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Assessment of Mercury in Diagnostic Biomaterials of Different Population Groups in Urban Areas of the Moscow Region

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Abstract

The results of a survey of different population groups in urban areas of the Moscow Region (industry workers, residents of areas with increased anthropogenic load of mercury and conditionally pristine areas, cohorts of pregnant women and children) to assess the contents of mercury in samples of various diagnostic biomaterials taken during the course of research are presented. Mercury concentrations in venous and umbilical cord blood, urine, hair, and nails were determined by means of instrumental neutron activation analysis and cold vapor atomic absorption spectrometry. Peculiarities of selection, transportation, storage, and preparation for analysis of samples of hair, nails, urine and umbilical venous blood are described. The determined concentrations of mercury in the studied diagnostic biomaterials are compared with normal and thresholds values. Although increased mercury concentrations were observed in some cases, no significant exposure of mercury was evident in general in the study towns. A positive correlation between the degree of consumption of seafood during pregnancy and a high content of mercury in the analyzed diagnostic biomaterials was shown.

Keywords

Mercury, Hair, Nails, Urine, Venous and Umbilical Cord Blood, The Impact, The Human

1. Introduction

Human elemental status regarding exposure to mercury can be assessed only by

direct determination of mercury content in human organs and tissues. To achieve this goal, the main task is to select the available biomaterials that most fully reflect the micro-elemental status of the organism. Short-term exposure and a significant amplitude effect are most contrastingly reflected in body fluids. When assessing the total exposure to elemental and inorganic forms of mercury, usually, the analysis of venous blood and urine is used, and to assess the impact of the organic form of mercury (in particular, methylmercury) the analysis of umbilical cord blood. These biomaterials are used primarily for clinical diagnostic purposes [1] [2] [3] [4] [5].

To assess the external effects of chemical elements, body tissues that are involved in the processes of their deposition and accumulation (hair, nails, bones) are more suitable. Available literature data show that the content of elements—toxicants in these tissues reflects the status of the organism, as a whole. These biomaterials are considered to be an integral indicator of mineral metabolism, which characterizes the effects of mercury over a fairly long period of time [5] [6] [7] [8] [9]. Thus, the most informative and accessible biomaterials in the process of evaluating the effects of mercury on the human body are blood (venous and umbilical cord), urine, hair and nails.

2. Materials and Methods

Research on the topic was devoted to assessing the possibility of using these biomaterials in studying the effects of mercury on various population groups living in the urbanized areas of the Moscow region. Sampling was carried out in medical institutions, industrial enterprises, schools, preschool institutions in Moscow, Moscow and Vladimir regions. A total of about 900 samples were selected. All procedures for the selection, transportation, preparation and analysis of biological materials were carried out taking into account the requirements of existing domestic techniques, international standards of the ISO system and the methods described in the Standard Operating Procedures (SOP) used in the DEMOCOPHES program and recommended for use by WHO [8] [9] [10]. The survey was randomly selected. All survey participants signed a voluntary informed consent to participate in the survey and completed individual questionnaires reflecting their social and professional status. The survey of children was carried out with the written consent of both parents, as well as with the filling of individual questionnaires.

Criteria for inclusion in the surveyed groups were:

- For working personnel—work experience in the enterprise for at least 3 years;
- For women in labor—stay in the maternity hospital no more than 14 days before and/or after childbirth, the birth of living children, the age of women is 18 years and older, the permanent residence in the service area of the maternity hospital for at least 3 years;
- For children—age 5 - 7 years, permanent residence in an area as close as possible to industrial areas.

Hair. Hair is a complex tissue of epidermal origin, consisting of several types of cells and a variety of chemical components. They are used for retrospective assessment of the effects of mercury on the body over a long period of time. The total content of trace elements in hair consists of concentrations of trace elements of endogenous and exogenous origin. To date, the IAEA protocol [11] remains the only recommendation in the field of hair selection and preparation for analysis. According to this document, it is necessary to select a strand of hair from the back of the head in close proximity to the skin (no further than 1 - 2 mm). For analysis, a proximal section with a length of at least 10 cm should be cut from the strand. If the length of the selected hair is less than 10 cm, a proximal section with a length of 5 cm is analyzed. Hair samples of 10 cm length are cut in half, and the resulting sections of 5 cm are analyzed as separate samples. At least 100 individual hairs should be selected from the head of one person. The sample length of 5 cm should be at least 20 mg (usually 100 mg). The hair samples are stored in clean plastic bags with a retainer-clasp. Preparation of hair samples for analysis is to remove surface contamination and degreasing. All options include washing the hair with detergent, repeated rinsing with distilled water, degreasing with an alcohol-ether mixture or acetone and air drying at moderate temperatures. Later, when performing instrumental neutron activation analysis (INAA), the sample is packaged and sent for irradiation and analysis without additional processing. When conducting any other methods of analysis, mineralization of hair samples is necessary. Hair mineralization is characterized by the same problems as for blood and urine mineralization, the main of which is the minimum value of the so-called "blank" experience. Acid mineralization of hair uses a different combination of reagents (HNO_3 and H_2O_2, HNO_3 and $HClO_4$, H_2SO_4 and H_2O_2, etc.) with heating at atmospheric or elevated pressure in combination with microwave or ultrasonic treatment of the sample. The analyzed sample is usually 100 - 300 mg, the volume of acids is 2 - 12 ml.

Nails. Since the nails, as well as the hair, are derived from skin cells, the selected nail samples were prepared for analysis according to the method proposed by the IAEA [11] for preparing hair samples. The nail sample was placed in a bottle with acetone of the mark of ultra pure and kept for 1 minute, after which the acetone was decanted and the sample was washed three times with distilled deionized water for 3 minutes. Then the sample was placed in an ultrasonic bath with acetone, where it was treated for 1 minute. Next, the sample was placed in an oven and at a temperature not higher than 500˚C, dried to constant weight (approximately 2 hours). If varnish was detected on the nails, the initial washing in acetone was carried out until the varnish was completely dissolved. In addition, in case of detection of surface contamination, mechanical cleaning of the nail was performed. Nail analysis was performed using the INAA method. On average, the mass of samples for analysis was 30 - 150 mg.

Blood. To assess the effects of mercury on the human body, venous and umbilical cord blood was sampled. The selection of venous blood was carried out in the conditions of a medical institution by trained personnel who are authorized

to work of this kind, according to the standard method of venous blood sampling [5] [8] [10] [12] [13]. Cord blood sampling was performed immediately after delivery in the delivery room. After separation of the umbilical cord from the child, it was clamped with a clamp. After that, all maternal blood from the umbilical cord at the venous puncture site was cleaned with a gauze cloth moistened with alcohol or an antiseptic liquid. Disinfection of the umbilical cord at the puncture site is very important because it helps to prevent any contamination of cord blood. Next, the vein was punctured in the sterilized area of the umbilical cord and blood was drawn into the syringe. If the blood flow stops, it is necessary to disinfect another area closer to the placenta and use the second needle for further blood collection. A cord blood sample was collected using a 20 ml disposable syringe (BD Discardit II, BD, Spain). Then, the selected blood was placed in 2 × 10 ml vacutainers containing heparin as an anticoagulant (BD Vacutainer, BD, UK) [6] [7], mixed thoroughly, frozen and stored until analysis at −20˚C for not more than 20 days. Transportation within 1 - 3 days can be carried out in specialized portable refrigerators filled with refrigerant or dry ice. To determine the concentration of mercury, the blood is diluted and partially or fully mineralized, depending on the method of analysis used in the future. In the analysis of the neutron activation method, the blood is simply dried at a temperature not exceeding 50˚C. When analyzing by cold vapor atomic absorption spectrometry, blood is diluted 3 - 20 times to reduce its viscosity. Full mineralization is carried out using nitric, perchloric acids and hydrogen peroxide by the open method or in a closed microwave system. The main problem of blood mineralization is to ensure the minimum value of the "idle" experience. When determining the low content of mercury in the blood, the probability of accidental contamination of the analyzed sample at various stages of sample preparation for analysis is particularly relevant. When using deionized water, as well as additionally purified nitric acid, the value of the "blank" test is significantly reduced.

Urine. Urine is the recommended material for assessing the effects on the body of inorganic mercury, since most of this form of mercury is excreted in the urine. Vessels for urine should be washed with a 10% solution of nitric acid to eliminate background contamination, then the vessels are kept in a tank with a solution of nitric acid for at least 3 hours (preferably overnight) [12] [14]. After that, the vessels are washed twice in deionized water and dried. To check for residual contamination by random sampling, 5% of all vessels are removed, filled with 200 ml of purified deionized water and shaken for 10 minutes. After that, this solution should be analyzed for mercury content. If there is any amount of mercury, the operation of washing the vessels is repeated. For direct collection of urine, at least 5 hours have passed since the last urination (the first-morning urine is preferable). A portion of the selected urine was placed in a 12 ml polypropylene tube containing 0.1 ml of a 20% sulfamic acid solution (Fluka, Korea) as a preservative, mixed thoroughly, frozen, and stored until analysis. Transportation of samples is possible at a temperature of 4˚C - 8˚C in an isothermal package. When preparing samples for neutron activation analysis, it is sufficient

to simply dry the samples at a temperature not higher than 50˚C. For the atomic absorption analysis with "cold" steam, the selected urine samples are mineralized. The mineralization of urine, as well as the mineralization of blood, is carried out by an open method using nitric and perchloric acids in flasks with a reflux refrigerator.

3. Results and Discussion

The results of determining the concentration of mercury in the hair of different groups of the population are shown in Table 1.

According to the WHO classification, there are two values of mercury concentration in the hair, considered as reference points—it is 0.58 μg/g and 2.5 μg/g. The concentration of 0.58 μg/g is the upper threshold of the normal (background) value and all values below are considered normal (background). According to the data given in [15] [16], at values higher than 0.58 μg/g, slight deviations in the intellectual development of children are detected. A concentration of 2.5 μg/g is the lower toxicity threshold, and values equal to or greater than 2.5 μg/g indicate the effect on the body of toxic doses of mercury.

The first line of Table 1 gives the values of mercury concentration in the hair of the working personnel engaged in the production of mineral fertilizers (Voskresensk, Moscow Region). This production is not related to the use of mercury in the process cycle. Average values of mercury concentration in the hair of workers do not exceed the normal value; nevertheless, 41% of the individual samples are characterized by a mercury concentration exceeding the normal (background) value. Excess toxic levels of concentration (2.5 μg/g) in the individual samples of the working staff were not detected. Data on the concentration of mercury in the hair of children 5 - 6 years old living in two industrialized cities of the Vladimir and Moscow regions show that the average concentration of mercury in the hair of children is two times lower than the upper threshold of the normal concentration (0.58 mg/g). Exceed this threshold less than 1% of individual samples. Excess toxic levels were not found in individual samples.

Table 2 shows the results of determining the concentration of mercury in the nails of workers employed in the production of mineral fertilizers and students of 1 - 3 grades in three Moscow schools. Since nails, like hair, are derived from skin cells, the criteria for estimating the concentration of mercury in the nails can be accepted as for hair.

Table 1. Hg content in the hair of various populations mkg/g (n is the number of samples).

Group	Min	Max	C_{av}	Median	STD
Workers not related to production of Hg, n = 134	0.15	1.81	0.58	0.53	0.36
Children of 5 - 6 years Gus-Crystalny, n = 85	0.11	0.65	0.23	0.23	0.13
Children of 5 - 6 years Podolsk, n = 40	0.08	0.57	0.23	0.21	0.12

Table 2. Content Hg nails of various populations mkg/kg (n is the number of samples).

Group	Min	Max	C_{av}	Median	STD.
Workers not related to production of Hg, n = 32	0.18	1.92	0.76	0.72	0.4
Pupils of 1 - 3 grades, Moscow, n = 137	0.05	0.29	0.12	0.12	0.06

Average values of mercury concentration in the nails of workers slightly exceed the threshold of normal (background) concentration. The calculation of the percentage distribution of mercury concentration values in the nails of workers shows that 41% of the total number of individual samples contains a normal amount of mercury, and in 59% the values exceed the threshold of normal concentration. Excess toxic levels—concentrations (2.5 μg/g)—in individual samples were not detected. In the nail samples of students of Moscow schools, the excess of the normal level of mercury concentration was not detected.

Table 3 shows the values of mercury concentrations in the venous blood of various population groups in Moscow and the region.

In the Russian Federation, a concentration of 1 - 5 μg/l [11] [15] is considered normal (background) (or according to some sources, 1 - 8 μg/l) [2] [9] [15] [16], and a mercury concentration of 10 μg/l is considered hazardous to health. The value of 5 μg/l is the concentration of mercury in human blood, below which, according to current data, there is no risk of adverse health effects. The value of 10 μg/l is the concentration of a substance in the blood, above which there is an increased risk of adverse health effects and, therefore, there is a need for measures to reduce or eliminate exposure. The data presented in Table 3 show that, on average, the concentration of mercury in the blood of workers does not exceed the normal (background) level. However, in 8.5% of individual samples from the entire sample, the mercury content exceeds the upper level of normal (background) concentrations.

The concentration of mercury in the blood exceeding the level of toxic effects (10 μg/l) in the studied samples was not detected. The data in the table show that the concentration of mercury in the blood of the population living in an industrial area is almost 2 times higher than the same concentration in samples from the "sleeping" area. Of the entire sample of individual samples of the population living in an industrial area in the zone of intensive anthropogenic impact, the level of normal (background) concentration of mercury in the blood was exceeded in 13.3%.

Excess toxic levels of mercury were not detected. The concentration of mercury in the blood of the population living in a relatively clean "bedroom" area is within the normal range.

Table 4 shows the values of mercury concentrations in cord blood, urine and hair samples taken in the perinatal centers of various cities of the Moscow region levels—concentrations Lyubertsy, Balashikha, Dolgoprudny, Vidnoe, Podolsk and Serpukhov.

These data show that the values of mercury concentration in cord blood are

Table 3. Hg content in venous blood of various populations mkg/l (n is the number of samples).

Group	Min.	Max.	C_{av}	Median.	STD
Workers. not related to production of Hg, n = 35	2.2	8.9	5.1	4.3	2.6
Population. living in industrial area of Moscow, n = 30	2.2	9.1	5.4	4.9	2.7
Population living in the "sleeping" area of Moscow, n = 30	1.2	4.3	3.1	3.1	1.3

Table 4. Hg content in biomaterials selected in maternity hospitals of the Moscow region (n = 120).

Biomaterial	Min	Max	C_{av}	Median	STD
Umbilical blood, mkg/l	0.34	2.47	1.06	0.95	0.59
Urtine, mkg/l	0.17	0.87	0.4	0.36	0.21
Hair, mkg/kg	84	600	249	241	140

significantly lower than in venous blood (Table 4) and completely (99.5%) are in the range of normal (background) concentrations. The mercury content in the urine of pregnant women is significantly lower than the values considered normal (2 - 5 μg/l), as well as similar values in umbilical cord blood. Excess of normal (background) level of mercury concentration in hair samples of pregnant women is observed in 3.3% of the total number of individual samples. Excess toxic levels were not detected in individual hair samples. Using average values of mercury concentration in urine, cord blood and hair, graphs of mercury distribution in these biomaterials for 6 cities of the Moscow Region were built (Figure 1).

The graphs shown in Figure 1 show that the pattern of distribution of mercury in hair and cord blood is almost identical, while the pattern of distribution of mercury in urine differs significantly from the first two. To check for possible dependencies, paired correlation coefficients between mercury concentration in cord blood, urine and hair in the specified cities were calculated. The results of calculations showed that the correlation coefficient between the concentration of mercury in the hair and in the urine is 0.29, in the blood and in the urine is 0.37, and in the umbilical cord blood and hair is 0.66.

Figure 2 shows the relationship between mercury concentration in cord blood and the hair of women. From this graph it is clear that this dependence is linear.

Comparing the concentration of mercury in cord blood and the urine of pregnant women shows that the content of mercury in cord blood characterizes the effects on the body, mainly in the form of methylated mercury (methyl mercury). This form of mercury enters the human body exclusively through the gastrointestinal tract with food and is removed from the body through the gastrointestinal

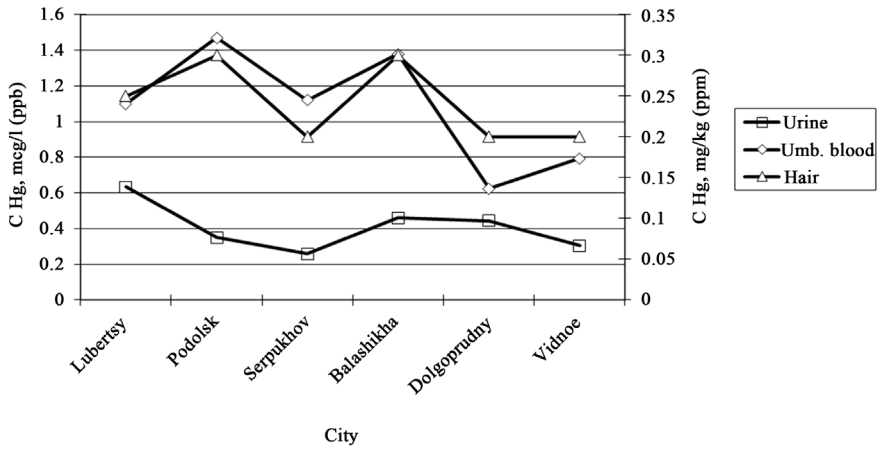

Figure 1. Distribution of blood and hair in pregnant women in different cities of the Moscow region.

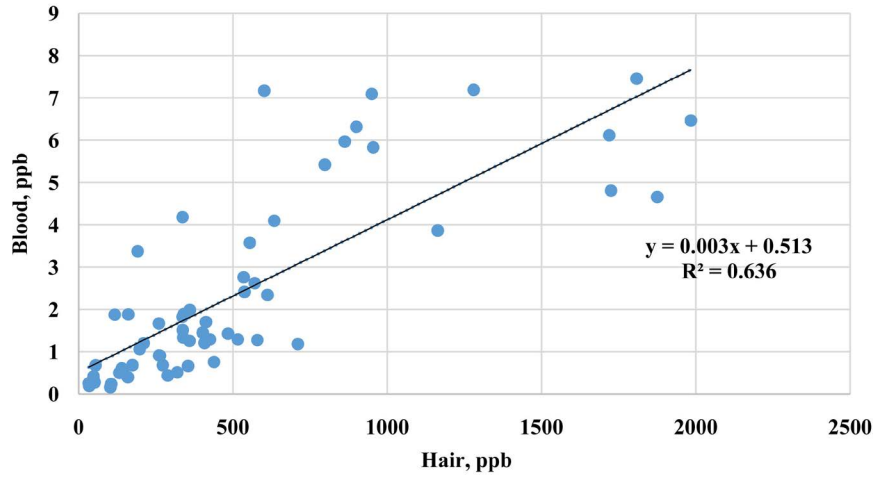

Figure 2. Correlation of mercury concentration in cord blood and the hair of women.

tract. Since it is believed that the main suppliers of methylmercury are fish and seafood, a survey was conducted of pregnant women on the frequency of consumption of fish and seafood.

Table 5 shows the average concentrations and main characteristics of the concentration distribution of the studied biomaterials depending on the frequency of consumption of fish products in the third trimester of pregnancy. These data show that there is a direct relationship between the frequency of consumption of all types of fish, crustaceans and algae by women in 1 - 2 trimesters of pregnancy and, especially, in the third trimester, and the level of mercury concentration in all studied biomaterials.

As noted above, the concentration of mercury in the urine reflects short-term and intense exposure to elemental and inorganic mercury. Therefore, it can be concluded that a low concentration of mercury in cord blood indicates low levels of mercury intake with food, and a low value of mercury concentration in urine indicates the absence of intense external exposure.

Table 5. The average content of mercury in the hair, cord blood and urine of women in labor depending on the frequency of consumption all types of fish and seafood in the third trimester of pregnancy(n is the number of respondents).

Frequency of eating fish and seafood	n	Med (SD)		
		Hair, mkg/g	umbilical blood, mkg/l	Urine, mkg/l
12 times in a week	21	0.30 (0.15)	1.18 (0.75)	0.43 (0.47)
		t = 3.11, p < 0.01	t = 2.69, p < 0.01	t = 3.18, p < 0.01
2 - 3 times a month	64	0.19 (0.13)	0.79 (0.54)	0.25 (0.20)
Less often	34	0.15(0.13)	0.71 (0.7)	0.25 (0.17)

4. Conclusions

1) The complex of biomaterials used in our studies reflects the level of entry into the human body of all forms of mercury and can be successfully used to assess the risks of its toxic effects. It should be noted that the data obtained in the process of carrying out the work did not reveal signs of the impact of high exposures of mercury on the human body in Moscow and cities of the Moscow region.

2) Hair and nail samples provide mutually replaceable analytical information, therefore, since nail analysis is associated with specific difficulties of sample preparation (in some cases it is impossible to completely clean the sample from external contamination), if there is a sufficient volume of hair samples, you can refuse to take nail samples and limit analysis of hair samples.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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