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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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
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 1409 ± 299 and a range from 712 to 2409 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 interactive 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 1647 ± 432 and a range from 552 to 3321 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 1653 ± 426; range from 778 to 3322 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**

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Male</th>
<th>Female</th>
<th>Parturient</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>126</td>
<td>96</td>
<td>188</td>
</tr>
<tr>
<td>Mean</td>
<td>1363</td>
<td>1470</td>
<td>1653</td>
</tr>
<tr>
<td>SD</td>
<td>287</td>
<td>304</td>
<td>426</td>
</tr>
<tr>
<td>( t = 2.67, \ P &lt; 0.01 )</td>
<td>( t = 4.17, \ P &lt; 0.0001 )</td>
<td>Male versus female</td>
<td>Female versus parturient</td>
</tr>
</tbody>
</table>
TABLE II
ALADH activity levels in healthy adults as a function of sex, racial groups and electrophoretic phenotypes

<table>
<thead>
<tr>
<th>Sex</th>
<th>Phenotypes</th>
<th>Racial groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
<td>Medium</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>1405</td>
</tr>
<tr>
<td></td>
<td>(53)</td>
<td>(29)</td>
</tr>
<tr>
<td>2-1</td>
<td>1313</td>
<td>1178</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(3)</td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
<td>1490</td>
</tr>
<tr>
<td></td>
<td>(66)</td>
<td>(47)</td>
</tr>
<tr>
<td>2-1</td>
<td>1348</td>
<td>1493</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(1)</td>
</tr>
</tbody>
</table>

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: $F = 0.7; P > 0.10$.

The 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 bleed 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 reticulocytic 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.

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