THE EFFECTS OF LEAD EXPOSURE ON IMMUNE CELLS IN UKRAINIAN CHILDREN

Hnidoi I.M.[1], Hnida N.I.[2]

[1]International Humanitarian University, Odesa, Ukraine
[2]Odessa National Medical University

Introduction

Lead (Pb) poisoning in children is considered as the longest-standing public health epidemic in U.S. history [1]. For low- and middle-income countries, similar problem is even more significant [2]. Lead has received extensive attention due to its multi-system toxicity in children, and the immune system is also one of the sensitive targets [1, 3]. Prove that excessive exposure to Pb has been associated with negative long-term effects on adaptive immune system in children [4]. The toxic effects of Pb exposure on the immune system of children ultimately lead to the development of a variety of diseases [3]. Environmental factor of heavy metal exposure also accelerates the risk of immune-mediated respiratory diseases, including asthma in children [5, 6]. The new review finds that exposure to heavy metals, particularly Pb, not only reduced lymphocyte numbers and suppressed adaptive immune responses in children, but also altered the innate immune response to impair the body's ability to fight pathogens [3]. Modern realities associated with the Covid-19 pandemic require that the level of not only specific, but also non-specific immune protection in the population is sufficient. At the same time, it is the pandemic that causes a decrease in the number of examinations of children for lead in the blood [7], which in turn does not contribute to timely preventive measures to reduce the immunotoxic effect of this heavy metal. In Ukraine, the situation is also aggravated by active hostilities [8], which, as a stress factor, also negatively affect the state of immunity, especially in children [9].

The morphological basis of the immune response in the body are immune cells [10]. According to a modern review [3], studies of the effect of lead in environmentally determined doses on immune cells in children are few and sometimes contradictory. There are even fewer such data from Ukraine.

The aim of this work was to study of the effect of lead in environmentally determined doses on immune cells in Ukrainian children.

Material & methods

100 random children aged 4 to 15 years were examined. The children were treated in a paediatric hospital in Odessa for various diseases. The involvement of children in the study was carried out subject to the informed consent of the parents of the children after providing detailed information about the procedure and purpose of the work.

The determination of lead was carried out in heparinized venous blood by atomic absorption spectrometry with electro-thermal atomization. Indicators of the cellular link of immunity were determined in capillary blood. Given the rather strong influence on the immunological parameters of circadian rhythms, meals and physical activity, blood was taken under certain standard conditions: at the same time of day (in the morning), on an empty stomach, before physical exertion, on the same day of the week. To establish the dependence of cellular immunity indicators on the blood lead level (BLL), children were divided into 4 groups: 1) BLL up to 4.9 μg/dl, n = 62; 2) BLL 5.0 – 9.9 μg/dl, n = 18; 3) BLL 10.0 – 14.9 μg/dl, n = 15; 4) BLL 15.0 μg/dl and above, n = 5. The first group of children served as control. Statistical processing of the obtained data was carried out by methods of variational statistics using the Student-Fischer T-criterion. It is known that not only the blood lead level can affect the studied indicators, but also age, sex of children, diseases they suffered, can affect. To establish homogeneity according to these factors of the selected groups of children, the χ^2 test was calculated. This method, which also called "analysis of proportions", allows to provide statistically evenness or unevenness of the distribution of certain features in groups. Rating χ^2 calculations showed that the groups were homogeneous, hence the differences in the immunity status could be explained in this case only by a different blood lead level.

Results & discussion

The results of the study of the state of the cellular link of immunity in the examined children of different groups are presented in table 1.

Table 1. Indicators of the cellular link of immunity in children of different groups, M±m

Indicators	A group of children depending on the BLL			
T-lymphocytes ($CD3^+$), %	55.39±0.78	51.94±1.42*	50.73±2.40*	48.80±2.69*
T-lymphocytes active ($CD3^+$+HLA-DR^-), %	41.98±0.85	38.44±2.03	34.07±1.38*	32.60±2.46
T-helpers ($CD4^+$), %	40.35±0.83	37.33±1.43	37.07±2.00	36.20±0.97
Cytotoxic T-cells ($CD8^+$), %	15.05±0.56	13.89±0.82	13.67±1.44	12.60±1.89
T-helpers / Cytotoxic T-cells index	2.98±0.15	3.00±0.38	3.17±0.29	3.06±0.29
B-lymphocytes ($CD19^+$), %	13.34±0.46	12.17±0.56	12.73±1.102	9.60±0.68*
NK sells ($CD16^+$), %	31.13±1.02	35.78±0.56*	36.53±3.16*	41.60±3.36*
Phagocytic neutrophil activity, %	59.92±1.09	64.61±3.83	62.73±4.23	58.40±6.09
Phagocytic neutrophil index, %	2.50±0.05	2.26±0.12*	2.13±0.15*	1.80±0.22*
Eosinophils, %	1.65±0.24	2.83±0.54*	2.73±0.92	1.80±0.92

Note. * - The difference is reliable ($p < 0.05$) with the indicator of the first group.

DOI: 10.5281/zenodo.7721509

Analysis of immunogramma indicators in children of different groups made it possible to identify certain differences depending on the range of Pb concentrations in the blood. The relative content of T-lymphocytes was significantly reduced in children of the second, third, and fourth groups, compared with the indicator of the first group, where BLLs were the lowest. This is consistent with the current data provided in the Zheng K. et al. (2023) review, that lower numbers of total T lymphocytes (CD3+) in German children were associated with higher BLLs [3].

In the third group of children, the content of active T-lymphocytes was also reduced, which also corresponds to modern literature data on the immunotoxic effects of lead.

Changes in the level of T-helpers, cytotoxic T-cells and their ratio in the surveyed children turned out to be unreliable, although a preliminary analysis of scientific studies prompted more significant changes. Thus, the researchers reported that children with high BLLs had significantly reduced numbers of Th cells (CD3+CD4+) and increased cytotoxic T cells (CD3+CD8+) compared to children in the control group [3]. Investigators found a negative association between the percentage of CD4+ T cells and BLLs ($r = -0.462$, $p < 0.01$), and that the ratio of CD4+/CD8+ was significantly decreased in children with high BLLs than in children in the control group (cit. for [3]). However, the same review suggests that the results may be inconsistent. Notably, researchers reported that BLLs in children were positively correlated with the percentage of CD4+ central memory T cells ($r = 0.312$, $p < 0.01$) and negatively correlated with the percentage of CD4+ naive T cells ($r = -0.317$, $p < 0.01$), suggesting that Pb may contribute to the biased development of CD4+ memory T cells in children (cit. for [3]). Considering this aspect, the results we obtained are consistent with the literature data.

In addition, it should be considered that the children we examined were on inpatient treatment for various diseases. Although the control group and the main groups had no differences in the distribution of these diseases, the very presence of various pathological conditions could somewhat distort the results of the effect on the immunity of lead. Therefore, the changes could not achieve a degree of reliability. In addition, the age of the children examined included the period of intersection of the leukocyte formula, which affected the data scattering, and therefore the achievement of the degree of reliability of the changes. In the future, this factor will need to be excluded.

In the fourth group of children, a decrease was observed compared to the control of the B-cell immunity index. This is consistent with the current data that lower numbers of B lymphocytes (CD19+) in German children were associated with higher BLLs (cit. for [3]).

The relative content of natural killer (NK) cells in the second, third and fourth groups of children, on the contrary, increased significantly compared to the indicator of the first group. Other researchers reported that an increase in BLL leads to a decrease in the percentage of NK cells [3]. In the children we examined, the changes are opposite. Perhaps this is also since both the control and the main groups of children themselves suffered certain diseases. It is known that NK cells are a distinct subset of lymphocytes with natural killer activity, and an increase in BLLs lead to a decrease in the percentage of NK cells [3]. During illness, this activity should increase. Obviously, the accompanying effects of lead cause NK hyper-reaction, which can further lead to an inadequate immune response or to depletion of immune system reserves.

Lead also has a certain effect on the state of the neutrophil link in the cellular defence of the body. The number of neutrophils has not changed significantly, which coincides with some data of the literature [6]. But the phagocytic neutrophil index was significantly reduced in the second and third groups of children compared to the first group. This indicates the inhibition of non-specific reactivity with an increase in the level of lead in the blood of children.

There is growing evidence that Pb also has toxic effects on innate immune cells [3]. Therefore, we analysed the content of these cells in the blood of the examined children. We didn't see such a clear connection when each 1 μg/dL increase in BLLs in preschool and school-aged children was associated with a 4.9 % (95%CI: 2.3, 7.6) increase in eosinophils (cit. for [3]). But we found that the relative content of eosinophils in children of the second group was significantly increased. This is consistent with data from other researchers that a significant increase in the number of eosinophils was associated with Pb exposure in children [3], and it's observed a strong positive correlation between BLLs and eosinophils ($r = 0.632$, $p < 0.001$) [11]. So Pb stimulates the production of innate immune cells that activate the innate immune response as the first line of defence in the immune response, suggesting that Pb exposure is associated with childhood infections, chronic inflammation, or allergic reactions [3, 11]. It may contribute to the development of allergic diseases such as asthma in children [3, 5, 6]. Several large-scale studies have shown that Pb induces allergic reactions in children [3]. In addition, childhood Pb exposure reduces antibody titers in specific vaccines, which increases the chance of vaccination failure (cit. for [3]). The changes we have identified at the cellular level can be the basis of this.

Conclusions

1. Lead in environmentally determined doses correlates with a decrease in the number of T- and B-lymphocytes in the examined Ukrainian children, which can lead to disruption of the process of adaptive immune response.
2. At the same time, the number of natural killers and eosinophils increased, which may be associated with the course of diseases on the background of elevated lead levels in children, and in the future can lead either to an inadequate immune response and the development of immune-mediated diseases, or to depletion of the reserves of the immune system and the development of chronic pathology.

DOI: 10.5281/zenodo.7721509

The effects of lead exposure on immune cells in Ukrainian children

Hnidoi I.M., Hnida N.I.

Introduction. The immune system is one of the sensitive targets for the toxic effects of lead. Even low, environmentally determined doses of this pollutant can affect the state of immunity, especially in children. For the public health of even a developed country like the United States, lead poisoning of children is the longest epidemic in history. In this case, first, attention is paid to the neurotoxic effect of lead. But there is growing evidence that the immunotoxic effect of this heavy metal is also significant. There are still relatively few epidemiological studies in this direction, as evidenced by the latest review of Zheng K. et al. (2023). But it has already been proven that lead in environmentally determined doses has a predominantly immunosuppressive effect, which leads to chronicity of inflammatory processes, to an increase in the prevalence of childhood infectious diseases. There is also evidence that excessive long-term exposure to lead can lead to distortion of the immune response, which is further realized by the development of immune-mediated diseases, including bronchial asthma in children. In addition, the immunotoxic effect of lead may be associated with the ineffectiveness of vaccinations, with a decrease in antibody titre. The problem is exacerbated by the fact that in low- and middle-income countries, to which Ukraine belongs, there are no such state programs for the primary and secondary prevention of lead lesions that operate in developed countries. Studies on populations of Ukrainian children are mostly isolated and scattered. **The aim** of this work is to study the effect of lead in environmentally determined doses on immune cells in Ukrainian children. **Material & methods**. 100 random children aged 4 to 15 years were examined. The children were treated in a paediatric hospital in Odessa for various diseases. The involvement of children in the study was carried out subject to the informed consent of the parents of the children after providing detailed information about the procedure and purpose of the work. The definition of venous blood lead concentration was by atomic-absorption spectrometry with electro-thermic atomization. Indicators of the cellular link of immunity were determined in capillary blood. **Results & discussion**. It was obtained the verified decreasing of relative number of T-lymphocytes ($p<0,05$) and active T-lymphocytes ($p<0,05$) in the children with increasing blood lead concentration, as compared with this index in the group of children with lead level up to 4,9μg/dl. The number of B-lymphocytes ($p<0,05$) also decreased. With it the relative number of NK-cells ($p<0,05$) was increased reliably in these children. It was shown also the verified decreasing of neutrophile phagocytosis index ($p<0,05$) in the same children. The number of erythrocytes ($p<0,05$) increased. The results obtained are mainly consistent with modern literature data. **Conclusions**. Lead in environmentally determined doses correlates with a decrease in the number of T- and B-lymphocytes in the examined Ukrainian children, which can lead to disruption of the process of adaptive immune response. At the same time, the number of natural killers and eosinophils increased, which may be associated with the course of diseases on the background of elevated lead levels in children, and in the future can lead either to an inadequate immune response and the development of immune-mediated diseases, or to depletion of the reserves of the immune system and the development of chronic pathology.

Keywords: ecology, lead, public health, children, immune cells.

References:

1. Del Rio M, Obeng A, Galkaduwa B, Rodriguez C, Costa C, Chavarria CA, Navarro EA, Avila J, Wekumbura C, Hargrove WL, Hettiarachchi G, Sobin C. An interdisciplinary team-based approach for significantly reducing lower-level lead poisoning in U.S. children. Toxicol Rep. 2022.10.76-86. doi: 10.1016/j.toxrep.2022.12.004.
2. WHO: Lead poisoning. 31 August 2022. URL: https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health
3. Zheng K, Zeng Z, Tian Q, Huang J, Zhong Q, Huo X. Epidemiological evidence for the effect of environmental heavy metal exposure on the immune system in children. Sci Total Environ. 2023. 868.161691. doi: 10.1016/j.scitotenv.2023.161691.
4. Huang W, Shi X, Wu K. Human Body Burden of Heavy Metals and Health Consequences of Pb Exposure in Guiyu, an E-Waste Recycling Town in China. Int J Environ Res Public Health. 2021.18(23).12428. doi: 10.3390/ijerph182312428.
5. Wang J, Yin J, Hong X, Liu R. Exposure to Heavy Metals and Allergic Outcomes in Children: a Systematic Review and Meta-analysis. Biol Trace Elem Res. 2022.200(11).4615-4631. doi: 10.1007/s12011-021-03070-w.
6. Zeng Z, Xu X, Zhu Y, Wang Q, Zhang Y, Huo X. Pb and Cd exposure linked with Il-10 and Il-13 gene polymorphisms in asthma risk relevant immunomodulation in children. Chemosphere. 2022. 294.133656. doi: 10.1016/j.chemosphere.2022.133656.
7. Courtney JG, Chuke SO, Dyke K, Credle K, Lecours C, Egan KB, Leonard M. Decreases in Young Children Who Received Blood Lead Level Testing During COVID-19. 34 Jurisdictions. January–May 2020. MMWR. 2021. 70(5).155-161.
8. Kumar BN, James R, Hargreaves S, Bozorgmehr K, Mosca D, Hosseinalipour SM, AlDeen KN, Tatsi C, Mussa R, Veizis A, Kállayová D, Blanchet K, Machado RS, Orcutt M, Severoni S. Meeting the health needs of displaced people fleeing Ukraine: Drawing on existing technical guidance and evidence. Lancet Reg Health Eur. 2022. 17. 100403. doi: 10.1016/j.lanepe.2022.100403.
9. He H, Tan Y, Li L. Meta-Analysis of Children's Acute Psychological Stress and Action Stress on Immune Function under Microscope Images. J Healthc Eng. 2022 .2022.6549805. doi: 10.1155/2022/6549805.
10. Delves PJ. Cellular components of the immune system. Modified Sep 2022. URL:

DOI: 10.5281/zenodo.7721509

https://www.msdmanuals.com/professional/immunology-allergic-disorders/biology-of-the-immune-system/cellular-components-of-the-immune-system
11. Rawat PS, Singh S, Zahid M, Mehrotra S. An integrated assessment of lead exposure in children: Correlation with biochemical and haematological indices. J Trace Elem Med Biol. 2021. 68.126835. doi: 10.1016/j.jtemb.2021.126835.

DOI: 10.5281/zenodo.7721509