BIOLOGICAL VARIATION OF PLASMA FERRITIN IN HEALTHY ADULT MALES IN SOUTH INDIAN POPULATION - A SAMPLE STUDY

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ABSTRACT

A sample study of biological variation of plasma ferritin in healthy adult males 19-25 years of age (n = 6) in the Indian population was determined. Venous blood was collected on 3 non-consecutive days during a 3 week period. Plasma ferritin was measured using enzyme linked immunoassay in an automated immunoassay system. Analytical and Biological variation was calculated. We found a mean biological variation of 21.64%. Thus, our results indicate that biological variation contributed most to the intraindividual variation.

KEY WORDS

Biological Variation, enzyme linked immunoassay

ABBREVIATIONS

IDA - Iron Deficiency Anaemia, EDTA - Ethylenediaminetetra-acetic acid, CV - Coefficient of Variation

INTRODUCTION

Anaemia affects approximately 42% of children younger than 5 years in developing countries and approximately 17% in industrialized countries (1). Although, there are many causes of anaemia such as poor nutrition and gastrointestinal infections, iron deficiency is often a major contributory factor of anaemia. The criteria for diagnosing Iron Deficiency Anaemia (IDA) are based on defining anaemia (blood hemoglobin level) and measures of iron status, metabolism and function, such as circulating plasma ferritin (reflecting the size of iron stores), serum transferrin (reflecting iron transport capacity), zinc protoporphyrin (reflecting defective hemoglobin synthesis), and serum transferrin receptors (reflecting cellular need for iron) (2).

Of all these parameters, plasma ferritin is the most specific biochemical test for iron deficiency because it correlates with total body iron stores, and a low plasma

ferritin concentration reflects depleted iron stores (3). However, there are many problems associated with the interpretation of plasma ferritin levels since apoferritin which is a precursor is an acute-phase reactant protein and is therefore elevated in inflammatory processes (4). Consequently plasma ferritin concentration in the normal range reflects adequate iron stores only in the absence of inflammation. Interpretation of plasma ferritin is also difficult at times of rapid systemic iron use, such as during rapid growth (5). Another important factor to be considered is the biological variation of plasma ferritin levels. Studies that have been carried out on biological variation of plasma ferritin in healthy men and women show that it varies from 14.5 to 18.1% (6-8). Apart from these studies, in a study done over a period of one year the biological variation of plasma ferritin was 71.1% (9). Thus biological variation is a major component of variability for plasma ferritin estimation and must be considered when used to assess iron status. Borel *et al.* (10) suggested that between 3 to 10 independent samples from an individual are necessary to accurately assess plasma ferritin. There is a lack of such data with regards to biological variation of plasma ferritin in the Indian population, where IDA is very much predominant. Thus in this study we focus on assessing the biologic variation of plasma ferritin for the Indian population.

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MATERIALS AND METHODS

Subjects

The sample study population consisted of 6 apparently healthy adult male subjects in the age group of 19-25 years, who were free from any inflammatory and or chronic diseases known to affect iron status on the basis of self-reported medical history, a complete biochemistry and hematological screening. Ethical approval for the study was obtained from Ethical committee of St. John's Medical College, Bangalore and all the subjects gave informed consent.

Experimental protocol

Venous blood was collected by a trained phlebotomist from each subject on 3 random nonconsecutive days during a 3 week period. All samples were drawn at a one particular time, at 8 am at each blood draw for each subject. Blood samples were collected in a EDTA vacutainer, mixed thoroughly by inverting the tubes gently. Plasma was separated by centrifugation at 4500 rpm for 15 minutes at 4°C and aliquot of samples were stored at -80°C for less than a month until analysis.

Before analysis, frozen samples were thawed to room temperature. Plasma ferritin was determined by an enzyme linked immunoassay method (Pathozyne Ferritin, Omega Diagnostics Limited, UK) using a completely automated immunoassay system (Alpha Prime, SFRI, France). The within run and between run assay CV% for plasma ferritin are 2.63% and 4.12% respectively. All samples were analyzed in a single batch to eliminate batch - to - batch variation.

Statistical analysis

Analysis was performed with SPSS (SPSS 10.1 for windows, SPSS Inc 2000). Results are reported as means with their variance. The biological within-subject coefficient of variation (CV) was calculated as the SD divided by the mean, expressed as a percentage.

RESULTS

The means, variance component, biological coefficient of variation for the sample study group is presented in Table 1. The mean plasma ferritin level of the study group was 44.33 ± 10.88 . The biological (intra individual) coefficient of variation for plasma ferritin varied from 5.37 % to 39.13 % with a mean variation

of 21.64 %.

DISCUSSION

The reliability of any laboratory result is influenced by preanalytical, analytical, and biological variations. Though preanalytic and analytic variables are factors to be considered, a more common problem when there are unanticipated discrepant results on serial testing is the failure of physicians to realize the extent to which biologic variation in the individual patient contributes to total test variation (11). Determining the within subject variability (Biological variability) of iron metabolism indices is also of practical value. When expressed as a standard deviation, it provides a significance criterion for changes in a given variable. This enables for example, a subject to be monitored with respect to the variable and clinically significant deviations from the normal state to be detected. The results from this study concur with other studies (6, 10) that biological variation is the main contributor to total intraindividual variation for plasma ferritin estimation in otherwise healthy Indians. The analytical variation was 2.63% while the biological variation was 21.64%. Thus at least 3 independent measurements are required to accurately determine plasma ferritin levels. Further with regards to Ferritin levels, women have higher biological variation than men (10) and this aspect should also be considered. Also when compared with other iron status parameters, Ferritin is one of the lowest biologically varying iron status markers (12), thus making it one of the most useful parameter. Thus a complete knowledge of the biological variation of this parameter is crucial for clinical diagnosis and treatment.

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Table 1. Means, variance component and biological coefficient of variation for plasma ferritin estimation

Mean	Variance	CV
44.33 ± 10.88 (g/L)	201.60	21.64%

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