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Impact of open manganese mines on the health of children dwelling in the surrounding area

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Introduction: Chronic manganese (Mn) exposure is a health hazard associated with the mining and processing of Mn ores. Children living in an area with increased environmental exposure to Mn may have symptoms of chronic toxicity that are different from adults who experience occupational exposure. The aim of the study was to compare health outcomes in a pediatric population living near open Mn mines with a group of children from a reference area and then to develop and implement preventive/rehabilitation measures to protect the children in the mining region.

Methods: After environmental assessment, a group of 683 children living in a Mn-rich region of Ukraine were screened by clinical evaluation, detection of sIgA (37 children), micronucleus analysis (56 children), and hair Mn content (166 children).

Results: Impaired growth and rickets-like skeletal deformities were observed in 33% of the children. This was a significantly higher percentage than in children in the reference region (15%). The children from the Mn-mining region also had increased salivary levels of immunoglobulin A (104.4 $\pm$ 14.2 mcg/ml vs. 49.7 $\pm$ 6.1 mcg/ml) among the controls ($p < 0.05$), increased serum alpha 1 proteinase inhibitor levels (4.93 $\pm$ 0.21 g/l compared with 2.91 $\pm$ 0.22 g/l for controls; $p < 0.001$) and greater numbers of micronuclei in the mucous cells of the oral cavity (0.070 $\pm$ 0.008 vs. 0.012 $\pm$ 0.009, $p < 0.001$).

Conclusions: These findings indicate the deleterious health consequences of living in a Mn-mining area. Medical rehabilitation programs were conducted and produced positive results, but further validation of their effectiveness is required. The study provided background information to formulate evidence-based decisions about public health in a region of high Mn exposure.

Keywords: *manganese*; *manganese exposure*; *children*; *chronic manganese toxicity*; *manganese mining*

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Manganese is one of the most abundant elements in the earth's crust and is widely distributed in soils, sediments, rocks, water, and biological materials (1, 2). It is an essential trace element for all known living organisms. Mn (II) ions function as cofactors for a number of enzymes in higher organisms. Mn is necessary for the formation of connective tissue and bone, for growth, embryonic development, and reproductive functions.

The major sources of man-made environmental Mn pollution arise from mining operations (3) and the manufacture of steel (4, 5). According to World Health Organization data, the daily intake of airborne Mn by the general population in areas without Mn-emitting industries is lower than 2 μg/day. In areas with major foundry facilities, intake may rise to 4–6 μg/day, and in areas associated with ferro- or silico-manganese industries, it may be as high as 10 μg/day, with peak values exceeding 200 μg/day (1).

Chronic Mn poisoning is a recognized health hazard associated with the mining and processing of Mn ores (6–9). Although the largest source of human exposure to Mn is via water and food, additional exposure may occur through air polluted with Mn emissions (1). Mn poisoning has been linked to impaired motor skills, cognitive disorders, and metabolic processes (10–15). Environmental

Mn pollution and its long-term effect on the health of the pediatric population living in Mn-mining areas is a major concern.

The hypothesis of our study was that children living in an area with increased environmental exposure to Mn will have symptoms of chronic Mn toxicity. The specific aims were: (1) to evaluate the environmental load of Mn in Ukrainian areas located near Mn mines, (2) to assess the risk of environmental Mn pollution on the health of the pediatric population living nearby, and (3) to develop and implement medical rehabilitation measures to protect children in the region. We also aimed to provide background information for health care authorities that would allow them to formulate evidence-based decisions about public health in the region.

Methods

Data were obtained from a sample of children residing in a Ukrainian region where Mn mining operations were active (near the cities Ordzhonikidze and Marhanets of Dnipropetrovsk [DNK] Oblast). These data were compared with records of children living in the territory of Novotroitskoye in the DNK region, an environmentally neutral region that served as a control, where the medical and health facilities are located. The children enrolled in the study were all Caucasian with similar socioeconomic status. They had similar sunlight exposure. They spent at least 8 hours each day, 5 days per week at daycare facilities, and during this time had at least two similar meals a day. The study was approved by the Research Board Committee at Dnipropetrovsk State Medical Academy.

Environmental assessment

Soil samples collected near a Mn ore agglomeration plant and a waste depository were analyzed at the Ecotoxicology Laboratory of DNK State Agrarian University. Soil was collected at depths of 0–10 cm and at distances of 0.1–0.2, 0.5, and 1.5–2 km from the ore agglomeration plant and the waste depository in the direction of the predominant winds in the area. Each soil specimen was formed by combining at least five samples of soil collected from different spots at the designated plots. Soil specimens were mixed and quartered. One kilogram specimens were prepared for chemical analyses using heavy metal extraction in ammonium acetate buffer (pH = 4.8, adjusted with 1N HCl). Atomic absorption spectrophotometry was used to detect metal and metalloid concentrations in the soil samples (16, 17). The same atomic absorption technique was used to measure the content of heavy metals in hair samples of 166 randomly selected children from the Mn area and 56 control children from the reference region. Parents were asked to wash the hair of their child the night before sampling. Hair was cut from the back of the head (occipital area), using stainless steel scissors, as close to the scalp as possible. When possible, 2 cm of hair closest to the scalp was used for testing. Children with short hair supplied less than a 2 cm length, but still a sufficient sample for analysis.

Clinical evaluation

Case data were collected from 683 preschool children (5–7 years old) living in the Mn mining region. The control data were collected from 56 children of the same age from the reference area. The children's previous medical histories were reviewed from the medical charts kept by the daycare facilities. Physical examinations were performed by researchers who travelled to the daycare facilities. Respiratory function was tested by a peak flow meter in the 166 children randomly selected for hair analysis (18). Children with chronic medical conditions were excluded from the study. The inclusion criteria were that each child was considered 'healthy', had lived in the area at least 3 years, and attended one of the randomly selected daycare facilities. The study areas had very low population migration rates.

Sample collection and detection of secretory immunoglobulin A (sIgA)

Saliva (1.5–2.0 ml) was collected from the anterior floor of the mouth with a sterilized syringe, placed in an airtight test tube, and transferred to the laboratory on ice. The samples were centrifuged at 3,000 rpm for 10 min. The supernatant liquid was diluted 10 times with normal saline and analyzed for sIgA using an enzyme immunoassay system (19). The test was performed on 37 children from one randomly selected daycare center from the Mn area and all 56 control children. The cost of analyses and limited budget determined the number of tests that could be performed.

Alpha-1 proteinase inhibitor (A1PI)

Serum samples (finger-pricks) were analyzed for A1PI using a semiquantitative procedure (20). The test was performed on the same children selected for sIgA analysis.

Cytogenetic monitoring

A micronucleus (mNUC) analysis in buccal cells for cytogenetic monitoring was employed to estimate the general mutagen background in a randomly selected group of 56 children from the mining region and 23 children from the control region. This test was used to screen environmental mutagens as previously described (21). At least 1,000 cells per child were analyzed.

Rehabilitation/prevention measures

The rehabilitation program tested in the mining region included the combined oral administration of humic

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substances (humics), carotene oil (pro-vitamin A), enterosorbents (pectin), and probiotics (acidophilus). All were officially approved and previously tested for food supplements (21, 22). A humic food additive in the form of a 0.05% solution of humic acid was used for 21 days according to instructions from the Pharmaceutical Committee of the Ukraine Ministry of Health. Treatment was provided for 2 months to the 37 children in the Mn mining area who were previously included in the hair, sIgA, and A1PI testing. All food supplement dosage levels were age-appropriate. Reassessments were conducted at the end of the 2-month treatment period. The children's legal guardians all gave verbal informed consent to allow their children to participate in the study.

Statistical analysis

Means and standard error of the mean were determined for each study variable. Results with *p*-values less than or equal to 0.05 were considered statistically significant. When the means of two independent samples (exposed vs. reference groups) were compared, unpaired *t*-tests were used. Paired *t*-tests were used to analyze values for the exposed group before and after rehabilitation measures were implemented, and to determine whether there were differences associated with the treatment. The simultaneous analysis of two variables was performed; correlations between hair concentration of Mn and various physical measures such as weight, height, BMI, and hyperplasia of the thyroid gland were also investigated.

Results

Environmental contamination

The distribution of Mn, Zn, and Cu in soil samples taken near the Mn ore agglomeration plant and Mn ore waste depository is shown in Tables 1 and 2. Both Zn and Cu were selected as indicators for comparative assessment. The black soil of the steppe was used as a measure of the Mn control level and averaged 220 mg Mn/kg soil.

Clinical findings

Among the 683 children in the Mn region, 53% had disproportional growth (deviation from normal height: weight charts), and 33% had rickets-like skeletal deformities versus 15% in the control area. Bowed legs, abnormal curvature of the spine, pelvic deformities, and breastbone projections were the most frequently observed skeletal abnormalities. Growth and developmental problems were significantly more common in the Mn mining area compared with the control region ($p < 0.05$). These findings correlated ($r = 0.41$) with Mn levels in the hair of the affected children. The degree of detectable hyperplasia of the thyroid gland, as determined by physical examination, also correlated with the level of Mn detected in hair ($r = 0.57$). The average amount of Mn in hair of children living in the contaminated region was 3.66±0.33 mcg/g versus 1.84±0.52 mcg/g for children in the control region ($p < 0.01$).

The chart review revealed that 53% of the mothers of the children enrolled in the Mn-affected area had chronic diseases, 46.5% of which involved a complicated pregnancy and/or delivery. These percentages were significantly higher than those observed in the control region ($p < 0.01$).

The children from the Mn mining region had a mean sIgA of 104.4±14.2 mcg/ml versus 49.7±6.1 mcg/ml among the control children ($p < 0.05$). Serum A1PI was also significantly higher in the Mn-mining region, at 4.93±0.21 g/l compared with 2.91±0.22 g/l for controls ($p < 0.001$). Although the average level of A1PI was higher among the group living in the Mn-affected area, 11% of the children in this group displayed A1PI levels below the average for the control children. Children with immunologic disturbances had peak flow meter readings 20% lower than the group average.

Cytogenetic data

The mean level of mNUC in the cells of children residing in the Mn-affected region was 5.8 times higher than that of the controls (Table 3).

Effects of rehabilitation

An antimutagen effect was observed in 87.5% of cases. The number of cells with mNUC decreased from 0.070±0.008 to 0.036±0.006 ($p < 0.01$). Following 2 months of rehabilitation treatment, the concentration of Mn decreased significantly in the hair of children living in the contaminated region—from 3.66±0.32 mcg/g to 2.68±0.35 mcg/g ($p < 0.05$). The level of A1PI also decreased from 4.93±0.21 g/l to 2.23±0.41 g/l ($p < 0.01$). The sIgA rose further, to 187.3±28.3 mcg/ml ($p < 0.05$), following the rehabilitation. At the end of the 2-month treatment period we did not observe any noteworthy changes in growth and/or skeletal deformities.

Discussion

Occupational exposure to Mn is characterized by contact with high concentrations of Mn in air for short periods of time. Chronic environmental exposure to Mn may consist

Table 1. Mn, Zn, and Cu distribution in soil samples taken near manganese ore agglomeration plant, (M±SEM), mg/kg

Distance from the ore agglomeration plant, m	Mn	Zn	Cu
100	535±12	8.9±0.8	7.6±0.1
500	900±19	9.2±0.5	7.6±0.2
1,500	535±15	8.5±0.6	7.9±0.2

Table 2. Mn, Zn, and Cu spreading in upper layer (0–10 cm) of arable lands neighboring manganese ore slime depository, (M ± SEM), mg/kg

Substrate	Location of sampling	Mn	Zn	Cu
Slime of processed manganese ore	Waste depository	700 ± 23	11.0 ± 2.2	11.3 ± 0.3
Black soil	200 m from depository	467 ± 10	22.2 ± 1.2	14.3 ± 0.2
	2 km from depository	427 ± 5	19.5 ± 1.0	14.3 ± 0.4

of low Mn concentrations in air over long periods, but food grown in Mn-rich soil can also be a major source of Mn for human populations (1).

The population evaluated in this study has a low migration rate, which led us to hypothesise that long-term, low-dose Mn exposure might produce observable effects on the growth and development of children living in the area. We suspected that children might be exposed to Mn through different routes such as food, water, and air. The level of Mn contamination in the study area was significantly higher than the control area. Reclamation of abandoned mined land in the region is critically important in order to reduce exposure levels for the local population (23, 24). The abandoned mined areas lower the water table and reduce crop yields in the areas surrounding the mines. Approximately 9,000 ha of mined land have been covered with soil, but most of these areas were inadequately reclaimed. Another 10,000 ha were left abandoned, with no reclamation, more than 30 years ago. An estimated additional 150,000 ha show obvious signs of adverse effects of being non-restored, mined land.

Through clinical evaluation of the children, we unexpectedly discovered that the major adverse effect of Mn exposure was on bone formation. We found that rickets-like skeletal deformities were prevalent in the area. Although we did not measure levels of vitamin D, calcium, or phosphorus, our control region, which had the same sunlight exposure, had fewer than half of the number of cases of rickets as in the area of Mn contamination. A literature search revealed the same phenomenon had been reported in experimental animals (25). Mn administered orally to young rats resulted in morphologic changes in the growth plate that histologically resembled rickets (25).

Table 3. An estimation of total mutagen background of the environment in Marhanets on mNUC test in cells of oral cavity mucosa of preschool-aged children

Areas	*n*	mNUC-index/cell
Mn laden	56	0.070 ± 0.008
Control	23	0.012 ± 0.009**

**$p < 0.001$.

We observed increased levels of sIgA and serum A1PI, which could represent compensatory protective mechanisms to counter Mn toxicity. The antibody sIgA plays a critical role in mucosal immunity (26), and the secretory component is directly involved in the sIgA function *in vivo* (27). Elevation of sIgA concentrations, as was found in our study, might reflect the intensity of the immune response needed to protect the body from external antigens. The A1PI is an anti-protease that can protect tissues from enzymes secreted by inflammatory cells (28). The concentration of A1PI can rise substantially in cases of acute inflammation (28). The precise protective mechanisms are not well understood. The 11% of children from the study region who displayed A1PI levels below the average for controls might have exhausted their protective mechanisms.

Measurement of mNUC has been accepted as an index of chronic mutagenic activity. It is a cost-effective, non-invasive method of detecting cytogenetic damage in an exposed pediatric population. Micronuclei in exfoliated buccal cells emerge during mitosis of the basal layer of the epithelium as extra-chromosomal DNA particles when chromosome fragments or whole chromosomes lag behind and fail to be included in the main nuclei of the daughter cells (29). The formation of micronuclei is induced by substances that cause breakage of chromosomes (clastogens), as well as by agents that affect the spindle apparatus (aneugens) (30). An increase in the number of cells with mNUC might be a characteristic of human cell genetic damage and reflect a mutagenic impact of the environment (31, 32).

The increased numbers of mNUC in preschool children from Mn mining areas is disturbing. It shows that exposure to Mn is associated with an increase in DNA damage compared with the reference population. The results of the rehabilitation studies demonstrated that natural adaptogens can promote a positive effect by decreasing the frequency of occurrence of genetic pathologies in somatic cells. The rehabilitation measures could not prevent the inhalation of Mn particles, but by giving exposed children enterosorbents, we may have prevented some enteric absorption of the metal, and facilitated excretion of Mn. Humics and carotene oil possess immuno-modulating effects that positively influenced the immunologic status of the children. Eubiotics support the stability of the enteric flora and have been found to be beneficial in a previous study in children with disturbed immunity (33, 34).

A limitation in our research was the absence of neurocognitive and neurodevelopmental measurements in the pediatric population. Future work in this area should evaluate the effects of Mn on the nervous system.

Conclusion

Our study demonstrates the observable influence of residing near open Mn mines on the health of a pediatric population. Major effects identified included deficits in bone growth and immune function and somatic cell mutation. These data contribute to a better understanding of Mn-related toxicity, and can be used by health authorities to make evidence-based decisions to improve the health of children living near Mn mines. The implementation of social and medical rehabilitation measures may protect the next generation living in unfavorable environmental conditions from developing the symptoms of chronic Mn toxicity.

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