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# EFFECT OF DIURNAL VARIATION ON THE MEASUREMENT OF SERUM IRON AND TIBC LEVELS IN HEALTHY POPULATION

Biochemistry

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## ABSTRACT

**Background:** It is well-known that deficiency or over exposure to various elements has noticeable effects on human health. The effect of an element is determined by several characteristics, including absorption, metabolism, and degree of interaction with physiological processes. Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport. **Material and Methods:** The Study was Conducted in department of Biochemistry Guru Gobind Singh Medical College Faridkot. Ethical clearance was taken from institutional ethical committee. Proper informed consent was taken from all the participants. Out of 60 healthy patients, 30 were males and 30 females. **Result:** The Correlation between iron in three time intervals on the same day (Morning – Evening and Morning – Afternoon )for morning – afternoon values, the p value was <0.001 and the r value was 0.920. For morning – evening, the p value was <0.001 and r value was 0.928, which shows statistically highly significant correlation. **Conclusion:** It was conducted that Iron levels showed significant diurnal variations on same day samples. The variation was statistically non significant for TIBC. Also no consistant pattern has observed for iron variation. So the time of day for Iron &TIBC estimation does not hold much value and only a fasting sample is required.

## KEYWORDS

Total Iron binding capacity, Serum Iron

## INTRODUCTION:

Serum iron, total iron-binding capacity (TIBC), and calculated transferrin saturation (TS) tests are used to screen for and monitor conditions of iron deficiency and iron overload. Although the usefulness of these tests for diagnosing iron deficiency can be debated,[1] they continue to be ordered with some frequency. The tests have proven useful for screening for hereditary hemochromatosis, and, because hemochromatosis is the most common single-gene inherited disorder in whites, many have advocated population-based screening.[2-3] Because we anticipated increased requests for serum iron studies, we reviewed our collection requirements for these analytes. Although the diurnal variation of iron is well documented, published studies offer conflicting conclusions about the pattern of variation. The prevailing opinion is that iron levels are higher in the morning than in the afternoon or evening.[4,5] However, some studies have shown iron levels to be higher in the afternoon or evening than in the morning,[6,7] and other studies have found significant variation but no systematic trend.[8,9] Our laboratory's iron reference range is based on specimens obtained in the morning. When we receive a request for an afternoon or evening test of iron levels for the investigation of iron deficiency, we contact the ordering physician, explain that afternoon or evening levels may be misleading, and suggest a ferritin level as an alternative test. Striving to streamline our processes, we questioned whether this practice, which introduces delays in specimen collection and interrupts the physicians, is necessary. Because many published studies were conducted a number of years ago and did not use state-of-the-art methods, we investigated the effect of time of day on iron, TIBC levels.

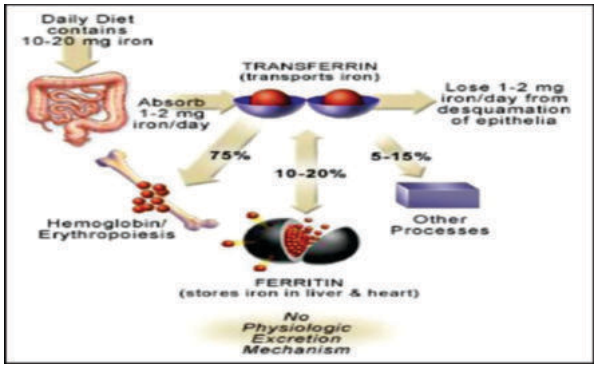


**Figure 1.** Iron is bound and transported in the body via transferrin and stored in ferritin molecules. Once iron is absorbed, there is no physiologic mechanism for excretion of excess iron from the body other than blood loss, that is, pregnancy, menstruation, or other bleeding

## MATERIAL AND METHODS;

The Study was conducted in department of biochemistry Guru Gobind Singh Medical College Faridkot. Ethical clearance was taken from institutional ethical committee. proper informed consent was taken from all the participants. Out of 60 healthy patients, 30 were males and 30 females.

**Inclusion criteria**:

Healthy volunteers of age group 18 -55 years were enrolled for the study.

**Exclusion criteria:**

Recent history of blood donation, intake of iron supplements, chronic liver disease, kidney disease.

**Sample Technique/ Method:**

After taking informed consent healthy volunteers were enrolled for the study. All participants were seated for 10minutes before collection and were informed to come in overnight fasting for collection of 8am samples. For subsequent samples, atleast 2hours fasting is required 3ml of the venous blood sample was drawn from each subject under aseptic conditions. After the formation of blood clot, the sample was centrifuged at 3000rpm for 10 minutes to separate serum. The serum was analysed for the biochemical investigation. Iron levels were determined by Ferrozine method.

## RESULTS AND OBSERVATIONS:

The Present study was conducted in Department of Biochemistry, Guru Gobind Singh Medical College, Faridkot with an objective to study the of effect of diurnal variation on the measurement of serum iron and TIBC levels in healthy population for this purpose, a total of 60 sample were taken.

**Table: 1 Distribution of Patients according to sex.**

| Sex | Number of patients | Percentage |
|---|---|---|
| Male | 30 | 50% |
| Female | 30 | 50% |
| Total | 60 | 100% |

Table 1 showing sex distribution of patients 30 males, 30 females were

enrolled in this study. The male and female ratio is 1:1

**Table: 2 Mean SD of age of patients**.

| No. of patients | Mean SD of age (year) | Range of age (year) |
|---|---|---|
| 60 | 32.67 5.80 | 19 -45 |

Table 2. showing the analysis of healthy individuals with mean age of 32.67 5.80 years and range 19 -45.

**Table 3. Descriptive Statistics of serum Iron levels in three time intervals on the same day.**

| Biochemical parameter | Mean ± SD ( µg/dl) | Range ( µg/dl) | Friedman analysis | Significance |
|---|---|---|---|---|
| Iron (Morning) | 64.73 ± 17.92 | 37 – 99 | <0.001 | HS |
| Iron (Afternoon) | 61.10 ± 16.77 | 32.5 - 96 | | |
| Iron (Evening) | 59.72 ± 16.02 | 30 – 98 | | |

Table 3 showed that the mean value of serum iron levels in morning 64.73 ± 17.92 with range 37 – 99 µg/dl and afternoonn were 61.10 ± 16.77 with the range of 32.5 – 96 µg/dl and evening were 59.72 ± 16.02 with the range of 30 -98 µg/dl. With p value <0.001 and it was statistically highly significant.

**Table 4. Descriptive statistics of serum TIBC levels in Three times intervals on the same day**

| Biochemical parameter | Mean ± SD ( µg/dl) | Range ( µg/dl) | Friedman analysis | Significance |
|---|---|---|---|---|
| TIBC (Morning) | 328.97± 49.21 | 245 - 420 | 0.562 | NS |
| TIBC (Afternoon) | 323.59 ± 52.04 | 212 – 420 | | |
| TIBC (Evening) | 320.93 ± 52.73 | 210 – 412 | | |

Table 4 shows that the mean values of serum TIBC in morning were 328.97 ± 49.21 µg/dl with the range of 245 -420 µg/dl and in afternoon were 323.59± 52.04 µg/dl with the range of 212 -420 µg/dl and evening were 320.93 ± 52.73 µg/dl with the range of 210 -412 µg/dl. This table shows friedman analysis that p value was 0.562, so it was statistically non significant.

**Table 5. Shows the mean value of serum iron and TIBC levels at three time intervals on the same day in males**

| Iron/TIBC levels in males | Mean ± SD ( µg/dl) | Range ( µg/dl) |
|---|---|---|
| Iron<br>TIBC<br>(Morning) | 78.56 ± 14.38<br>342.79 ± 41.50 | 45 – 99<br>245 – 420 |
| Iron<br>TIBC<br>(Afternoon) | 7 3.37 ± 13.69<br>332.96 ± 49.63 | 40 – 96<br>245 -412 |
| Iron<br>TIBC<br>(Evening) | 71.3 ± 13.57<br>327.13 ± 52.52 | 35 – 98<br>210 – 410 |

Table no. 5 shows that the mean value of serum iron and TIBC levels in males, the mean value of iron in morning were 78.56 ± 14.38 µg/dl with the range of 45 -99 µg/dl, and mean of TIBC 342.79 ± 41.50 µg/dl with range of 245 -420 µg/dl. and afternoon iron levels were 7 3.37 ± 13.69 µg/dl with the range of 40 -96 µg/dl, & TIBC levels were 332.96 ± 49.63µg/dl wth the range of 245 -412 µg/dl and evening serum iron levels 71.3 ± 13.57µg/dl with the range of 35 -98 µg/dl & TIBC levels were 327.13 ± 52.52µg/dl with the range of 210 - 410µg/dl.

**Table 6. Shows the mean value of serum iron and TIBC levels at three time intervals on the same day in females.**

| iron/ TIBC Levels In Females | MEAN ± SD (µg/dl) | RANGE (µg/dl) |
|---|---|---|
| Iron<br>TIBC<br>(Morning) | 51.05 ± 8.83<br>315.00 ± 52.47 | 37 – 77<br>245 – 420 |
| Iron<br>TIBC<br>(Afternoon) | 48.63± 8.86<br>314.92± 54.96 | 32.5 – 70<br>212 – 420 |
| Iron<br>TIBC<br>(Evening) | 48.30± 1.67<br>315.68 ± 50.38 | 34 – 74<br>242 -412 |

Table no. 6 Shows that the mean value of serum Iron and TIBC levels in females, the mean value of iron in morning were 51.05 ± 8.83µg/dl with the range of 37 – 77 µg/dl, & mean value of TIBC were 315.00 ± 52.47µg/dl with the range of 245 - 420µg/dl, and afternoon levels of iron were 48.63± 8.86µg/dl with the range of 32.5 - 70µg/dl & TIBC levels were 314.92± 54.96µg/dl with the range of 212 – 420µg/dl, and evening levels of iron were 48.30± 1.67µg/dl with the range of 34 – 74µg/dl & TIBC levels were 315.68 ± 50.38µg/dl with the range of 242 - 412µg/dl.

**Table 7. Correlations between morning iron values with afternoon and evening values.**

| Duration of day | Mean ± SD (µg/dl) | r – value | p - value | Significance |
|---|---|---|---|---|
| Morning | 64.73± 17.92 | - | - | - |
| Afternoon | 61.10±16.77 | 0.920 | <0.001 | HS |
| Evening | 59.72± 16.02 | 0.928 | <0.001 | HS |

Table no. 7 Showing the Correlation between iron in three times intervals on the same day (morning – evening and morning – afternoon). For the morning – afternoon values, the p value was <0.001 and r value was 0.928 which shows statistically highly significant correlation.

## DISCUSSION:

In the past at our institution, blood samples for routine laboratory testing typically were obtained in the morning and analyzed in batches, and the results were reported in the afternoon. Most reference values were determined based on an overnight fast and morning sample collection. Random access instrumentation now allows continuous analysis and reporting throughout the day. This improvement makes it possible for specimens to be obtained at times more convenient to patients and for physician appointments to be more frequent and efficient. However, interpretation of test results for analytes with significant diurnal variation may be problematic if collection times change.

The within-day and between-day variations in iron levels seen in our study are well documented and limit the diagnostic usefulness of iron measurements[9,10]. Although the diurnal variation seems unpredictable and may be outside the laboratory's control, control of dietary factors may reduce iron variability. Statland et al[11] reported a statistically significant increase in the iron level 2 hours after subjects had eaten. Lammi-Keefe et al[12] demonstrated a reduction in the between-day iron coefficient of variation from 26.1% to 16.6% after fasting. In our study, the variability seen in specimens obtained at noon and 4 PM (at least a 2-hour fast) was not significantly greater than the variability seen in 2 fasting collections. When TS is used to screen for hemochromatosis, the fasting state has been shown to decrease the number of false-positive TS screens.[13] Published screening algorithms recommend repeated testing on a fasting specimen if the initial screen is abnormal.[14,15] Others have reported that diet has little effect on iron levels.[7,16].

The present study was conducted in department of Biochemistry, Guru Gobind Singh Medical College, Faridkot with an objective to ascertain the type of effect of diurnal variation on the measurement of serum iron and TIBC levels in healthy population for this purpose, a total of 60 sample were taken. The present study was done 30 males and 30 females with mean of age (year)is 32.67 ± 5.804 and with range of age (year) is 19 -45.

In the present study, the table no. 3 showed that the mean value of serum iron levels in morning 64.73 ± 17.92 with range 37 – 99 µg/dl and afternoonn were 61.10 ± 16.77 with the range of 32.5 – 96 µg/dl and evening were 59.72 ± 16.02 with the range of 30 -98 µg/dl.

In the present, the table no. 4 shows that the mean values of serum TIBC in morning were 328.97 ± 49.21 µg/dl with the range of 245 -420 µg/dl and in afternoon were 323.59± 52.04 µg/dl with the range of 212 -420 µg/dl and evening were 320.93 ± 52.73 µg/dl with the range of 210 -412 µg/dl.

## CONCLUSION:

It was conducted that iron levels showed significant diurnal variations on same day samples. The variation was statistically non significant for TIBC. Also no consistant pattern has observed for iron variation. So the time of day for iron &TIBC estimation does not hold much value and only a fasting sample is required.

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