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Original Article

Evaluation of Oxidative Stress Biomarkers in Acute Mercury Intoxication

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Abstract

Introduction: Very few studies have evaluated the association between mercury exposure and oxidative stress in humans, particularly in children.

Aim: This is the first report where we aimed to determine the oxidative stress status of children who were accidentally exposed to elemental mercury.

Materials and methods: In the present study, the study group was composed of 86 randomly selected children poisoned by mercury; the control group was composed of 78 children who had no history of mercury exposure. At admission, blood samples were collected. Blood superoxide dismutase activity, catalase enzyme activity, and glutathione peroxidase activity were measured by Fridovich, Beutler, and Lawrence Burk methods respectively, and the results were given as U/g Hb. Malondialdehyde level was measured by Ohkawa methods, and the results were given as mmol/ml.

Results: Catalase activity was significantly lower in the patient group compared to the control group (1.28±0.62 vs. 3.90±0.86 U/g Hb, p=0.010). In exposed children, SOD activity was significantly higher than the controls (5936±810 vs. 2226±464 U/g Hb, p=0.03), while the GSH-Px activity was significantly lower (13.01±3.21 vs. 34.97±7.32 U/g Hb, p=0.013). The MDA levels of the mercury group were significantly higher than the MDA levels of the control group (2.85±0.84 vs. 2.05±0.79 mmol/ml, p=0.04).

Conclusions: The results of the present study showed that acute mercury poisoning causes an alteration of oxidative stress status in children exposed to elemental mercury.

Keywords

catalase, glutathione peroxidase, intoxication, malondialdehyde, mercury, superoxide dismutase

INTRODUCTION

Mercury (Hg) is a toxic heavy metal that can be classified into three groups; organic, inorganic, and elemental (metallic mercury).[1] Elemental mercury can evaporate at room temperature. The vapour can be rapidly absorbed from the lungs and distributed to the central nervous system. Elemental mercury exposure occurs frequently and is potentially toxic, especially in children. It is easily absorbed from the skin. Unprotected touch to mercury can lead to serious poisoning. Elemental mercury crosses the blood-brain barrier[2] and accumulates in the brain tissue. Elemental mercury is very attractive to children because of its bright, bullet-like appearance and the tendency to create beads.[3] Clinical manifestations of mercury intoxication vary depending on its form, concentration, route of ingestion, and duration of exposure.[1,4,5] Although there is important literature that describes the clinical outcome of acute mercury poisoning, only a small number of studies have examined the possible mechanism of mercury toxicity.[2,6] Excessive reactive oxygen species (ROS) adversely affect proteins, lipids, carbohydrates, and nucleic acids (DNA, RNA). The role of RNAs, especially the noncoding ones, plays a pathogenic role in the process of oxidative stress.[7] Antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GR) control the production and amount of ROS to maintain an appropriate cellular redox balance. High ROS levels and/or reduced antioxidant levels lead to oxidative stress by altering the redox balance of the cell.[8] Previous in vivo and in vitro studies have shown that exposure to mercury leads to an increase in ROS. Increased ROS has been suggested to inhibit antioxidant enzymes (SOD, CAT, GSH-Px), depletion of glutathione (GSH), and oxidative stress in biological systems due to the formation of reduced sulfhydryl groups (-SH).[5,8,9] Nevertheless, very few studies have evaluated the association between Hg exposure and oxidative stress in humans, particularly in children.[1,10-12]

AIM

We aimed to determine the oxidative stress status of children who were accidentally exposed to elemental mercury.

MATERIALS AND METHODS

In February 2012, accidents involving mercury contact occurred in two different schools and provinces of Turkey. All children patients who were exposed to elemental mercury were referred to the hospital. Children whose serum mercury levels were measured and mercury poisoning was confirmed were included in the study. The control group consisted of healthy sex- and age-matched subjects from our Pediatric Primary Care Center. Healthy status was determined through the subjects' medical history and either a parental report or self-report to rule out the presence of chronic or acute diseases. Upon admission, a questionnaire to assess any clinical symptoms related to mercury exposure was given, and physical examination was performed. To promote urinary excretion of mercury, patients received the oral therapy [D-penicillamine (50 mg/kg per day divided into four doses) or with 2,3-Dimercaptopropanesulfonic acid (DMPS) (20 mg/kg per day divided into four doses)] available at the Toxicology Center. DMPS is the drug of choice for patients with neurological symptoms. N-acetylcysteine (15 mg/kg per day) was added to the treatment regimen of some patients. To assess mercury exposure, 24-h urine samples were collected daily, and mercury levels greater than 10 μg/L were considered toxic. The criteria for discharge were mercury levels lower than 10 μg/L in 24-h urine samples and being asymptomatic.

At admission, blood samples were collected. All samples were stored at +4°C until biochemical analysis. Urinary mercury levels were studied by inductively coupled plasma-mass spectrometry (ICP-MS) method. Blood SOD activity, CAT activity, and GSH-Px activity were measured by Fridovich, Beutler, and Lawrence Burk methods, respectively, and the results were given as U/g Hb.[13-15] MDA level was measured by Ohkawa methods, and the results were given as mmol/ml.[16] The research was approved by the local ethical committee (2012/09-2).

Statistical analysis

The data were transferred to the computer environment. SPSS v.20 software package was used for all statistical analyses. Student's t-test was used to compare the means of two samples. Mann Whitney-U test, a non-parametric method, was used to compare the mean of two independent samples without normal distribution. The data were expressed as mean ± SD. The level of significance was set at $p<0.05$.

RESULTS

The study group was composed of 86 patients (M/F=37/49) with a mean age of 11.1±1.7 years. The control group was composed of 78 healthy children (M/F=32/46) with the mean age of 10.4±1.3 years. There was no statistically significant difference between the two groups concerning sex and mean age. Seventeen children had both contacted and played with mercury and inhaled its vapour, while 62 children had only inhaled mercury vapour. Six children had only contacted mercury, and only one child reported having tasted mercury. The mean duration of exposure was 13.0±3.2 (min 10, max 20) minutes. Twenty-nine children were asymptomatic. In symptomatic patients headache was the most common presenting complaint. The results

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of neurological and physical examinations were normal in 34 children (39.5%). Mid-dilated/dilated pupils were the most common neurological abnormality, and this sign was present in 52 children (60.5%) **(Table 1)**. The mean urinary mercury concentration was measured as 42.60 µg/L (min: 2.10, max: 2366.0).

There was no significant difference between the groups in mean of hemoglobin (11.6±3.2 vs. 12.4±2.5, p=0.73) WBC ($6.1\times10^3\pm1.7103$ vs. $5.6\times10^3\pm1.4\times10^3$, p=0.58), PLT ($188.8\times10^3\pm22.20\times10^3$ vs. $167.55103\pm21.3\times10^3$, p=0.61), urea (36.2±3.6 vs. 30.8±4.2, p=0.72) creatinine (0.57±0.04 vs. 0.49±0.03, p=0.61), AST (36.2±3.4 vs. 31.4±4.4, p=0.69), ALT (37.1±2.7 vs. 34.1±1.9, p=0.73). CAT levels were significantly lower in the patient group compared to the control group (1.28±0.62 vs. 3.90±0.86 U/g Hb, p=0.010). In exposed children, SOD activity were significantly higher than in the controls (5936±810 vs. 2226±464 U/g Hb, p=0.03), while the GSH-px activity was significantly lower (13.01±3.21 vs. 34.97±7.32 U/g Hb, p=0.013). MDA levels of the mercury group were significantly higher than the MDA levels of the control group (2.85±0.84 vs. 2.05±0.79 mmol/ml, p=0.04) **(Table 2)**.

Table 1. Demographic data of patients and control groups, distribution of signs and symptoms

	Patient (n=86)	Control (n=78)
Sex (male), n (%)	37 (43)	32 (41)
Mean age, (years)	11.1±1.7	10.4±1.3
Route of exposure		
Dermal contact, n (%)	6 (6.9)	
Inhalation, n (%)	62 (72.1)	
Dermal contact + inhalation, n (%)	17 (19.7)	
Oral, n (%)	1 (1.1)	
Neurological examination		
Normal, n (%)	34 (39.5)	
Mild dilated pupil, n (%)	52 (60.5)	
Peripheral neuropathy, n (%)	4 (4.6)	

DISCUSSION

Antioxidant defence systems of the body include enzymatic and nonenzymatic mechanisms. SOD, CAT and GSH-Px are important enzymatic antioxidant enzymes. SOD is a major antioxidant enzyme that catalyses hydrogen peroxidation of superoxide radicals.[17,18] The most important task of CAT is to remove toxic H_2O_2 from cells. GSH-Px provides considerable protection against free oxygen radicals, peroxides, and carcinogens.[19] Under normal conditions, there is a balance between the formation and elimination of reactive oxygen species. When the balance is impaired in favour of ROS, oxidative stress occurs. The structures which are affected by ROS are lipids, proteins, carbohydrates, nucleic acids. As a result, oxidative damage and oxidative stress occurs. Moreover, MDA is the most important marker of oxidative stress.[20] Although several in vivo and in vitro studies have suggested that exposure of animals to inorganic or organic forms of mercury is associated with the induction of oxidative stress, human studies are rare in the literature.[21-23] In the present study, we found altered oxidative stress status in children exposed to elemental mercury, which was characterized by decreased CAT, GSH-Px activities, and increased MDA level and SOD activity, compared to the controls. GSH-Px is an important selenium (Se) dependent enzyme which can reduce hydroperoxides. Since GSH-Px is a Se-dependent enzyme, reduced GSH-Px activity may be explained by the formation of a complex between Se-Hg in the active site of the enzyme. Mercury can also modify the tertiary and quaternary structures of GSH-Px, thereby diminishing the enzyme activity.[24] On the other hand, Abdel-Hamid et al. reported that mercury directly inhibits CAT activity. Our result also supports this finding.[25] MDA is the product of the decomposition of large chain reactions leading to oxidation of polyunsaturated fatty acids and

Table 2. Comparisons of blood laboratory parameters and CAT, SOD, GSH-Px and MDA levels between groups

	Patient (n=86) Mean ± SD	Control (n=78) Mean ± SD	p
Hemoglobin (gr/dl)	11.6±3.2	12.4±2.5	0.73
WBC (cell/µL)	$6.1\times10^3\pm1.710^3$	$5.6\times10^3\pm1.4\times10^3$	0.58
PLT (cell/µL)	$188.8\times10^3\pm22.20\times10^3$	$167.5510^3\pm21.3\times10^3$	0.61
Urea (mg/dl)	36.2±3.6	30.8±4.2	0.72
Creatinine (mg/dl)	0.57±0.04	0.49±0.03	0.61
AST (U/L)	36.2±3.4	31.4±4.4	0.69
ALT (U/L)	37.1±2.7	34.1±1.9	0.73
CAT (U/g Hb)	1.28±0.62	3.90±0.86	0.010
SOD (U/g Hb)	5936±810	2226±464	0.03
GSH-Px (U/g Hb)	13.01±3.21	34.97±7.32	0.013
MDA (mmol/ml)	2.85±0.84	2.05±0.79	0.04

is an important parameter of oxidative stress.[26] Our results showed that MDA levels were higher in mercury exposed children compared to the controls. In the present study, the patients were admitted to the hospital within two weeks period and the blood samples for analysis were collected at admission. The duration of exposure of patients was variable. Both of these two factors may have affected the study results. Grott et al. investigated the relationship between mercury exposure and oxidative stress in the communities of the Brazilian Amazon; their findings showed decreased CAT and GSH-Px activities.[6] Samir and Aref reported that the GSH-Px and SOD activities in blood were significantly decreased in exposed dental personnel compared with controls.[27] Pinheiro et al. reported increased glutathione levels and decreased CAT activity in women residing in the Amazon and suggested that increased glutathione may have been a response to oxidative stress. At this point, it is speculated that the increased SOD activity revealed in the present research is also a response to oxidative stress.[28]

Al-Saleh et al. investigated the effect of mercury dental amalgam fillings on renal and oxidative stress; they reported that dental amalgam fillings affected kidney tubular functions in children. In our study none of the children suffered from renal dysfunction.[29]

The most frequent presenting complaint of these patients was headache, with mid-dilated/dilated pupils being the most common neurological abnormality. The central nervous system is one of the main targets of mercury accumulation and damage. Whether the primary cellular target of mercury is astrocytes or neurons, mercury can disturb cellular function through various mechanisms. In astrocytes, mercury inhibits the uptake of the excitatory neurotransmitters glutamate and aspartate. Mercury further increases the extracellular levels of these amino acids by inducing their release from intracellular stores. Such an increase in extracellular glutamate and aspartate can cause the N-methyl-d-aspartate receptors to overactivate, which could cause the cell to enter into an excitotoxic cascade.[5]

The present study has several limitations. First of all, the age range of the participants was not wide to interpret the results for all pediatric age groups. Secondly, the severity of the toxicity, length of hospital stays, and the need for treatment were not evaluated.

Another point to consider is that we should be aware that if the urine mercury concentration exceeds 100 μg/L, neurological symptoms may develop and the level of 800 μg/L or above can be fatal.[30]

CONCLUSIONS

Mercury could be toxic when inhaled, ingested, or absorbed through skin. Mercury toxicity involves the production of reactive oxygen species that in return damage lipids in membranes, proteins, or enzymes. The results of the present study showed that acute mercury poisoning causes alteration of oxidative stress status in children exposed to elemental mercury.

Compliance with the ethical standards

All human studies were approved by the appropriate Ethics Committee and were therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All persons gave their informed consent before their inclusion in the study.

Conflict of Interest

Authors have no conflict of interest to declare.

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Оценка биомаркеров окислительного стресса при острой интоксикации ртутью

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Резюме

Введение: В очень немногих исследованиях оценивалась взаимосвязь между воздействием ртути и окислительным стрессом у людей, особенно у детей.

Цель: Это первый отчёт, в котором нашей целью было определить состояние окислительного стресса у детей, случайно подвергшихся воздействию элементарной ртути.

Материалы и методы: В данном исследовании исследуемая группа состояла из 86 детей, отобранных случайным образом и отравленных ртутью; контрольную группу составили 78 детей без воздействия ртути в анамнезе. Образцы крови были взяты при поступлении. Активность супероксиддимутазы (СОД) в крови, активность фермента каталазы и активность глутатионпероксидазы (GSH-Px) измеряли методами Фридовича, Бейтлера и Лоуренса Берка, соответственно, и результаты были представлены как U/g Hb. Уровни малонового диальдегида (МДА) измеряли по методам Окавы и результаты были представлены в mmol/ml.

Результаты: Активность каталазы была значительно ниже в группе пациентов по сравнению с контрольной группой (1.28±0.62 против 3.90±0.86 U/g Hb, p=0.010). У облученных детей активность СОД была значительно выше, чем в контроле (5936±810 против 2226±464 U/g Hb, p=0.03), в то время как активность GSH-Px была значительно ниже (13.01±3.21 против 34.97±7.32 U/g Hb, p=0.013). Уровни МДА в группе, подвергшейся воздействию ртути, были значительно выше, чем уровни МДА в контрольной группе (2.85±0.84 против 2.05±0, mmol/ml, p=0.04).

Заключение: Результаты настоящего исследования показывают, что острое отравление ртутью вызывает изменение статуса окислительного стресса у детей, подвергшихся воздействию элементарной ртути.

Ключевые слова

каталаза, глутатионпероксидаза, интоксикация, малоновый диальдегид, ртуть, супероксиддисмутаза