Delta-Aminolevulinic Acid Dehydratase Activity in the General Population of Southern Minas Gerais, Brazil

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Abstract: Blood samples were collected from 113 subjects (56 males and 57 females) living in the district of Alfenas, in southern Minas Gerais state, Brazil, to establish reference values for delta-aminolevulinic acid dehydratase activity (ALA-D, EC 4.2.1.24). The state of health of the population was confirmed by hematological and biochemical parameters analyzed in blood and urine samples. ALA-D determination was performed according to the Berlin & Schaller spectrophotometric method. Distribution may be regarded as according to normal distribution and reference values obtained, in µmol. min⁻¹ L⁻¹ erythrocytes, were: mean (± SD) = 54.5 (± 9.8); 95% confidence interval = 52.7–56.4; lower reference value (mean–2 SD) = 34.9. Mean ALA-D activity was higher than any other published elsewhere and the reference values established are useful as a baseline for evaluating ALA-D activity when monitoring persons exposed to lead. Age, gender, drinking, or smoking did not significantly alter (Student t-test, p≤0.05) the reference values for ALA-D.

Key words: Delta-aminolevulinic acid dehydratase, Reference value, Gender, Age, Drinking, Smoking, Blood ALA-D

Introduction

Markers of lead intoxication have been developed based on their capacity to identify lead intoxication at a preclinical, i.e., biochemical stage of manifestation. δ-Aminolevulinic acid dehydratase [EC 4.2.1.24], a polymorphic enzyme¹,² that converts aminolevulinic acid to porphobilinogen in the heme biosynthetic pathway, is an effective biomarker of exposure to lead regarded as highly sensitive to inhibition from lead and is an indicator of recent exposure to lead³,⁴.

For proper interpretation of biomonitoring results, it is necessary to establish reference values for the internal dose and effect biomarkers in a population that is not occupationally exposed⁵–⁸.

The main purpose of establishing reference values is to obtain guideline values, which may be used to identify subjects with increased internal exposure to a defined environmental chemical, i.e., subjects whose level of internal dose is above the range of background exposure for the population in general⁹. In this case, the reference values resulted from the sum of the baseline concentration (background levels regulated by physiological mechanisms) and, possibly, of the amount of analyte originated from environmental of individual exposure. The occupational exposure can then be demonstrated when the value recorded for the determinants exceeds the reference values⁹.

A variety of factors that can influence these values must be considered for evaluation of reference values, such as: age, gender, consumption of alcoholic drinks and tobacco, ingestion of medicines, hobby activities, place for living and working, among others, that may imply in alterations of basal bioindicator levels⁸,¹⁰,¹¹.

This study was designed to examine ALA-D activity in the population in general of the district of Alfenas, in southern
Minas Gerais state (Brazil) that is, to establish reference values for this enzyme. The effects of some biological (gender and age) and of some personal habits (drinking and smoking) on ALA-D background levels were also evaluated.

Materials and Methods

Blood samples were obtained from 128 volunteers who were healthy adults (64 men and 64 women), without occupational exposure to lead and ages between 18–60 years old (mean ± SD = 33.5 ± 8.4) and residents in the district of Alfenas (southern Minas Gerais state, Brazil). This region is an important agricultural area with a low density of traffic and little industry. The samples were drawn by venipuncture using disposable heparinized syringes and placed in polypropylene tubes until the analysis, performed after a maximum of two hours subsequent to blood collecting.

All individuals were requested to fill out a questionnaire to collect information about their personal habits (smoking, alcohol intake, hobby activities), place of residence, age, type of work, food intake, ingestion of pharmaceutical products, among others. The subjects were classified by gender, age and smoking and drinking habits. We classified people smoking more than 5 cigarettes a day as smokers (21 men and 8 women) and those consuming some kind of alcohol beverage at least 4 times a week (minimum 20 g alcohol a week) as drinkers (47 men and 13 women).

Some volunteers were excluded from the study: those who presented any complaints as to their state of health (5 subjects) and those with altered biochemical parameters (blood glucose, total cholesterol, bilirubin, AST and ALT, urea, triglycerides, total proteins, creatinine in serum and urine and urinalysis - qualitative analysis of non-normal elements) and hematological parameters (hemogram) (10 subjects), with 113 subjects representing the reference population (57 women and 56 men). Table 1 summarizes demographic data of the population. The research protocol was approved by the Ethical Committee for Research from the University.

ALA-D activity was measured using the European standardized method with spectrophotometric determination carried out at 555 nm. The Student t-test was used to verify statistical differences between subgroups (using statistical software Statgraphics, Statistical Graphics System, STSG) subsequent to finding a normal distribution (Shapiro-Wilk) of the values. Differences were considered as statistically significant at p ≤ 0.05.

Results

Reference values of ALA-D activity were expressed in µmol. min⁻¹.L⁻¹ erythrocytes as mean values (± standard deviation), 95% confidence interval, lower/upper reference value, experimental range in the total population (n=113) and in subgroups according to gender and age of the subjects (Table 2). Table 3 shows the results of ALA-D according to age group, smoking and drinking habits for men and for women. ALA-D was not significantly different in males and in females gender nor according to the age group of the volunteers. There was no difference in ALA-D results for non-smokers and for smokers, nor for non-drinkers and for drinkers (Student’s t-
Discussion

The population was selected at random and answered to the recommended characteristics necessary to establishing reference values\(^1\), sufficiently large to cover a representative part of the population in general of the district of Alfenas and to afford an evaluation of the effect of relevant confounders at the level of the bioindicator. Besides, these were healthy people, not exposed occupationally to metals, ranging from 18 to 60 years, both male and female.

Reference values can be expressed in different ways depending on a distribution of results (normal, non-normal) and on the purpose in applying them in biomonitoring programs\(^6\,7\). In a Gaussian distribution of the results, they are expressed as mean ± standard deviation, confidence range and reference values (mean ± SD)\(^10\). For practical purposes, reference values are intended to characterize the lower or upper margin of current background levels of the bioindicator in the population in general\(^9\).

When evaluating ALA-D activity, that is inhibited in
workers exposed to lead, it would seem more reasonable to use the lower reference value, expressed as mean – 2 SD (34.9 µmol. min⁻¹. L⁻¹ erythrocytes) to evaluate individual data in distinguishing occupational/non-occupational exposure (normal distribution). In biological monitoring of workers exposed to lead on a group basis, one has to consider the comparison of the distribution frequencies of ALA-D activity in both groups, exposed/non-exposed rather than mean or reference interval.

In the 113 volunteers in this study, the mean ± SD ALA-D activity was 54.5 ± 9.8 µmol. min⁻¹. L⁻¹ erythrocytes. A slightly lower mean was reported by Schuhmacher et al.¹³, 43.5 ± 8.7 µmol ALA-D. min⁻¹. L⁻¹ erythrocytes in the Tarragona Province, in Spain (two areas, industrialized and predominantly agricultural). An investigation conducted by Fernicola & Azevedo¹⁴, in the polluted metropolitan area of São Paulo city, Brazil, found a “normal” mean for ALA-D, of 47.2 ± 9.5 µmol. min⁻¹. L⁻¹ erythrocytes (n=56). Perhaps the differences found may be attributed to different levels of exposure to environmental lead in the regions mentioned and/or other factors owing to personal habits, lifestyles, and hobby activities, among others.

Men made up 49.55% of the population studied, and women 50.44%. The mean age was 33.5 ± 8.4 (range 18–60) years. ALA-D activity did not differ statistically for males and females, nor between the two age groups studied (18–36 years and >36 years old). There is lack of data pertaining to the influence of age or gender on ALA-D activity in the population in general, not occupationally exposed to metals. In a study on the inhabitants of an industrialized city, Sechi & Alessio⁵) reported that increasing age decreases ALA-D activity, especially for women over 60 years of age. Unless the mean ALA-D in older group (37–60 years) had been slightly lower than the observed in younger group (18–36), no statistically significant difference was detected.

The percentage of total smokers was only 25.7% (37.5% of the men and 14.0% of the women) and no significant difference was found in enzyme levels for smokers and non-smokers, regardless of the number of cigarettes consumed daily or the gender (Table 2). Tola et al.¹⁶) reported no influence from smoking on ALA-D activity in workers with similar levels of lead in the blood. Perhaps in a larger group of smokers, some effect from Pb would influence ALA-D activity; nevertheless, no data about alterations to background enzyme activity owing to tobacco have been reported.

Low-to-moderate alcohol intake was reported by 53.1% of the volunteers, with greater prevalence in men (83.9%) as compared to women (23.2%). In Brazil, men customarily drink beer and/or a sugar-cane distilled drink at meals. Drinking did not show any significant alteration to ALA-D levels in both genders. It is important to note that the number of volunteers in both subgroups with different lifestyles related to gender was too different to come to a clear conclusion about the influence of alcohol on reference values for ALA-D (Table 2). Moore et al.¹⁷) reported that ALA-D activity returns to normal levels about 10 h after drinking. The lag between drinking (at dinner) and collecting blood, the next morning (12–16 h after), probably explains the results found in our study.

This work establishes that background activity of ALA-D did not vary significantly according to gender, age, alcohol and smoking habits of people from general population.

References


