Clinical Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Influence of diurnal variation and fasting on serum iron concentrations in a community-based population

Leonard T. Nguyen[a], Joshua D. Buse[a,b], Leland Baskin[a,b], S.M. Hossein Sadrzadeh[a,b], Christopher Naugler[a,b,c,*]

[a] Department of Pathology and Laboratory Medicine, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr NW, Calgary, Alberta T2N 4N1, Canada
[b] Calgary Laboratory Services, 3535 Research Rd NW, Calgary, Alberta T2L 2K8, Canada
[c] Departments of Family Medicine and Community Health Services, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr NW, Calgary, Alberta, Canada T2N 4N1

ARTICLE INFO

Keywords:
Serum iron
Diurnal variation
Fasting
Postprandial
Blood specimen collection
Reproducibility of results

ABSTRACT

Objectives: Serum iron is an important clinical test to help identify cases of iron deficiency or overload. Fluctuations caused by diurnal variation and diet are thought to influence test results, which may affect clinical patient management. We examined the impact of these preanalytical factors on iron concentrations in a large community-based cohort.

Design and Methods: Serum iron concentration, blood collection time, fasting duration, patient age and sex were obtained for community-based clinical testing from the Laboratory Information Service at Calgary Laboratory Services for the period of January 2011 to December 2015.

Results: A total of 276,307 individual test results were obtained. Iron levels were relatively high over a long period from 8:00 to 15:00. Mean concentrations were highest at blood collection times of 11:00 for adult men and 12:00 for adult women and children, however iron levels peaked as late as 15:00 in teenagers. With regard to fasting, iron levels required approximately 5 h post-prandial time to return to a baseline, except for children and teenage females where no significant variation was seen until after 11 h fasting. After 10 h fasting, iron concentrations in all patient groups gradually increased to higher levels compared to earlier fasting times.

Conclusions: Serum iron concentrations remain reasonably stable during most daytime hours for testing purposes. In adults, blood collection after 5 to 9 h fasting provides a representative estimate of a patient's iron levels. For patients who have fasted overnight, i.e. ≥ 12 h fasting, clinicians should be aware that iron concentrations may be elevated beyond otherwise usual levels.

1. Introduction

Iron plays multiple essential physiological functions including enzyme catalysis, oxidative phosphorylation, and oxygen transport. Iron deficiency is often suspected in patients experiencing fatigue and headaches, and low levels of iron are linked to poor health outcomes such as weakened immunity, developmental problems in children and decreased energy metabolism [1–3]. It is the most common nutritional deficiency and cause of anemia, which affects a quarter of the global population [4–6]. As well, overabundant iron levels are of medical concern as iron accumulation can result in the formation of reactive oxygen species that are capable of initiating oxidative stress with eventual damage to vital organs [7]. Furthermore, the body has a limited dietary intake of iron and little ability to excrete excess iron, therefore its iron stores is maintained within a narrow equilibrium.

While serum iron clinical tests continue to be routinely ordered by physicians, their usefulness in providing a true assessment of a patient's iron levels remains greatly debated [8]. Iron concentrations are susceptible to preanalytical factors such as diurnal variation, diet and exercise [9–12]; however, conclusions regarding their degree of impact differ [13,14]. Clinical laboratories generally recommend blood collection to be performed in the morning when iron levels are thought to be high, sometimes following either 6, 8 or 12 h of fasting prior to sample collection. In this retrospective study, we examined the effect of time of collection and length of fasting on serum iron concentration using population-level laboratory data from Calgary, Alberta. Similar

Abbreviations: CLS, Calgary Laboratory Services; TIBC, total iron-binding capacity

* Corresponding author at: Diagnostic and Scientific Centre, C262, 9, 3535 Research Road NW, Calgary, AB T2L 2K8, Canada
E-mail addresses: leonard.nguyen@cls.ab.ca (L.T. Nguyen), joshua.buse@cls.ab.ca (J.D. Buse), leland.baskin@cls.ab.ca (L. Baskin), hossein.sadrzadeh@cls.ab.ca (S.M.H. Sadrzadeh), christopher.naugler@cls.ab.ca (C. Naugler).

http://dx.doi.org/10.1016/j.clinbiochem.2017.09.018
Received 5 May 2017; Received in revised form 15 September 2017; Accepted 21 September 2017

previous analyses focusing on the serum levels of vitamin B_{12}, prostate-specific antigen, and lipids have shown that these analytes have little to no significant association with fasting duration, therefore fasting for these clinical tests was concluded to be unnecessary [15–17].

2. Materials and methods

As this is a quality improvement initiative, ethics approval was not required for this study. Data on serum iron concentration, patient age, sex, blood collection time and fasting time were extracted for community-based patients from January 2011 to December 2015. Testing was carried out by Calgary Laboratory Services (CLS) and all data stored in CLS's Laboratory Information System. CLS is the sole supplier of laboratory testing services in Calgary, Alberta, Canada, and its surrounding area, with annual volumes of 30 million tests for a population of > 1.8 million persons.

Sample collection times were recorded and rounded to the nearest hour. Patients were asked to recall the amount of time since their last meal. These fasting times were rounded and categorized into 1 h intervals. Fasting times ranged from 0 h, for patients reporting 0 h or 0.25 h, to > 16 h. Serum iron results were excluded in cases of missing fasting time, sex or age. In cases of repeat testing for a patient, the earliest result was retained.

At CLS, iron is measured by the Ferrozine method on Roche Cobas 8000 analyzers. In this assay, ferric iron (Fe^{3+}) is released from transferrin in the serum sample using citric acid and detergent. Fe^{3+} is reduced by ascorbate to ferrous iron (Fe^{2+}), which is then complexed with Ferrozine reagent for colorimetric detection at 570 nm [18]. Ferrozine is the recommended chromogen for the measurement of serum iron by the Iron Panel of the International Committee for Standardization in Hematology [19]. Reference intervals were defined by CLS as follows: 5–25 μM for children and teenagers aged < 18, 6–28 μM for adult females, and 8–30 μM for adult males. Given their distinct reference limits and sex- and age-specific differences [20], analyses were conducted separately for children (ages 0–13), teenage males and females (ages 14–17), and adult males and females (ages 18 +).

Statistical analyses were conducted by univariate analysis in the general linear model using the SPSSv19 statistical package (IBM).

3. Results

In total, 276,307 test results were retrieved from the Laboratory Information System at CLS, separated into five patient categories: adult male (109,087 results, 39.5%, median age 48), adult female (157,356 results, 56.9%, median age 45), teenage males (2181 results, 0.8%, median age 16), teenage females (3130 results, 1.1%, median age 16) and children < 14 years of age (4553 results, 1.6%, median age 10). Iron concentration results for blood collection times from 06:00 to 22:00 are summarized in Table 1 and test volume distributions according to collection and fasting times are shown in Fig. 1. The highest test volumes occurred in the morning from 08:00 to 10:00, accounting for 49.5% of tests taken. 78.6% of iron tests were taken for patients who had fasted for ≥ 12 h. Results ranged from 1 to 97 μM with 8 to 9 μM interquartile ranges. The median iron concentrations were 18 μM for adult and teenage males, 16 μM for adult females, and 15 μM for teenage females and children.

Mean serum iron concentrations were observed to be highest through most daytime hours from 8:00 to 15:00 with many of the collection time points being within each other's 95% confidence intervals (Fig. 2). Teenage patients had sustained high levels of iron into the afternoon hours with maximum mean iron at 14:00 and 15:00 for males and females, respectively, compared to 11:00 for adult males and 12:00 for adult females and children. The lowest iron levels were observed with collection times past 16:00, which have large 95% confidence intervals that are partly due to small sample sizes.

The influence of fasting on serum iron results is summarized in Fig. 3. Mean iron concentrations at each fasting time deviated < 20% from the overall group means. For adults and teenage males, the lowest concentrations occurred at fasting times between 4 and 9 h. By contrast, there were elevated concentrations for teenage females and children at 6 h and 7 h fasting. Past 12 h fasting, the increasing iron means exceeded those reported for patients who had eaten ≤ 15 min prior to specimen collection (0 h). Linear regression analyses were performed for each patient group (Table 2). Despite low coefficient of determination values (R^2) due to high variance in the cohort datasets, overall fasting-dependent increases were observed for iron levels in all patient groups ($p < 0.001$).

Rates of iron deficiency and elevated iron were plotted for different fasting times (Fig. 4). These were determined from the recommended clinical reference intervals at CLS assigned to each patient group. Teenage males showed pronounced elevated iron rates and low deficiency, and teenage females also showed high elevated rates. Spikes in deficiency were observed for adult females and teenage males at 7 h fasting. Elevated rates showed continuous increases at fasting times after 12 h, reaching 28.6% for teenage males with > 16 h fasting.

4. Discussion

Iron regulation is a dynamic process that requires a careful balance between its physiological functions and its toxicity [7]. Therefore, the 1–2 mg absorbed daily from the diet has to maintain the 3–4 g total within the average adult body against daily losses via cell sloughing, sweat or blood loss, especially during menstruation or childbirth for women [3]. Following absorption in the duodenum, iron is transported by transferrin to its recipient cells and tissues [21]. Most of the iron in the human body is associated with heme proteins, e.g. hemoglobin, myoglobin and cytochrome *c*, as well as storage proteins, e.g. hemosiderin and ferritin [21]. A patient's iron status may be evaluated through a panel of four clinical tests. Serum ferritin is the best indicator of iron deficiency while serum iron, total iron-binding capacity (TIBC), and transferrin saturation are good indicators of iron overload [22,23]. Serum iron is considered to be the least reliable of these measures despite continued high test order volumes [24,25].

To the best of our knowledge, the pre-analytical effects of blood collection time and fasting on serum iron test results have yet to be examined at a population level. In this cross-sectional retrospective study, 276,307 test results were obtained within a five-year period from community patients categorized as children, or teenage and adult males and females.

As expected, iron levels were higher in men than women or children. CLS, the medical diagnostic laboratory servicing Calgary and its surrounding area, uses the same reference interval for patients under 18 years old. However, a recent pediatric study shows that separate reference intervals are more appropriate for 14–17 year old male and female patients rather than grouping them with patients aged 13 and under [20]. Our results demonstrate that the iron levels of teenage males are much closer to those of adult males than children.

Diurnal patterns for several haematological parameters are set by the hypothalamic suprachiasmatic nuclei, which generate the body's circadian rhythm [26,27]. Case-control studies have found peak iron concentrations occurring either in the morning [28,29], the afternoon [11,30], or even inconsistently between test subjects [13]. Furthermore, patterns of iron variation can be disrupted by changes in sleeping schedule or deprivation [31,32]. Generally, iron levels are believed to peak at early morning hours and decrease in the afternoon [33]. In our study, the highest iron concentrations were observed from 11:00 to 12:00 for adults and children, and teenage patients showed high iron levels into the afternoon as late as 15:00. Overall, the 08:00 to 15:00 time interval covers a wide window of high iron status where the mean concentrations at each hour interval remain within 10% of each other across all patient groups. However, the stability of iron during this time period and furthermore, slight fluctuations around the clinical cutoffs