SERUM FERRITIN AND ITS BIOLOGICAL VARIATION IN HEALTHY ADULT MALES

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Abstract:
Iron Deficiency Anemia (IDA) is the most common nutritional deficiency worldwide. Serum Ferritin is the most specific biochemical test for diagnosing Iron Deficiency Anemia. Biological variation is an important factor to be considered in assessing Serum Ferritin levels. AIM To determine Biological Variation of Serum Ferritin in Healthy Adult males. METHOD The study population consisted of 8 apparently healthy adult male subjects in the age group of 19-25 years. Venous blood was collected from each subject on 3 random non-consecutive days during a 3 week period. Serum ferritin was determined by an enzyme linked immunoassay method. RESULT The mean serum ferritin level of the study group was 25.75 gl. The biological (intra individual) coefficient of variation for serum ferritin varied from 15.50 to 38.37 with a mean variation of 26.22. Thus, our results indicate that biological variation contributed most to the intraindividual variation. Keyword: Ferritin, Biological variation

INTRODUCTION:
Iron is one of the most important minor elements required for normal biochemical function of various tissues. Iron deficiency anemia (IDA) is the most common nutritional deficiency worldwide. Global prevalence of anemia in adult men are 40.2% of which south east Asia contributes to 4.2%. Iron deficiency results when iron demand by the body is not met by iron absorption from the diet. Indians are more prone to develop iron deficiency anemia since Indian diet contain lot of inhibitors of iron absorption. Patients presenting with IDA may have varied reasons like inadequate dietary intake, impaired absorption, or physiological/ pathological blood loss, known or occult. IDA causes reduced work capacity in adults and affects motor and mental development in children and adolescents. Diagnosing IDA is the primary step, evaluating a cause is next in the ladder, because IDA is not an end diagnosis.
FERRITIN -SPECIFIC ASSAY:
The Diagnosis of Iron Deficiency is based on a battery of laboratory measurements like defining anaemia (blood hemoglobin level) and measures of iron status, metabolism and function, such as circulating Serum Iron level, Percent Iron Saturation, Serum Ferritin (reflecting the iron stores), Serum Transferrin (reflecting iron transport capacity), Zinc protoporphyrin (reflecting defective hemoglobin synthesis), and Serum Transferrin Receptors (reflecting cellular need for iron). Measurement of hemoglobin or hematocrit is the most cost efficient factor used for screening anemia. Measurement of Serum Iron or Percent Iron Saturation may be an unreliable index of iron stores due to wide physiological variation. In normal individuals, concentration of serum ferritin shows good correlation to body iron stores and can detect even mild iron deficiency without clinical evidence of anemia. Serum ferritin is also used for differentiating between the anemias of chronic disease and iron deficiency anaemia. Hence it is considered to be the best clinical measure of body iron content.

STRUCTURE OF FERRITIN
The iron storage protein ferritin consists of a protein shell called apoferritin with a molecular mass of about 500 kDa composed of 24 sub-units. The protein shell encloses a core of ferric-hydroxy-phosphate which can hold up to 4,000 atoms of iron. Ferritin of 1 µg/l is approximately equivalent to 10mg of storage iron. Normal range of Serum ferritin concentration is 15–300 µg/l, whereas in patients with iron deficiency anaemia, it is typically less than 12–15 µg/l.

LIMITATION OF FERRITIN MEASUREMENT:
There are many problems associated with the interpretation of serum ferritin levels. Biological variation of serum ferritin is absence of inflammation, since Apoferritin, is an acute-phase reactant protein. Serum ferritin concentration in the normal range reflects adequate iron stores only in the Starvation or even fasting alcohol intake can cause an increase in the serum ferritin concentration. Severe exercise also leads to an increase its concentration due to muscle damage and inflammatory reactions.

Serum ferritin concentration decreases with blood donation, vitamin C deficiency. Interpretation of serum ferritin is also difficult at times of rapid body growth due to increased systemic iron use. Among these, biological variation is an important preanalytical variant to be considered in assessing serum ferritin levels.

BIological VARIATION
Different results are usually observed when a quantity is measured in different specimens from the same individual obtained over a time span. For an individual, this variation is due to the imprecision of the measurement procedure (i.e.) the metrological variability, as well as to the rhythmic and random fluctuations of the quantity value around a virtual homeostatic set point (i.e.) the intra-individual biological variability. On the other hand, when studying the intra-individual biological variation of a quantity, a mean value is estimated for each individual participating in the study. The variation among these mean values is due to the inter-individual biological variability. Inherent fluctuation of the concentration of body fluid components around the homeostatic setting point is called Within-subject (intra individual) biological variation. This Difference in concentration of the components of biological fluids among persons is called Between-subject biological
Data on the components of biological variation is important and can be applied to set quality specifications for Precision, Bias, Total error allowable, The allowable difference between methods, In proficiency testing programs or external quality assessment schemes (EQAS), and Reference methods. Data on biological variation can be used to report best test results, the best sample to collect, and the best test procedure. Generation and application of data on biological variation is an essential prerequisite in the evolution of any new test procedure.

**DATA FROM VARIOUS STUDIES**

Studies that have been carried out on biological variation of serum ferritin in healthy men and women shown that it varies from 14.5 to 18.1%. Study done in India has shown biological variation of 21.71%. There is a lack of such data with regards to biological variation of serum ferritin in the South Indian population, where IDA is very much predominant. Thus this study focuses on assessing the biologic variation of serum ferritin for our laboratory population.

**AIM**

To determine the Biological Variation of Serum Ferritin in Healthy Adult males

**MATERIALS AND METHODS**

**Subjects**
The study population consisted of 8 apparently healthy male subjects in the age group of 19-25 years

**Exclusion criteria**
Subjects with inflammatory and or chronic diseases known to affect iron status on the basis of self-reported medical history.

**Study protocol**
Venous blood was collected from each subject on 3 random non-consecutive days during a 3 week period, all samples were drawn at one particular time, at 10 am. Serum was separated by centrifugation at 4500 rpm for 5 minutes and aliquot of samples were stored at -20°C until analysis. Before analysis, frozen samples were thawed to room temperature. Serum ferritin was determined by an enzyme linked immunoassay method (Pathozyme Ferritin, Omega Diagnostics Limited, UK) using a semi automated immunoassay system. The coefficient of variation (CV%) for the kit ferritin is less than or equal to 10%. All samples were analyzed in a single batch to eliminate batch to batch variation. The assay is based on noncompetitive sandwich method, by using immobilized monoclonal antibodies and enzyme labelled forms against the human ferritin.

**RESULT**

Results were reported as means with their variance. The biological within-subject coefficient of variation (CV) was calculated as the Standard Deviation divided by the mean, expressed as a percentage. The mean, variance component, biological coefficient of variation for the sample study group is presented in Table 1. The mean serum ferritin level of the study group was 25.75µg/l. The biological (intra individual) coefficient of variation for serum ferritin varied from 15.50% to 38.37% with a mean variation of 26.22% Table 1 shows Mean, Variance, Standard deviation, Biological Coefficient of Variation for the
**DISCUSSION:**

To evaluate if a test can meet clinical needs, the bias and imprecision of the method are needed. Determining the biological variability of iron status indices enables a subject to be monitored with respect to the variable and clinically significant deviations from the normal state. Thus a complete knowledge of the biological variation of the parameter is essential for reporting a test result, for clinical diagnosis and treatment. Vernon A. Pillon et al reported observations on day-to-day variation in serum ferritin, in 13 healthy subjects during five weeks. The average intrasubject coefficients of variation were 14.5%. Gnanou et al in a study reported a mean biological variation of 21.64% which was done in Bangalore in healthy adult males. In a study Ford et al reported the individual variability for ferritin in 60 stable hemodialysis patients ranged between 2–62% measured over an initial two-week period and from 3–52% when factored over a six-week period. He concluded that single serum ferritin values should not be used to guide clinical decisions regarding treatment.

Jane C. Dale, studied between-day variations of ferritin levels in 20 healthy adult volunteers. He revealed that for determining iron status, ferritin is the best single test. Also concluded between-day variations of ferritin were smaller than those of iron. Borel et al reported gender has an influence on the day-to-day variability of serum ferritin concentrations, with women having a greater CV than men (26.8% vs 15.2%). The results from this study concur with study of Borel et al, Gnanou et al that biological variation is the main contributor to total intraindividual variation for serum ferritin estimation in healthy individuals. In this study analytical variation was 10% while the biological variation was 26.22%. In a review Ricos et al recommended that at least 10 subjects and 5 samples to be used to study biological variability. The analytical method, instrument and reagents used in the study make no difference on biological variability estimates. Database has been currently available for around 320 analytes which is updated every two years for laboratory use.

<table>
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<th>Patient</th>
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<th>Variance</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (CV)</th>
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Thus at least 3 independent measurements are required to accurately determine serum ferritin levels according to Borel et al [24]. Also when compared with other iron status indices, Ferritin is one of the lowest biologically varying best parameter[15] On the basis of our data we suggest that the assessment of an individual’s iron status may be misinterpreted if only a single blood sample is collected because of the biological variation of serum ferritin.

CONCLUSION:
Serum ferritin being an important parameter for assessing iron status due to its biological variability either minimum of three measurements of serum ferritin[15] has to be considered or it has to be combined with other iron status indices like transferrin saturation [26] for clinical evaluation. In spite of consequences like requiring serial measurements and/or combination with other indicators of iron status like transferrin saturation the Pros of ferritin include inexpensive and easily measurable, allowing for frequent monitoring. Limitation: this study was carried out only in small sample with inadequate sampling and males only. Further studies have to be carried out in various age groups in both sexes and on focussing short term and long term biological variability.

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