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Granulocyte viability test in children from an environment with heavy metal pollution

Bozena Hager-Malecka, Aldona Lukas, Z Szczepanski, J Frydrych, F Sliwa, W Lukas, Krystyna Romanska

Department of Paediatrics, Silesian Medical Academy, Zabrze, Third Department of Paediatrics, Silesian Medical Academy, Bytom, and Laboratory of Clinical Pathology, Outpatient Clinic of the Tarnowskie Gory-Miasteczko Slaskie Zinc Plant, Poland

In a group of 43 school-children living near a zinc plant, the test of granulocyte viability by the method of Cocchi et al, the lead level by flame atomic absorptiometry, and the level of erythrocyte zinc-protoporphyrin by the fluorimetric method were determined in peripheral blood.

The mean result of the granulocyte viability test was 6.49%. In 18 children, the proportion of abnormal granulocytes was raised abnormally above 6.0%. The lead level was in the range from 14.1 to 53 μg/dl, and the level of erythrocyte ZPP was from 0.3 to 2.1 μg/g Hb. No correlation was found between the results of the granulocyte viability test and the blood lead level. The result of a comparison with the ZPP value was of borderline significance ($p = 0.05$).

The criteria of lead poisoning of children with environmental exposure are not sufficiently clear. New laboratory diagnostic tests are being introduced and the greatest advance in the field was achieved by introducing determinations of the blood lead level and tests for its toxic effects on biochemical processes [3].

Lead poisoning may not manifest itself clinically and the symptomless period may precede for a long time the appearance of clinical symptoms. Heme biosynthesis is particularly sensitive to the effects of lead [6, 8]. One of the earliest signs of damage to erythrocyte functions is a rise in the level of zinc-protoporphyrin (ZPP) due to a direct action of lead on mitochondria [4, 6, 8].

The aim of this work was to evaluate the disturbances of intracellular metabolic processes in granulocytes, using the method of granulocyte viability test. Studies on the life span of the circulating leucocytes allow the detection of abnormalities in their function [2]. In addition, the cause of the increased incidence of respiratory infections in children living in a highly industrialized area was also studied [1, 4, 8].

Material and Methods

The study was carried out in 43 healthy children aged 8—10 years living in a town 0.5 to 2 km from a zinc plant. Samples of 6 ml venous blood were taken into heparinized test tubes, and peripheral blood cell counts were done.

The granulocyte viability test was carried out by the method of Cocchi et al [2]. A drop of 1% aqueous eosin was placed on a slide and left to dry, then a drop of blood

was placed on it and mixed. Then the slide was put into a humid chamber at room temperature for 2 minutes. The proportion of granulocytes containing the red dye was calculated by microscopic examination of the sample. Since damaged granulocytes are unable to remove the absorbed eosin in its ionised form, this remained in the cell and stained diffusely its cytoplasm. The acceptable proportion of abnormal granulocytes should be 0 to 6% according to Cocchi et al [2].

The lead level in whole blood was determined by flame atomic absorptiometry according to Whiteside [9]. The measurements were performed with a Sp-2900 Pye Unicam atomic absorption spectrophotometer. The results were expressed in μg/dl. Values above 30 μg/dl were regarded as raised blood lead level. Levels of blood lead from 30 to 69 μg/dl suggested an increased absorption of lead. Lead poisoning was diagnosed when the blood lead level exceeded 70 μg/dl [8].

Determination of zinc-protopophyrin (ZPP) in the erythrocytes was based on measurements of the fluorescence of zinc-protoporphyrin III complex, using a Manual Hematofluorometer Model ZPP Meter 210 (Aviv) apparatus. The relationship of ZPP to haemoglobin in the tested blood sample was expressed as μg/g Hb. The meannormal result according to Stankiewicz and Frydrych [6] was 1.1 ± 0.2 μg/g Hb. and the acceptable value in children was 4.4 μg/g Hb.

The results were subjected to statistical analysis using Student's t test and the chi square test with Yates' modification.

Results

The mean proportion of granulocytes with abnormal viability was $6.49 \pm 10.3\%$ in peripheral blood of the children (Table I). The children were then divided into two groups, group I with the proportion of abnormal granulocytes exceeding 6% (the normal value of viable granulocytes), and group II with that proportion below 6%. The first group comprised 18 children with raised proportion of abnormal granulocytes, mean value $13.3 \pm 12.98\%$ (Table II). In the second group of 25 children, granulocyte viability was normal, the mean value was $1.64 \pm 1.76\%$ (Table II).

The serum lead level was from 14.1 μg/dl to 53.0 μg/dl, with a mean of 29 ± 49 μ/dl (Table I). In the group with abnormal result of the test the blood lead level was $25.81 \pm \pm 6.91$ μg/dl, while in the group with a normal result of the test the mean was 31.3 ± 10.51 μg/dl. The difference was not significant statistically.

The mean zinc-protoporphyrin level measured in the erythrocytes was in these children 1.23 ± 0.55 μg/g Hb, with a range of 0.3 to 2.1μg/g Hb (Table I). The results in the first group compared with the second group showed a difference of borderline statistical significance, being 1.42 ± 0.53 μg/g Hb, and 1.1 μg/g Hb in the first and second groups, respectively ($p=0.05$) (Table II).

The blood cell count was normal in all the 43 children, the mean haemoglobin concentration was 13.53 ± 1.36 g/dl, the mean white blood cell count was 4.200 ± 1.100 cu, mm.

Physical examination failed to demonstrate in the studied children any abnormalities in the osteoarticular, respiratory, circulatory and nervous systems.

TABLE I

Mean results of granulocyte viability test (g. v.), blood lead level, and erythrocyte zinc-protoporphyrin (ZPP) in children with environmental lead exposure

n = 43	g.v.%	Pb μg/dl	ZPP μg/g Hb
$\bar{x}$	6.49	29	1.23
SD ±	10.3	9.49	0.55
range	0—62	14.1—53.0	0.3—2.1

TABLE II

Granulocyte viability test (g. v.) blood lead level and erythrocyte zinc-protoporphyrin (ZPP)

Group	No of patients		Pb μg/dl	ZPP μg/g Hb	g. v. per cent
			1/	2/	
normal g. v.	25	$\bar{x}$	31.3	1.1	1.64
		SD ±	10.51	0.53	1.75
		range	14.1—53	0.1—2.0	0—5
abnormal g. v.	18	$\bar{x}$	25.81	1.42	13.3
		SD ±	6.91	0.53	12.98
		range	17.5—37.2	0.4—2.1	6—62

1.t = 1.94 0.1 p 0.05
2.t = 2.0 p = 0.05

DISCUSSION

The results showed that in children living under environmental exposure to lead the number of granulocytes with reduced viability is increased in the peripheral blood. The mean proportion of granulocytes with reduced viability (6.49 ± 10.3%) was significantly higher than the mean value of 1.7 ± 1.5% obtained by Cocchi et al in a group of healthy children aged 1—6 years [2]. Impaired function of the granulocytes was observed in the neonatal period during septicaemia or malnutrition, and the toxic effect of lead on biochemical processes is also known; of particular importance for the assessment of these effects is the determination of zinc-protoporphyrin. After excessive absorption of lead the blood level rises to 30—69 μg/dl and biochemical disturbances develop. These changes may appear without any clinical symptoms or signs [8].

In our material the mean blood lead level was 29 ± 9.49 μg/dl (from 14.1 to 53.0 μg/dl). In 17 children the level ranged from 31.0 to 53.0 μg/dl. The results obtained showed a wide

range of blood lead values which may be still within the physiological variability or may be caused by severe exposure to lead from time to time [7, 8].

In children lead is absorbed mainly through the digestive tract with soil, wall paints and from the surface of toys. The blood lead level corresponds to the degree of exposure. One of the important indicators of lead poisoning are changes of erythrocyte zinc-protoporphyrin. Its level in the children with environmental exposure was in the range from 0.3 to 2.1 μg/g Hb, with a mean of 1.23 $\pm$ 0.55 μg/g Hb, and in 17 children with raised serum lead level it ranged from 0.3 to 1.8 μg/g Hb.

Many authors stressed the lack of a correlation between ZPP and the blood lead level [4, 6, 8]. A raised ZPP level indicated a toxic effect of lead on heme synthesis, confirming the presence of disturbances of intracellular metabolism. It may be assumed then that demonstration of a toxic effect of lead on blood elements in children is sufficient for considering them to be at risk of lead poisoning. A moderate rise of the blood lead level (30—69 μg/dl) does not seem sufficient for this purpose [8]. The absence of a significant correlation between the serum lead level and the increase in the proportion of abnormal granulocytes and a simultaneously demonstrated correlation between these values and the results of ZPP determinations might confirm this concept.

The presented results suggest that during excessive absorption of lead by the organism of a child, despite the absence of clinical signs of lead poisoning, certain metabolic disturbances develop. The value of ZPP in the erythrocytes rises, the survival of granulocytes is shortened.

Summing up, in children living in an area with atmospheric lead pollution the test of granulocyte viability is often abnormal. Besides, a correlation exists between the erythrocyte zinc-protoporphyrin concentration and the result of the granulocyte viability test in these children.

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L Aldona MD
ul. Batorego 15
Bytom, Poland