Clinica Chimica Acta, 194 (1990) 229–234
Elsevier

CCA 04887

Short Communication

The contribution of sex, electrophoretic phenotype, pregnancy and race to the variability of delta-aminolevulinate dehydrase (ALADH) levels in human erythrocytes. A study in Black mixed Brazilians

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(Received 15 June 1990; revision received 24 September 1990; accepted 25 September 1990)

Key words: Variability; ALADH-level; Sex; Pregnancy; Electrophoretic-phenotype; Race

Introduction

Delta-aminolevulinate dehydrase (EC 4.2.1.24) (ALADH) catalyzes the condensation of delta-aminolevulinate to form porphobilinogen in the porphyrin biosynthesis pathway [1]. In human erythrocytes ALADH exhibits great variability due both to genetic and environmental causes [2–6]. Genetic studies of human ALADH showed similar activity in twins and siblings [2,4], autosomal recessive inheritance of deficiency [6,7], depression of activity in heterozygotes for the deficient allele [7], and no activity difference between the two common alleles ALADH*1 and ALADH*2 [8]. Environmental studies, revealed that ALADH is very sensitive to inhibition by lead, making ALADH measurement an indicator of lead poisoning [9,10].

We investigated the role of sex, pregnancy, race and electrophoretic phenotypes on the variability of ALADH in human erythrocytes.

Subjects and methods

Healthy adults

University students and blood donors without history of occupational exposure to lead, were sampled. Subjects with history of alcoholic beverage ingestion within

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the last twenty-four hours were excluded [5]. Information on health status, sex and age were obtained. Black admixture was subjectively estimated from the degree of skin pigmentation, hair type and facial features [11]. The efficiency of this classification has been critically reviewed and substantiated [12]. Thus, each subject was assigned to one of five groups in a scale ranging from the lightest to the darkest as follows: White, Light Mulatto, Medium Mulatto, Dark Mulatto and Black.

Parturients

This sample was collected at a public maternity hospital for indigent patients. Criteria for sampling parturients were the same as those for healthy adults, plus a history of normal gestation and delivery, no recent blood transfusion or medical treatment by drugs. Interviews and blood collections were performed within the first day of delivery.

Laboratory procedures

Blood samples were collected in plastic tubes containing dry heparin, and refrigerated upon collection. ALADH assay was carried out within six hours after blood drawing following a procedure described elsewhere [3]. Dithiothreitol (DTT) was added to cell lysates to eliminate any possible inhibition of ALADH by lead which may have been present in the urban environment [3,6].

Each blood sample was simultaneously assayed in duplicate. The difference between the two assays was used to estimate the coefficient of repeatability as described elsewhere [13]. On eight occasions out of 420 (1.9%), the difference between the two assays was greater than the coefficient of repeatability. These eight samples were excluded. Hematocrit was done by the traditional Wintrobe method and ALADH activity was expressed as nmol of porphobilinogen generated per ml of blood cells per hour at 37°C (nmol PBG/ml RBC/hour) [3]. Starch gel electrophoresis for ALADH phenotype was carried out according to methods previously described [8].

Statistics tests were performed in a PCxt II ITAUTEC microcomputer using analysis program from SPSS/PC + System.

Results

Healthy adults

The distribution of ALADH activity in the blood of 223 adults (96 females), age 14 to 50, followed a normal curve (Kolmogorov–Smirnov Goodness of fit test = 0.675; $P > 0.10$) with a mean value of 1404, standard deviation of 309 and a range from 213 to 2409 nmol PBG/ml RBC/hour. A unique observation, of activity as low as 213, in a 36-year-old Dark Mulatto male, was considered to be a heterozygote for the enzyme deficiency for the following reason: the histogram of ALADH activity levels showed that this case was 3.8 standard deviations below the

mean. All statistical tests were performed after exclusion of this deficient sample. Thus, the distribution of activity had a mean of 1 409 ± 299 and a range from 712 to 2 409 nmol PBG/ml RBC/hour.

Analysis of variance (ANOVA) showed no contribution of racial groups to the enzyme activity variability ($F = 0.7$; $P > 0.10$) but disclosed a significant sex effect ($F = 7.8$; $P < 0.005$), higher in females, and ALADH electrophoretic types ($F = 6.2$; $P < 0.05$) higher in ALADH 1 phenotype. However, there is no interative effect between sex and phenotype ($F = 0.003$; $P > 0.90$). Finally, a regression analysis of enzyme activity on age yielded non significant results ($r = 0.04$; $P > 0.50$).

Parturients

ALADH enzyme activity in 189 parturients followed a normal distribution (Kolmogorov–Smirnov Goodness of fit test – 0.59; $P > 0.80$) with a mean of 1 647 ± 432 and a range from 552 to 3 321 nmol PBG/ml RBC/hour. A possible case of heterozygosity for ALADH deficiency was observed in a 17 years old, Light Mulatto female. Her ALADH level was 2.5 SD below the mean. Excluding this unique observation, the distribution did not change significantly: mean 1 653 ± 426; range from 778 to 3 322 nmol PBG/ml RBC/hour.

Table I shows that ALADH activity is higher in the female than in the male and even higher in parturients than non-parturient (healthy adult) females.

Activity within phenotypes

Table II shows the average enzyme activity as a function of electrophoretic phenotype, sex and racial groups. Considering the wide range of enzyme activity and the rather small frequency of ALADH 2-1 phenotypes, the five racial groups were pooled into three groups: White + Light Mulatto = *Light*; Medium Mulatto = *Medium* and Dark Mulatto + Black = *Dark*.

The results given in Table II show that individuals of ALADH 1 phenotype have higher enzyme activity than carriers of ALADH 2-1 regardless of sex and racial group. Within the ALADH 1 phenotype group there is a peculiar effect of the

TABLE I

ALADH enzyme activity in healthy adults males and females, and in female parturients

	Sample type		
	Male	Female	Parturient
n	126	96	188
Mean	1363	1470	1653
SD	287	304	426
	$t = 2.67$; $P < 0.01$ Male versus female	$t = 4.17$; $P < 0.0001$ Female versus parturient	

TABLE II

ALADH activity levels in healthy adults as a function of sex, racial groups and electrophoretic phenotypes

Sex	Phenotypes	Racial groups		
		Light	Medium	Dark
Male	1	1405	1359	1316
		(53)	(29)	(34)
	2-1	1313	1178	954
		(4)	(3)	(2)
Female	1	1490	1515	1541
		(66)	(7)	(8)
	2-1	1348	1493	–
		(12)	(1)	

ANOVA: $F = 4.4$; $P < 0.01$; for sex: $F = 7.8$; $P < 0.005$; for phenotype: $F = 6.2$; $P < 0.05$; for racial groups: $F = 0.7$; $P > 0.10$.

proportion of Black ancestries which has opposite direction in females compared to males: the higher the proportion of black ancestries in females, the higher the ALADH activity while in males it is the reverse (Table II).

Finally, the activity of ALADH in parturients, contrasts to that of adult females for not showing the effect of phenotype ($F = 0.86$; $P > 0.10$). Probably, the cause of enzyme rising in parturients is strong enough to supplant the phenotype effect.

Discussion

Identification of the causes of heterogeneity in red blood cell ALADH requires the collection of a population sample which reflects all possible sources of variation. Therefore, we explored variables such as sex, age, electrophoretic phenotype, pregnancy and proportion of black racial admixture.

The effect of sex

Higher hepatic ALADH activity in female than in male siblings has been described in mice and attributed to hormonal control [14]. The male–female differences that had been reported in human erythrocytes ALADH were attributed to a secondary effect of lead exposure [15]. In the present paper, however, the possibility of a similar secondary effect of lead is excluded for two reasons: first, there is no known environmental exposure among the tested university students either male or female; second, the ALADH assays were carried out in the presence of DTT. Thus, the association of the female sex with elevated ALADH activity may be due to sex hormones, such as pregnandiol, since the latter one is known to have indirect effect on ALADH levels through the induction of ALA synthetase [16,17].

The effect of pregnancy–delivery

Pregnancy in mice increases hepatic ALADH activity by 50 to 60 percent due to increased hematopoiesis or hormonal control or both [14]. The association of increased hematopoiesis and increased ALADH activity is well established in bled rabbits and the enzyme activity is directly related to the reticulocytes count [18]. In human patients with Addisonian or pernicious anaemia, the increased ALADH enzyme activity associated with a response to vitamin-B12 therapy also appears to be closely related to the reticulocyte count [18]; recent haemorrhage or hemolysis frequently leads to an increase in the enzyme activity [5]. Thus, the present finding of considerable higher levels of ALADH activity in parturients within the first day of delivery compared to other females, may result from a joint effect of pregnancy, through erythropoiesis which remains increased in the postpartum period [19,20], plus the effect of haemorrhage [5] occurring during delivery. In the absence of ALADH activity studies during pregnancy, specially in the pre-partum days, makes it impossible to assess the specific contribution of pregnancy and delivery to the elevation of ALADH activity in parturients.

The effect of phenotypes

Out of eight population studies determining the ALADH electrophoretic phenotypes, only two carried out the enzyme assay simultaneously [8, present data]. The two results are contradictory. Battistuzzi et al. [8] observed no effect of ALADH phenotypes on enzyme activity while the present results showed higher levels in carrier of ALADH 1 phenotype (Table II). Overall, the means of ALADH activity for phenotypes 1 ($n = 197$) and 2-1 ($n = 22$) were 1 420 and 1 289, respectively. However, there is a joint effect of sex, racial group and phenotypes which is shown in Table II and deserves further investigation.

Acknowledgements

This work was partly supported by CNPq National Research Council research grants (PIG/CNPq 40.0340/85) and by CNPq Research Scholarships: CNPq 301.748-76/GE (ESA) CNPq 30.5857/85-1/GE (MGFS) and CNPq 800.398/87-4 (AFBGD).

References

1 Gibson KD, Neuberger A, Scott JJ. The purification and properties of δ-aminolaevulic acid dehydrase. Biochem J 1955;61:618–629.
2 Sassa S, Granick S, Bickers DR, Levere RD, Kappas A. Studies on the inheritance of human erythrocyte δ-aminolevulinate dehydrase and uroporphyrinogen synthetase. Enzyme 1973;16:326–333.
3 Granick JL, Sassa S, Granick S, Levere RD, Kappas A. Studies in lead poisoning. II. Correlation between the ration of activated to inactivated δ-aminolevulinic acid dehydratase of whole blood and the blood lead level. Biochem Med 1973;8:149–159.

4 Sassa S, Granick S, Kappas A. Effect of lead and genetic factors on heme biosynthesis in the human red cell. Ann NY Acad Sci 1975;244:419–439.
5 Morgan JM, Burch HB. Erythrocyte delta-aminolevulinic acid dehydratase activity as a measure of lead exposure. J Lab Clin Med 1975;85:746–755.
6 Bird TD, Hamernyik P, Nutter Y, Labbe RF. Inherited deficiency of delta-aminolevulinic acid dehydratase. Am J Hum Genet 1979;31:662–668.
7 Doss M, Benkmann HG, Goedde HW. δ-Aminolevulinic acid dehydrase (porphobilinogen synthase) in two families with inherited enzyme deficiency. Clin Genet 1986;30:191–198.
8 Battistuzzi G, Petrucci R, Silvagni L, Urbani FR, Caiola S. δ-aminolevulinate dehydrase: a new genetic polymorphism in man. Ann Hum Genet 1981;45:223–229.
9 Hernberg S, Nikkanen J. Enzyme inhibition by lead under normal urban conditions. Lancet 1970;1:63–64.
10 Oyanguren C, Oyanguren H, Guzman E, Vega F, Labarca Y. δ-aminolevulinico dehidrasa y protoporfirina eritrocitarias en la exposicion a plomo. Rev Med Chile 1982;110:439–443.
11 Krieger H, Morton NE, Mi MP, Azevêdo ES, Freire-Maia A, Yasuda N. Racial admixture in northeastern Brazil. Ann Hum Genet 1965;29:113–125.
12 Azevêdo ES. Subgroup studies of black admixture within a mixed population of Bahia, Brazil. Ann Hum Genet 1980;44:55–60.
13 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;8:307–310.
14 Russel RL, Coleman DL. Genetic control of hepatic δ-aminolevulinate dehydratase in mice. Genetics 1963;48:1033–1039.
15 Fernícola NAGG, Azevêdo FA. Níveis de chumbo e atividade da desidratase do ácido δ-aminolevulínico (δ-ALAD) no sangue da população da Grande São Paulo, Brasil. Rev Saúde Públ 1981;15:272–282.
16 Williams WJ, Beutler E, Erslev AJ, Rundles RW. Hematologia Rio de Janeiro: Ed Guanabara Koogan 1976;97.
17 Wintrobe MM, Lee GR, Boggs DR, Bithele TC, Athens JW, Forster J. Hematologia Clinica Tomo I. Buenos Aires: Ed Intermedica. 4th ed. 1979;171.
18 Battistini V, Morrow JJ, Ginsburg D, Thompson G, Moore MR, Goldberg A. Erythrocyte delta-aminolaevulic acid dehydrase activity in anaemia. Br J Hematol 1971;20:177–184.
19 Imaz FU. Obstetricia Practica. Buenos Aires: Ed Intermedica. 4th ed 1977;88.
20 Levin J. Distúrbios hematológicos da gravidez. In: Burrow G, Ferris TF. Complicações clínicas na gravidez. Rio de Janeiro: Ed Roca, 1st ed. 1983;60.