially by inhibition of key antioxidant enzymes or consumption of the intracellular antioxidant glutathione. Mercury causes this by increasing the production of ROS. Among the heavy metals, mercury is an important metal in terms of its pro-oxidant effect.[7,8]

Nitric oxide is a biological mediator involved in various physiological and pathophysiological processes that can be found in almost every part of the organism.[9] While other free radicals are harmful at any concentration, NO is involved in important physiological events at low concentrations. NO is a bidirectional molecule. It is prooxidant since it creates peroxynitrite-mediated lipid oxidation reactions in cells, and also an antioxidant with its capacity to prevent formation of lipid radical chains.[10] However, uncontrolled and excessive NO synthesis is harmful for cells. Thanks to these properties, NO becomes an ideal physiological messenger molecule.[11] NO reacts with the superoxide radical and turns into

peroxynitrite and plays an important role in cellular damage in high concentration.[12] Peroxynitrite forms 3-Nitrotyrosine by adding a nitro group to the phenolic ring of proteins or free form of tyrosine. This reaction can occur spontaneously, or it can be catalyzed by transition metals, SOD, CO2 and myeloperoxidase.[13–15] Since nitrotyrosine is the stable end product of peroxynitrite oxidation, measurement of nitrotyrosine has been reported to be a useful marker for detecting NO-dependent in vivo damage.[16] While in normal individuals 3-NT levels undetectable in plasma and tissues, it increases significantly in conditions associated with increased NO production and oxidative stress, such as inflammatory and degenerative processes. Although nitrotyrosine and nitric oxide are found in many cells, they have been an effective marker in showing brain damage in recent years. In one study, pregnant mice were exposed to mercury during fetal brain development and checked for nitrergic activity. It was observed that nitrogenous activity increased in the moleculer layer of Dentate Gyrus, Stratum Lacunosum Moleculare, and the Stratum Radiatum.[17] High levels of 3-nitrotyrosine have been reported in Atherosclerosis, Multiple Sclerosis, Alzheimer's disease, Parkinson's disease and animal models, cystic fibrosis, asthma, lung diseases, myocardial failure, stroke, amyotrophic lateral sclerosis, chronic hepatitis C, cirrhosis, and diabetes.[18,19]

In a study conducted by Sumathi et al.[20], they gave methyl mercury orally to male visar rats for 21 days. The rats in the control group were given orally Bacopa Maniere extracts (a plant used as a neuroprotective in alternative medicine in India) in addition to methyl mercury. After the applications, they determined that the erythrocyte SOD, CAT and GPx activities were significantly reduced in the group given only methyl mercury. They found that NO2 - and NO3 - levels increased after methyl mercury administration (they thought that these metabolites are a marker of free radical damage and NO production could be effective in oxidative damage), while in the group given Bacopa Maniere extracts together with methyl mercury, oxidative damage associated with methyl mercury in the brain decreased.

In a study by Moneim[21], it was investigated whether berberine plant is protective in neurotoxicity and oxidative damage caused by mercury. Adult male albino rats were injected with HgCl2 for 7 days. In this study, oxidative stress occurred by increasing NO production and decreasing antioxidant enzymes after mercury exposure. Conversely in those given beriberine plant before exposure, glutathione increased and NO and lipid peroxidation decreased.

In a study by Karapehlivan[22] et al. investigated the protective effect of omega-3 fatty acids on HgCl2 toxicity in mice. In this study, Malondialdehyde (MDA) levels, glutathione, nitric oxide (NO) and total sialic acid (TSA) levels were examined and histopathological changes in selected organs were examined. 28 mice were divided equally into 4 groups. 1. Group; Intraperitoneal saline injected. Group 2; 0.4 mg / kg / day intraperitoneal mercury chloride injected. Group 3; 0.4 mg / kg / day mercury chloride intraperitoneally and simultaneously subcutaneously 0.5 g / kg / day, omega-3 fatty acid applied. Group 4; only 0.5 g/kg/day omega-3 fatty acid applied. In this study, all applications continued for 7 days. Compared to Group 1, MDA, NO and TSA levels were found to be higher in group 2 and lower in group 3 and 4. The highest GSH level was found in Group 4. Histopathologically, severe degeneration of liver and

kidney was observed in Group II animals. In conclusion, with this study Karapehlivan et al. showed that omega 3 fatty acid can reduce mercury chloride-induced toxicity by improving the antioxidant system and acute phase response in mice.

Durak[23] et al. evaluated MDA and SOD, CAT and GPx activities by incubating erythrocytes which obtained from healthy male volunteers, for 60 minutes at 37°C in 3 separate groups. First with only HgCl2, second with only vitamins (vitamin A and E) and lastly both HgCl2 and vitamins. While MDA levels increased in erythrocytes incubated with HgCl2 alone, SOD, CAT and GPx activities were found to decrease ($P < 0.05$).

In the literature that we can find, it has been shown that NO and Nitrotyrosine levels increase in almost all of the studies showing the relationship between exposure to mercury and NO, Nitrotyrosine levels. In a study by Kim et al[24], they treated rat macrophage cells with mercury, in the presence and absence of lipopolysaccharide, and they found that low-dose mercury reduces lipopolysaccharide-induced NO production in mouse macrophages and may impair immunity by reducing host defense cells. However, in this study by Kim et al., the NO production induced by lipopolysaccharide in macrophages with mercury exposure was examined, the NO levels in the blood is not studied. In our study, the effect of mercury toxicity on NO and Nitrotyrosine, which are blood oxidative stress biomarkers, is investigated in children. In our literature review, we found that the relationship between mercury toxicity and oxidative stress biomarkers was mostly shown in animal studies. Only handfull studies were conducted in humans. We found that NO and Nitrotyrosine levels increased in children diagnosed with mercury intoxication, and we thought that the application of antioxidant therapy to these children in the acute period can reduce oxidative stress.

Our study included 65 patients diagnosed with mercury poisoning as the patient group and 23 outpatients without a history of mercury exposure as the control group. In our study, the effect of mercury toxicity on NO and Nitrotyrosine, which are blood oxidative stress biomarkers, is evaluated in children. In our literature review, we found that the relationship between mercury toxicity and oxidative stress biomarkers has been shown mostly in animal studies, and only a few studies have been conducted in humans. We found that NO and Nitrotyrosine levels increased in children diagnosed with mercury intoxication. Based on our study, we thought that oxidative stress can be reduced by applying antioxidant therapy to these children in the acute period, in addition to chelator therapy in mercury intoxication.

Table 1
Correlations mercury exposure and NO levels

			NO level	Nitrotyrosine level	Mercury level
Spearman's rho	NO_level	Correlation Coefficient	1,000	,605**	,179
		Sig. (2-tailed)	.	,000	,153
		N	88	88	65
	Nitrotyrosine level	Correlation Coefficient	,605**	1,000	,029
		Sig. (2-tailed)	,000	.	,819
		N	88	88	65
	Mercury level	Correlation Coefficient	,179	,029	1,000
		Sig. (2-tailed)	,153	,819	.
		N	65	65	65
**. Correlation is significant at the 0.01 level (2-tailed).					

Table 2
NO levels in the patient and control groups.

NO (µmol/lt)			
Group	Mean ± sd	min- max	P
Control group (n = 23)	5,6452 ± 2,3446	1,68 – 10,32	< 0,001
Patient (n = 65)	12,8420 ± 3,5968	7,20–20,40	
*Statistically significant value: (p < 0.05). sd = standard deviation.			

Table 3
Nitrotyrosine levels in the patient and control groups.

Nitrotyrosine (nmol/lt)			
Group	Mean ± sd	min- max	p
Control group (n = 23)	707,1335 ± 167,2	438,90–996,40	< 0,001
Patient (n = 65)	1004,9655 ± 129,78	641,30-1436,30	
*Statistically significant value: (p < 0.05). sd = standard deviation.			

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