Erythrocyte arginase activity as an indicator of lead exposure

K FUKUMOTO,1 I KARAI,2 Y NISHIKAWA,3 AND S HORIGUCHI3

From the Osaka Prefectural Institute of Occupational Health,1 Osaka, 540, the Department of Preventive Medicine and Public Health,2 Osaka City University Medical School, Osaka, 545, and the Department of Clinical Chemistry,3 Osaka Prefectural Hospital, Osaka, 558, Japan

ABSTRACT A semi-automated method has been developed for the determination of the arginase activity of erythrocytes using dried blood spots, which are easy to prepare on site in a factory for later laboratory analysis. The mean arginase activity of erythrocytes in 49 men occupationally exposed to lead was 62.9 IU/g-Hb (SD, 14.4 IU/g-Hb); in 45 men not exposed to lead the mean was 44.6 IU/g-Hb (SD, 11.6 IU/g-Hb). A significantly higher mean arginase activity was found in the specimens from lead-exposed workers (p < 0.001). The correlation coefficient between blood lead and erythrocyte arginase was r = 0.67 (p < 0.001). The degree of correlation between blood lead and lead indicators including arginase was r = 0.75 for urine δ-aminolaevulinc acid, r = 0.67 for erythrocyte arginase, r = 0.66 for urine lead, and r = 0.63 for coproporphyrin. Erythrocyte arginase showed no significant correlation in the liver function tests, GOT, GPT, and albumin in serum. When 40 μg/100 g of blood lead concentration was fixed as the basic value and 56.2 IU/g-Hb of erythrocyte arginase activity was set as the screening value in lead-exposed workers, the sensitivity and specificity of the arginase test were 0.96 and 0.65, respectively. Thus the validity of the test was calculated to be 1.61. These results show that the arginase level of erythrocytes can be considered to be one of the significant indicators of occupational exposure to lead because it reflects well the dose-response relationship of lead in the human body. Our method allows rapid analysis of erythrocyte arginase and thus should be useful in screening for lead exposure.

Arginase (EC 3531) which catalyses the hydrolysis of arginine to ornithine and urea is present in the urea cycle. Arginine, an enzyme activated by a binary cation,1 is present mainly in liver, kidney, and erythrocytes in man. Familial hyperargininaemia is well known as a disorder of urea synthesis with erythrocyte arginase deficiency.3 Arginase activity in erythrocytes increases in pernicious anaemia and thalassaemia major4 5; serum arginase activity increases in liver diseases.6 7 Chmielnicka4 reported recently that serum arginase is an indicator of liver damage caused by exposure to lead, the effect of exposure to lead on erythrocyte arginase activity has not been fully discussed. In this study erythrocyte arginase, blood lead, and other lead indicators were analysed in lead-exposed and lead-unexposed workers. Using the suggestion by Zielhuis and Verberk5 who have introduced the concept of validity of biological tests in epidemiological toxicology, we studied the sensitivity (SE), specificity (SP), and validity (V) of the lead indicator tests, fixing the basic value of blood lead as 40 μg/100 g. The screening value of arginase activity was set to show the maximum SE and V from the concept of a screening test. The SE and V of the arginase test are compared with those of other lead indicator tests and the usefulness of the arginase test as a lead indicator is discussed.

Subjects

Forty-nine male workers (aged 25–70, mean 46) exposed to lead in a scrap lead refining factory were studied. Forty-five workers (aged 26–56, mean 42) free from lead exposure in a well-equipped insecticide factory were studied as controls. A health questionnaire was submitted to both groups and clinical laboratory tests were performed before the experiment. Workers with abnormal liver function test results and endocrinological disorders were excluded.

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Samples of blood and urine were collected from each worker. For the erythrocyte arginase assay, three drops of heparinised blood were spotted on to a filter paper and allowed to dry at room temperature. Three discs (6 mm in diameter) of a sample, a blank, and a sample for Hb determination were punched from the dried blood spots with an office puncher.

**Methods**

**ASSAY OF ARGINASE ACTIVITY**

**Reagents**
Distilled water free of ammonium ion was used. Enzymes and coenzyme were purchased from Boehringer, Mannheim, FRG. All chemicals were of the highest purity commercially available.

**Glycine buffer**—Dissolve 7.51 g of glycine in distilled water, adjust the pH to 9.50 with sodium hydroxide (1 mol/l), and dilute to one litre with distilled water.

**Arginine solution**—Dissolve 15.8 g of arginine monohydrochloride (Merck) in distilled water, adjust the pH to 9.50 with sodium hydroxide (1 mol/l), and dilute to 100 ml with distilled water.

**MnCl₂ solution**—Dissolve 5 g of MnCl₂·4H₂O in distilled water and dilute to 100 ml.

**Perchloric acid**—Dilute 3-6 ml of 60% HClO₄ to 100 ml with distilled water (0.33 mol/l).

**2-Oxoglutaric acid sodium salt in phosphate buffer**—Dissolve 20-97 g of Na₂HPO₄·12H₂O, 1-18 g of KH₂PO₄, and 0-6 g of NaOH with distilled water, add 1-19 g of 2-oxoglutaric acid sodium salt, adjust the pH to 7.50, and dilute to one litre with distilled water.

**Urease solution**—5 mg of urease (from jack beans, type S) with 50% glycerol.

**Working reagent for urea determination**—Mix together 3-0 mg of NADH, 18 mg of adenosine-5-diphosphate, 0-5 ml of urease, and 0-05 ml of glutamate dehydrogenase (1200 U/ml 50% glycerol) with 12 ml of phosphate buffer containing 2-oxoglutaric acid sodium salt.

**Urea standard solution**—Dissolve 21-42 mg of urea with 100 ml of distilled water.

**Procedure**
Each disc was placed in a test tube containing 0-15 ml of glycine buffer and 0-05 ml MnCl₂, and this mixture was activated at 37°C for 30 minutes. Next, 0-1 ml of arginine solution was added and the mixture was incubated for 10 minutes. The reaction was stopped by adding 0-2 ml HClO₄ (0.33 mol/l). For the blanks, arginine was added after HClO₄. The mixture was centrifuged for 10 minutes at 3500 rpm. The urea concentration in the supernatant was measured with a centrifugal analyser, CentriflChem 400. The analyser pipette was programmed for 5 μl of sample, 50 μl of diluent water, and 350 μl of reagent. After a lag period of one minute, the rate of decrease of absorbance at 340 nm was measured for one minute at 30°C and compared with that of the 3-57 mmol/l of urea standard solution. The cyanmethaemoglobin method was used for Hb determination. A disc was placed in 5 ml of the reagent and shaken slowly for 40 minutes, the absorbance at 540 nm was then measured.

**Calculation**

\[(U_s - U_b) \times 0.5 \times 10^{-3} \times Hb(g)^{-1} IU/g·Hb\]

where, \(U_s\) and \(U_b\) are urea concentrations in the sample and the blank, respectively. One unit is the activity producing 1 μmol urea for one minute at 37°C. The activity is expressed in units per gram of Hb.

The reproducibility and linearity of the method and the influence of the storage condition on arginase activity were studied.

**OTHER CLINICAL TESTS**
Lead concentrations in blood and urine were measured by anodic stripping voltammetry after the samples had been dried and ashed at 450°C.⁹ Urine δ-aminolaevulinic acid was measured by the method of Tomokuni and Ogata.¹¹ Urine coproporphyrin was measured by the method of Sano and Granick.¹² GOT, GPT, and albumin in serum were measured by the Reitman-Frankel method and the BCG method using an automated analyser (Hitachi, Ltd, Japan).

**Results**
Within-run precision studies showed a 4.09% coefficient of variation (n = 10). Arginase activity showed linearity up to 180 IU/g·Hb and no change in activity at room temperature (25°C) for one week.

The percentage of the relative cumulative frequency of erythrocyte arginase activity in 45 control workers not exposed to lead was plotted on normal probability paper (fig 1). The distribution was of a normal type and the mean arginase activity of erythrocytes was 44.6 ± 11.6 IU/g·Hb. The 49 men occupationally exposed to lead had an activity of 62.9 ± 14.4 IU/g·Hb, the difference in the mean activity between the two groups was significant (p < 0.001).

Table 1 shows the data of lead indicator tests with lead-exposed and lead-unexposed workers. In all tests the values in the lead-exposed group were from three to eightfold higher than those in the controls. The correlation between blood lead and lead indicators was studied and SE, SP, and V in each test were calculated, fixing the basic value of blood lead as 40 μg/100 g.

Figure 2 shows the correlation between blood lead
Table 1  Results from lead indicator tests with lead-exposed subjects and controls

<table>
<thead>
<tr>
<th>No of subjects</th>
<th>Lead workers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 49)</td>
<td>(n = 45)</td>
</tr>
<tr>
<td></td>
<td>GM</td>
<td>DF</td>
</tr>
<tr>
<td>Blood lead (µg/100 g)</td>
<td>33.0</td>
<td>1.5***</td>
</tr>
<tr>
<td>Urine lead (µg/l)</td>
<td>8.6-2</td>
<td>2.6***</td>
</tr>
<tr>
<td>Urine δ-ALA (mg/dl)</td>
<td>6.0</td>
<td>0.032***</td>
</tr>
<tr>
<td>Urine coproporphyrin (µg/l)</td>
<td>48.1</td>
<td>3.7***</td>
</tr>
</tbody>
</table>

ALA=δ-aminolaevulinic acid.
***Significant difference at p < 0.001.

Fig 1  Relative accumulation frequency of erythrocyte arginase activity in workers not exposed to lead.

Fig 2  Correlation between blood lead and erythrocyte arginase. I; \( Y = 0.55X + 40 \), II; \( X = 0.81Y - 13 \).

Fig 3  Correlation between blood lead and urine lead. I; \( \log Y = 0.017X + 1.3 \), II; \( X = 25.9 \log Y - 14 \).

and erythrocyte arginase. The coefficient of correlation was significant at \( r = 0.67 \) (p < 0.001). When 56.2 (mean + 1 SD) IU/g-Hb of erythrocyte arginase activity was set as the screening value in fig 2, SE and SP were 0.96 and 0.65. Therefore V equalled 1.61 (SE + SP). Figure 3 shows the correlation between blood lead and urine lead. The coefficient of correlation was \( r = 0.66 \) (p < 0.001). When 40 µg/l of urine lead concentration was set as the screening value in fig 3, V was calculated to be 1.26 because SE and SP were 0.95 and 0.31. Though SE of urine lead and erythrocyte arginase had the same value, SP and V of urine lead were less than those of arginase.

Fig 4  Correlation between blood lead and urine coproporphyrin. I; \( \log Y = 0.022X + 0.89 \), II; \( X = 18.0 \log Y + 6.30 \).
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Figure 4 shows that the coefficient of correlation between blood lead and urine coproporphyrin was \( r = 0.63 \) (\( p < 0.001 \)). When 100 \( \mu \)g/l of coproporphyrin was set as the screening value, SE, SP, and V were 0.52, 1.00, and 1.52, respectively. Though SE and V of CP were less than that of arginase, SP was better than that of arginase. Figure 5 shows the case of urine \( \delta \)-aminolaevulinic acid (ALA-U). The coefficient of correlation between blood lead and ALA-U was the highest, \( r = 0.75 \). When 5 mg/dl of ALA-U was set as the screening value, SE, SP, and V were 0.73, 0.89, and 1.62, respectively. The V of ALA-U was almost the same as that of erythrocyte arginase.

Table 2 shows the coefficients of correlation between erythrocyte arginase and lead indicators and liver function tests. The highest correlation was found between erythrocyte arginase and blood lead. The degree of correlation with erythrocyte arginase was blood lead > ALA-U > urine lead > coproporphyrin. No correlation was found between erythrocyte arginase and liver function tests such as GGT, GPT, and albumin in serum.

Discussion

Tests of urine lead, urine coproporphyrin, and ALA-U have been traditionally used to evaluate lead toxicosis and lead exposure.\(^{13-15}\) In these tests the screening values are set in relation to the basic value of blood lead.\(^{16,17}\) This paper compares the usefulness of the erythrocyte arginase test with other lead indicator tests.

The degree of correlation with blood lead was ALA-U > erythrocyte arginase > urine lead > urine coproporphyrin. The coefficient of correlation between blood lead and ALA-U was high, \( r = 0.75 \). When 40 \( \mu \)g/100 g of blood lead concentration was fixed as the basic value and 5 mg/dl of ALA-U was set as the screening value, SE and SP were 0.73 and 0.89. Therefore this showed that ALA-U is an efficient indicator for lead exposure because V = 1.62 (SE + SP) (fig 5). The coefficient of correlation between blood lead and erythrocyte arginase was good, \( r = 0.67 \) (\( p < 0.001 \)). The mean arginase activity and SD in controls were 44.6 ± 11.6 IU/g-Hb. If the screening value was set as the mean arginase activity plus 2 SD it was calculated to be 67.8 IU/g-Hb. In this case SE, SP, and V were 0.61, 0.88, and 1.49, respectively. The SE and V in this case were less than those of ALA-U. When the mean arginase activity plus 1 SD (56.2 IU/g-Hb) was set as the screening value for lead exposure, however, SE, SP, and V were 0.96, 0.65, and 1.61, respectively. In this case, SE of the arginase test was higher than that of the ALA-U test. Though there was a 4% probability of overlooking abnormal activity of erythrocyte arginase in a group with blood lead above 40 \( \mu \)g/100 g, the probability of overlooking an abnormal value of ALA-U was 26%.

In the relation between urine coproporphyrin and erythrocyte arginase, SE of the erythrocyte arginase test (0.96) was higher than that of the urine coproporphyrin test (0.52). But SP of urine coproporphyrin (1.00) was higher than that of the arginase test (0.65). Therefore, erythrocyte arginase is more efficient for screening for lead exposure than urine coproporphyrin. By comparison with urine lead, SP and V of the arginase test were higher than those of urine lead though SE in the two tests were the same. The degree of correlation with erythrocyte arginase was blood lead > ALA-U > urine lead > urine coproporphyrin (table 2), which shows that erythrocyte arginase activity reflects well the dose-response effects of lead in the human body.

The serum arginase concentration is reported to be useful as the basis of a liver function test.\(^{18}\) Bremner et
reported that plasma arginase activities in sheep increased with liver damage caused by copper intoxication. As erythrocyte arginase showed no correlation with GOT, GPT, and albumin in serum, it has no relation with liver diseases.

Determination of erythrocyte arginase activity by our method is rapid. Also, dried blood spots are easy to prepare and transport. As the erythrocyte arginase concentration reflects the reaction of the human body against lead, it may be efficiently used in screening for lead exposure. We suggest that 56-2 IU/g-Hb of erythrocyte arginase activity be used as the screening value of lead exposure.

References