Int Arch Occup Environ Health (1987) 59:613–621

International Archives of
Occupational
and
Environmental
Health

Short communication

Zinc protoporphyrin as an indicator of lead exposure: precision of zinc protoporphyrin measurements

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Summary. In lead-exposed workers many factors affect the relationship between the levels of lead and of zinc protoporphyrin (ZPP) in blood. When the zinc protoporphyrin level is used to assess the lead in the blood level, the analytical accuracy of the ZPP and the PbB determinations should be known. Also the variability of lead exposure over time is an important parameter of this assessment. The absolute and relative precision of ZPP measurements with two brands of ZPP meters (AVIV and ESA) were compared. The absolute precision of the ZPP measurements is not constant for the AVIV meter, but is constant for the ESA meter. The relative precision for both brands of meters decreases with increasing ZPP levels. Between the AVIV and the ESA meters, a significant difference in response, irrespective of the kind of anticoagulant used, was observed. The regression equations between PbB and ZPP levels were studied. Different factors can affect the relationship between PbB and ZPP, e.g. variability in lead exposure, the time-lag between the increase of PbB and ZPP, and the precision and accuracy of PbB and ZPP measurements.

Key words: Precision – Accuracy – Standardization – Zinc protoporphyrin – Lead in blood

Introduction

In addition to the lead in blood concentration (PbB), some biological effect parameters are used both for monitoring the state of health and/or for assessment of the health risk. Some indicators of a biological effect on the heme synthesis are the activity of the enzyme 5-aminolevulinic acid dehydratase in blood, the concentrations of 5-aminolevulinic acid and coproporphyrin in urine and of porphyrins in blood [15]. It has been shown that the concentration of porphyrins in blood offers the best predictor of the individual lead in blood level in workers

with a PbB level, which was rather constant over a period of at least six months (PbB 1–4 μmol/l) [5]. Among these porphyrins, zinc protoporphyrin (ZPP) is the most simple to determine by means of a solid state type fluorometer. The ZPP level can be measured frequently in a drop of blood, with little inconvenience to the worker and at low cost. When the estimated ZPP does not exceed a predetermined, acceptable level, determination of PbB may be omitted. Therefore, routine monitoring of ZPP in blood may be regarded as an effective tool in the prevention of overexposure to lead, both in occupational and in environmental health. However, it should be kept in mind that, when PbB levels are estimated from ZPP, an error of ± 0.5 μmol/l Pb/l blood may be made by this estimation [5].

Beside this problem, standardization of the ZPP measurement is necessary to minimize the variability of the dose (PbB)-effect (ZPP) relationship. This variability is due to three errors:

- the error in the PbB determination (the precision of the PbB determination);
- the error in the ZPP determination (the precision of the ZPP determination); and
- biological variation in the relationship between PbB and ZPP.

When the precision of the PbB and/or ZPP determinations are poor, the variability of the relationship is also influenced; however, this is usually not the case [5].

The accuracy of the PbB determination can be checked by round-robin studies and the use of control samples. This aspect will not be dealt with in this study.

The object of the present study was to:

- improve the precision and the accuracy of the ZPP measurements; and
- present a critical evaluation of the relationship between PbB and ZPP levels under conditions of stable occupational exposure to lead. As criterion of stability, the analytical precision of the PbB-value was used; if the second measurement of PbB was within the range of the first PbB ± SD (standard deviation), then exposure was stated to be stable [5].

Materials and methods

Comparison of ZPP meters

ZPP was measured by using two brands of meters: the AVIV Hematofluorometer and the ESA Model 4000. Both fluorometers measure the ratio of ZPP to Hb.

AVIV meter

The AVIV meter is provided with a set of three calibration glasses, colored with Rhodamine B. The values of the glasses are given by AVIV as:

(1) 2.1 ± 0.5 μg ZPP/g Hb;
(2) 7.4 ± 0.5 μg ZPP/g Hb; and
(3) 33.5 ± 1.0 μg ZPP/g Hb.

With each glass, 100 measurements were made with increasing ZPP levels and with random sequences.

ESA meter

The ESA meter is also provided with a set of three calibration glasses, colored with Rhodamine B in Krylon No. 1602 Ultra Flat Black. The values of the glasses are given by ESA as:

(1) "green" $-0.7 \pm 1\%$ µg ZPP/g Hb,
(2) "yellow" $10.7 \pm 1\%$ µg ZPP/g Hb, and
(3) "red" $29.5 \pm 1\%$ µg ZPP/g Hb.

With each glass, 99 measurements were made with increasing ZPP levels and with random sequences.

Anticoagulants

Blood of six healthy non-exposed volunteers was treated with three different anticoagulants: heparin, Na-K-EDTA and Na-citrate. The ZPP value was measured with both meters on the same day, one week after the collection of blood.

Results

Precision

From the measurements with the AVIV meter, the following conclusions could be drawn:

— The sequence of measurements affects the ZPP value, i.e. when measuring subsequently increasing levels, the ZPP levels were slightly lower than when measuring in random order (Table 1a).

Table 1. Influence of sequence of measurements on the ZPP-value. ZPP in µg/g Hb

(1a) AVIV meter

Calibration glass	Sequence						t	P
	Increasing			Random				
	$\bar{x}$	SD	n	$\bar{x}$	SD	n		
1	1.9	0.2	45	2.0	0.2	55	−2.20	<0.05
2	7.3	0.4	45	7.4	0.4	55	−1.40	0.10–0.20
3	39.6	1.0	45	40.5	2.2	55	−1.73	0.10

(1b) ESA meter

Calibration glass	Sequence						t	P
	Increasing			Random				
	$\bar{x}$	SD	n	$\bar{x}$	SD	n		
"Green"	−0.7	0.1	45	−0.8	0.1	54	1.67	0.10
"Yellow"	10.8	0.1	45	10.7	0.2	54	0.64	>0.20
"Red"	29.1	0.3	45	29.1	0.2	54	0.39	>0.20

Table 2. Relative standard deviation (RSD) per calibration glass. Measurements were made in random order

(2a) AVIV meter

Calibration glass	μg ZPP/g Hb			
	$\bar{x}$	SD	n	RSD (%)
1	2.0	0.22	55	11.0
2	7.4	0.42	55	5.8
3	40.5	1.75	55	4.3

(2b) ESA meter

Calibration glass	μg ZPP/g Hb			
	$\bar{x}$	SD	n	RSD (%)
"Green"	−0.8	0.10	54	12.5
"Yellow"	10.7	0.14	54	1.3
"Red"	29.1	0.18	54	0.6

- When the meter is used for longer than 20 min, the time between two succeeding measurements must be prolonged from the proposed time of 30 s to 45 to 60 s; if not, too low values will be measured.
- The mean SD increases with increasing ZPP levels; the absolute precision is not constant and also the relative precision decreases with increasing ZPP-values (Table 2a).

From the measurements with the ESA meter, the following conclusions could be drawn:

- The sequence of measurements does not affect the ZPP value (Table 1b), with exception of the negative value.
- The duration of the warming-up period does not influence the ZPP values.
- The absolute precision is good, 0.1 to 0.4 μg ZPP/g Hb; the relative precision decreases with increasing ZPP values (Table 2b).

Influence of anticoagulants

The ZPP values of blood samples mixed with different anticoagulants ranged from 0.8 to 2.5 μg ZPP/g Hb (Table 3); the precision of the values' SD was 0 to 0.21 μg ZPP/g Hb. Comparing the influence of the anticoagulants, the following conclusions can be drawn:

- When using citrate, the ESA meter yields considerably higher ZPP values than the AVIV-meter: citrate $ZPP_{ESA} = (0.70 \pm 0.23) + ZPP_{AVIV}$ (μg ZPP/g Hb).
- When using heparin and EDTA, the differences between the ZPP-values are small: heparin $ZPP_{ESA} = (0.11 \pm 0.11) + ZPP_{AVIV}$ (μg ZPP/g Hb), EDTA $ZPP_{ESA} = (0.28 \pm 0.12) + ZPP_{AVIV}$ (μg ZPP/g Hb).

Table 3. ZPP values in μg/g Hb (arithmetic average and SD) for different anticoagulants

Sample	Heparin		EDTA		Citrate	
	ESA	AVIV	ESA	AVIV	ESA	AVIV
1	1.63 ± 0.06	1.58 ± 0.09	1.83 ± 0.06	1.48 ± 0.14	2.50 ± 0.17	1.80 ± 0.00
2	1.17 ± 0.06	1.10 ± 0.00	1.27 ± 0.06	0.88 ± 0.09	1.70 ± 0.10	0.97 ± 0.12
3	1.03 ± 0.06	1.00 ± 0.00	1.23 ± 0.06	0.83 ± 0.09	1.43 ± 0.06	0.98 ± 0.09
4	1.50 ± 0.00	1.50 ± 0.00	1.67 ± 0.12	1.55 ± 0.07	1.73 ± 0.06	1.27 ± 0.06
5	1.68 ± 0.09	1.38 ± 0.06	1.70 ± 0.10	1.48 ± 0.06	2.28 ± 0.17	1.53 ± 0.15
6	1.22 ± 0.09	1.00 ± 0.00	1.32 ± 0.09	1.15 ± 0.07	2.10 ± 0.10	1.02 ± 0.14

- A paired Student's *t*-test was performed on the ZPP values acquired with both meters, with the following result:
  for heparin $t = 2.28$, $0.05 < P < 0.10$
  for EDTA $t = 5.39$, $P < 0.05$
  for citrate $t = 5.94$, $P < 0.05$
  The criterion used for equality was $P < 0.20$. A significant difference in response between the ESA and the AVIV meters exists, irrespective of the kind of anticoagulant used.
- With the ESA meter differences are also found between ZPP values in heparin and EDTA blood, in heparin and citrate blood and in EDTA and citrate blood. Using the AVIV-meter, no differences are observed.

Accuracy

In a previous experiment [6], the ZPP levels in EDTA blood of 20 exposed workers were measured on the same day with AVIV and ESA meters. The range was 2 to 30 μg ZPP/g Hb. Considerable differences in the measured values were observed: $ZPP_{AVIV} = 0.09 + 0.78\ ZPP_{ESA}$ (unexplained variance: $1 - r^2 = 0.011$). The systematic difference in ZPP values between measurements with the AVIV and ESA meters was 22 to 24%, although the correlation was excellent.

Discussion

Different ZPP-meters may considerably differ in measured ZPP levels, depending on the calibration of the meters; the relative standard deviation may reach 22 to 24%. Moreover, an absolute difference of up to 0.3 μg ZPP/g Hb may exist for the most commonly used anticoagulant, EDTA. Therefore, there is a need for standardization.

Several studies on the relationship between PbB and porphyrins have been published as shown in Table 4. Different units were used, i.e. for PbB: μg/100 ml or μg/l; for ZPP μg/100 ml whole blood, μg/100 ml RBC, μg/g Hb or μmol/mol Hb. The first step for standardization should be to use the same, pre-

Table 4. Regression equation of the relation between PbB- and ZPP-levels in male lead workers

Reference		Equation	Correlation (r)	SEE[a]	n	Units		Range PbB (μg/100 ml)
						PbB	ZPP	
[1]		$\log ZPP = 0.97 + 0.02\ PbB$	0.84		95	μg/100 ml	μg/100 ml blood	10–90
[3]		$\log ZPP = 2.5 \log PbB - 2.28$	0.60		144	μg/100 ml	μg/100 ml blood	30–110
[4]	a	$\log ZPP = 1.04 + 0.023\ PbB$	0.91			μg/100 ml	μg/100 ml blood	20–80
	b	$\log ZPP = 1.017 + 0.018\ PbB$	0.74					
[5]		$\log ZPP = 1.16 + 0.00153\ PbB$	0.70	0.223	55	μg/l	μg/100 ml RBC	5–90
[7]		$\log ZPP = 0.839 + 0.014\ PbB$	0.77		72	μg/100 ml	mmol/mol Hb	10–100
[8]		$\log ZPP = 0.54 + 0.017$	0.87		35	μg/100 ml	μg/100 ml blood	20–100
[9]		$ZPP = 9.49\, e^{0.04\ PbB}$	0.83		211	μg/100 ml	μg/100 ml blood	10–90
[12]		$\log ZPP = 0.89 + 0.019\ PbB$	0.87	0.232	142	μg/100 ml	μg/100 ml blood	5–80
[13]		$\log ZPP = 0.44 + 0.052\ PbB$	0.88		707	μg/100 ml	μg/100 ml blood	—

[a] SEE: Standard error of the estimate

Table 5. Availability of data on precision and accuracy of PbB and/or ZPP

Reference	PbB		ZPP	
	Precision	Accuracy	Precision	Accuracy
[1]	+	−	+	+
[3]	−	−	−	−
[4]	−	−	−	−
[5]	+	+	+	+
[7]	−	+	−	−
[8]	−	−	−	−
[9]	−	−	−	−
[12]	+	−	+	−
[13]	−	−	−	−

−: No information given
+: At least qualitative information

Table 6. Calculated ZPP values (μg/100 ml) of two different PbB levels, using the equations presented in Table 4

Reference		PbB (40 μg/100 ml blood) (1.93 μmol/l)	PbB (60 μg/100 ml blood) (2.90 μmol/l)
[1]		59	148
[3]		53	146
[4]	a	91	263
	b	55	125
[5]		27	55
[7]		68	130
[8]		17	36
[9]		47	105
[12]		45	107
[13]		44	479

ferably Système International (SI) units for PbB μmol/l and for ZPP μmol/mol Hb.

To predict PbB values from ZPP values, one needs a measure of uncertainty of the prediction, e.g. standard error of the estimate SEE), confidence interval width, etc. However, only two studies presented the SEE.

Table 5 shows that most authors did not present data on the accuracy and precision of the analytical methods. Moreover, to predict PbB, there is a need for a population with a rather stable exposure, selected on the basis of stable lead levels in blood. This was only the case in two studies [4, 5].

The usually assumed hematocrit value of 42% is questionable with reference to lead-exposed workers and controls with different ethnic origins [3, 13].

The type of exposure and/or the type of exposed groups probably differ in some of the studies. This lack of standardization leads to different ZPP concentrations calculated from the same PbB (Table 6), assuming that the precision and accuracy of the PbB level was similar in all studies.

Differences in sex may lead to differences in the relationship between PbB and ZPP levels [11], probably due to a sex-related difference in the endocrine system [14]. Other factors which affect the PbB-ZPP relationship are the time-lag between increase of PbB and of ZPP [2], the stability of Pb-exposure expressed as PbB and the duration of Pb-exposure [10], although the last was not confirmed in one study [5].

Conclusion

It can be concluded that the ZPP measurement should be standardized or at least compared in interlaboratory comparison studies. The absolute precision of the ZPP is not constant for the measurements with the AVIV meter, while the absolute precision is good for the ESA meter measurements. The relative precision for both brands of meters decreases with increasing ZPP levels.

The systematic difference in ZPP values in EDTA blood between measurements with the AVIV and ESA meters was 22 to 24%. As an anticoagulant, EDTA or heparin can be used, although heparin should not be used in comparison studies between the two brands of ZPP meters.

In comparing regression equations between PbB and ZPP levels and in estimating PbB levels from ZPP, many requirements have to be fulfilled:

- the accuracy of the PbB determination should be known from interlaboratory comparison studies; and
- the exposure should be stable. This can be checked by an intra-individual comparison of PbB levels. Preferred units are for PbB: μmol/l, and for ZPP μmol/mol Hb.

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Received September 29, 1986 / Accepted April 24, 1987