Hemoglobin Vs Serum Ferritin as Assessors of Iron Deficiency Anemia, Preference for Age and Gender Groups, Using Inferential, Binary Logistic Regression and Bivariate Correlation Statistics

Hamzullah Khan1*, Neelum Shehzadi2, Khalid Khan3, Mohammad Khalid4, Saadullah Afridi5 and Sarwat6

1Associate Professor of Hematology, Nowshera Medical College, Nowshera, Pakistan
2Associate Professor of Gynecology and Obstetrics, Nowshera Medical College, Nowshera, Pakistan
3Assistant Professor of Pediatrics, Nowshera Medical College, Nowshera, Pakistan
4Associate Professor of Medicine, Nowshera Medical College, Nowshera, Pakistan
5Director Public Health, Sarhad University, Peshawar, Pakistan
6Assistant Professor of Gynecology and Obstetrics, Bacha Khan Medical College, Mardan, Pakistan


Abstract

Objectives: To assess the hemoglobin vs serum ferritin as predictors of Iron deficiency anemia, using binary logistic regression, bivariate correlation and inferential statistics.

Materials and Methods: This cross sectional study was conducted in the Pathology department, Qazi Hussain Ahmed Medical Complex, Medical Teaching Institution Nowshera, from 20th February 2019 to 21st January 2020. Relevant information’s were entered in SPPS Version 25th for descriptive and inferential analysis.

Results: Out of 381,149 patients enrolled with Hb < 11 g/dl. Twenty (13.4%) were males and 129 (86.6%) females. Mean age with SD was 30 ± 6.2 years. The Mean Ferritin level with SD was 64.35 ± 33.45. The Mean Hemoglobin level with SD was 9 ± 1.8 g/dl. There was a statistically significant difference in Mean ± SD hemoglobin between gender groups (8.5 g/dl ± 1.43-Males) (9.14g/dl ± 1.86-Females) (p = 0.053) on Independent Sample Test and vice versa for ferritin. Ninety (60.4%) patients had serum ferritin less than 15 ng/ml. The Positive Predictive Value (PPV) of Hemoglobin as predictors of IDA was 39.1%, while 60.4% for serum ferritin. There was a significant positive relationship between Hb% and ferritin (p = 0.01, r = 0.4). There was an inverse relation of Hb% and ferritin with an increase in age (p = 0.185, r = -0.1), (p = 0.38, r = -.07) respectively. Odds ratio of 3.2 times was noted for low iron stores in patient with low hemoglobin (p = 0.01, OR-3.2). The probability of ferritin less than 15ng/ml was 2.7 times in female gender (p = 0.022, OR = 2.7).

Conclusion: We concluded that the PPV of serum ferritin is higher than Hb% for prediction of iron deficiency anemia.

Keywords: Hb% vs Ferritin; Iron Deficiency Anemia; Predictor of IDA

Introduction

In Pakistan the iron deficiency anemia is most prevalent of all types of anemia accounts for 83%of all anemia as reported in literature [1]. Iron deficiency anaemia (IDA) is a global issue more common in the third world countries including Pakistan. Iron deficiency anemia is the major cause of anaemia [2]. Centers for Disease Control and Prevention (CDC) reported the prevalence of iron deficiency anemia positively correlated with increase in gestation, noting 8%, 12%, and 29% in all three trimesters respectively [3].

Another trial reported females in their reproductive age and children in category of under 5 years have been reported to have IDA in studies reported from Pakistan [4]. Iron is important micro-nutrient responsible for synthesis of Hemoglobin. If there are low iron stores in the body the production of hemoglobin will decrease. There are number of factors contributing in IDA like age, gender, Number of gravid and para, socioeconomic conditions and intake of iron in diet etc [5].
Ferritin is a protein which stores iron and indicates the iron storage/reservoir in the body. The depletion/exhaustion of these stores can lead to sever IDA. Serum ferritin is the main storage form of iron, therefore is the best indicator for assessment of iron deficiency [6].

Hemoglobin is a predictor of anemia. Literature have shown its correlation with IDA in reticulocyte hemoglobin (RET Hb). RET-Hb is statically significant correlation with serum ferritin and is best predictor of IDA [7].

Pregnancy is most often associated with exhaustion of iron store and if not given iron therapy can result in morbid outcome in the form of iron deficient babies. Serum ferritin acts as predictor of iron deficiency in anemic pool [8]. The concentration of ferritin is directly proportional to the total iron stores in the body. The difference of iron stores in males and females reflects the iron in their body and indirectly reflects the anemic status of these individuals.

Iron deficiency is diagnosed when there is low Hb%, low Hematocrit and low serum ferritin. But IDA can also occur even at normal Hb% but low serum ferritin, that triggers the serum ferritin as standard indicator/predictor of IDA [9]. It has been reported that no single test is diagnostic of iron deficiency anemia unless the serum ferritin is below normal and transferrin saturation is low with high iron binding capacity, that again prefers the low serum ferritin as predictor of IDA [9].

Present study was conducted to assess the hemoglobin vs serum ferritin levels as predictors of Iron deficiency anemia, using binary logistic regression, bivariate correlation, inferential statistic along with other statistical tools in a hospital based study.

Materials and Methods

This cross sectional study was conducted in department of pathology Qazi Hussain Ahmed Medical Complex Nowshera in collaboration with department of Gynecology and obstetrics and Research and Development NMC. Sample size was calculated using open-epi software with Absolute precision of (6%) and Confidence level at 95% at 381, taking the anticipated proportion of serum ferritin at 45.5% [10]. Out of total 149 was selected to be followed with Hb < 11 g/dl and those advised further with serum ferritin.

The inclusion criteria were irrespective of age and gender with Hb% < 11g/dl as per WHO Guidelines [2], while the exclusion criteria were all candidates taking oral or iv iron therapy for their already diagnosed IDA or with Hb% > 11 g/dl.

Blood samples were collected from these women. The technique for the blood sampling collection was to collect 3 ml of venous blood by veine-puncture using disposable syringes under aseptic techniques. The blood was added in an EDTA vacutainer at concentration of 1.5 mg/ml and mixed gently. Complete blood count was calculated on hematology analyzer. Anemia was labeled when the pregnant women had a hemoglobin level of < 11 g/dl in accordance with the definition of World Health Organization [11].

Similarly, 2 ml blood was drawn from each individual, by veni-puncture under aseptic condition. Blood in plain bottles was stored for ferritin analysis. Serum Ferritin was measured by electro-chemiluminescence immunoassay using Roche Cobas E360 Chemistry Analyzer for which commercial kits of Roche diagnostics were used as per the instructions of the manufacturer. For calibration, we used 6 ferritin standards (corresponding to ferritin levels of 0, 10, 50, 250, 500, and 1000 ng/ml) as per the instructions of manufacturer.

We categorized our patients in three categories on the basis of serum ferritin levels. Analysis of serum ferritin levels based on the recommendations of the WHO [12]:

1. Sever IDA: Less than 15 ng/ml.

Ethical approval for the survey was obtained from the Ethical Review Committee of Nowshera Medical College/QHAMC.

Data was entered on 25th SPSS Version. Using descriptive statistics the mean, SD, range and percentile were calculated. Independent T test was used to show the statistical significance between the two groups/genders taking Mean values with 95% confidence interval with p-value at (0.05). Logistic regression for Odds ratio with their 95% CIs were reported to test the probability of acquiring IDA in both genders.

Independent t-test was used for hemoglobin and ferritin levels in gender groups to show the significance of the specific tool/indicator in prediction of IDA.

Pearson correlation, spearman ranked correlation and logistic regression were used to show the relationship and probability of occurrence.

Descriptive statistics were used for numerical variables to show the frequency, mean and Standard Deviation for different numerical variables.

**Results**

Total number of patients was 149. The gender wise distribution was females 129 (86.56%), and males 20 (13.42%). The age range 20 - 30 years covering 65% of the study population followed by age range 30.1 - 40 years age (28.19%). Ninety (60.4%) cases had serum ferritin less than 15 ng/ml.

Mane age with SD was 30 ± 6.2 years. The minimum age was 20 years and maximum 55 years with range of 35. The Mean Hemoglobin with SD was 9.06 ± 1.82.

The mean ferritin level with SD was 64.35 ± 33.45. The minimum ferritin recorded was 1.72 to a maximum of 993.4 ng/ml with range of 991ng/ml (Table 1).

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Age</th>
<th>Hemoglobin</th>
<th>Serum Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Valid</td>
<td>149</td>
<td>149</td>
<td>149</td>
</tr>
<tr>
<td>N Missing</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>30</td>
<td>9.06</td>
<td>64.35</td>
</tr>
<tr>
<td>Median</td>
<td>30</td>
<td>9.8</td>
<td>10.14</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>6.258</td>
<td>1.82</td>
<td>33.45</td>
</tr>
<tr>
<td>Range</td>
<td>35</td>
<td>7.3</td>
<td>991.68</td>
</tr>
<tr>
<td>Minimum</td>
<td>20</td>
<td>3.7</td>
<td>1.72</td>
</tr>
<tr>
<td>Maximum</td>
<td>55</td>
<td>11</td>
<td>993.4</td>
</tr>
</tbody>
</table>

**Table 1:** Descriptive statistics of age, hemoglobin and serum ferritin levels.
The difference in Mean ± SD hemoglobin of the male gender (8.5 g/dl ± 1.43) was statistically significant with the Mean ± SD hemoglobin of the female gender (9.14 g/dl ± 1.86) with p-value of 0.053 (Independent Sample Test).

The difference in Mean ± SD of serum ferritin of the male gender (43.1 ng/ml ± 18.7) was not statistically significant with the Mean ± SD ferritin of female gender (67.65 ng/ml ± 16.95) with p-value of 0.446 (Independent Sample Test) (Table 2).

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
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</thead>
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<tr>
<td>Hb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>8.5</td>
<td>1.43</td>
<td>0.32159</td>
</tr>
<tr>
<td>Female</td>
<td>129</td>
<td>9.14</td>
<td>1.86</td>
<td>0.16444</td>
</tr>
<tr>
<td>Ferritin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>43.1</td>
<td>18.7</td>
<td>24.30691</td>
</tr>
<tr>
<td>Female</td>
<td>129</td>
<td>67.65</td>
<td>16.95</td>
<td>12.05831</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F</th>
<th>Sig.</th>
<th>t</th>
<th>df</th>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>2.084</td>
<td>0.151</td>
<td>-1.482</td>
<td>0.053</td>
<td>-0.6473</td>
<td>-1.5107 0.21607</td>
</tr>
<tr>
<td>Ferritin</td>
<td>0.489</td>
<td>0.486</td>
<td>-0.764</td>
<td>0.446</td>
<td>-24.55</td>
<td>-88.019 38.9188</td>
</tr>
</tbody>
</table>

**Table 2**: Difference of Hb% and ferritin levels in gender groups using independent T test.

We categorized our patient in anemic and non-anemic group on the basis of serum ferritin levels as defined by WHO [1]. The frequency of the patients with serum ferritin less than 15 ng/ml was 90 (60.4%), while 59 (39.6%) had serum ferritin more than 15 ng/ml.

The relationship of age, Hemoglobin and serum ferritin was assessed using Pearson correlation, and was found that there was a moderate uphill positive statistically significant relation between Hemoglobin and serum ferritin levels with (p-value 0.01, r = 0.4). There was an inverse (negative) relation of Hb and ferritin with an increase in age, but not statistically significant (p-value 0.185, r = -0.1), (p-value = 0.38r = -.07) respectively (Table 3).

<table>
<thead>
<tr>
<th>Ferritin</th>
<th>Pearson Correlation</th>
<th>1</th>
<th>.385**</th>
<th>-0.11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>149</td>
<td>149</td>
<td>149</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hb</th>
<th>Pearson Correlation</th>
<th>.385**</th>
<th>1</th>
<th>-0.071</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. (2-tailed)</td>
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<td>0</td>
<td>0.386</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>149</td>
<td>149</td>
<td>149</td>
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<table>
<thead>
<tr>
<th>Age</th>
<th>Pearson Correlation</th>
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<th>-0.071</th>
<th>1</th>
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<tbody>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.18</td>
<td>0.386</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>149</td>
<td>149</td>
<td>149</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3**: Correlations statistics of hemoglobin and serum ferritin levels, age and category of ferritin less than 15 ng/ml, using Pearson correlation test on 95% confidence interval.

Then we categorized patient on basis of serum ferritin with more or less than 15ng/ml and tried to seek its Pearson relation with Hb%. It was noted that there was a statistically strong significant relation of Hemoglobin with Category 1 (serum ferritin less than 15 ng/ml) with $p = 0.001$, $r = 0.6$.

We studied through logistic regression analysis of anemic patients based on ferritin levels with hemoglobin and age numerical variables. We found that probability of decrease in Hb is 3.2 times when ferritin decreases to less than 15 ng/ml ($p = 0.01$, $r = 3.2$), however no such relation of low ferritin was found with age (OR = 1.1).

We further studied through logistic regression analysis the relation and exposure of patients with low serum ferritin level (less than 15 ng/ml) with gender and were statistically significant. The odds ratio further stamped that probability of female gender is 2.7 times more to have low serum ferritin levels. ($p = 0.022$, OR = 2.7) (Table 4).

### Classification Table

<table>
<thead>
<tr>
<th>Observed Ferritin Category</th>
<th>Predicted Percentage Correct</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ferritin &lt; 15 ng/ml</strong></td>
<td></td>
</tr>
<tr>
<td>Step 1</td>
<td></td>
</tr>
<tr>
<td>Ferritin &lt; 15 ng/ml</td>
<td>67</td>
</tr>
<tr>
<td>Ferritin &gt; 15 ng/ml</td>
<td>23</td>
</tr>
<tr>
<td><strong>Ferritin &gt; 15 ng/ml</strong></td>
<td></td>
</tr>
<tr>
<td>Step 1</td>
<td></td>
</tr>
<tr>
<td>Ferritin &lt; 15 ng/ml</td>
<td>14</td>
</tr>
<tr>
<td>Ferritin &gt; 15 ng/ml</td>
<td>45</td>
</tr>
</tbody>
</table>

### Variables in the Equation

<table>
<thead>
<tr>
<th>Variables in the Equation</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
<th>Exp (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>1.182</td>
<td>0.217</td>
<td>29.557</td>
<td>1</td>
<td>0.000</td>
<td>3.262</td>
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<tr>
<td>Gender</td>
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<td>0.744</td>
<td>1.797</td>
<td>1</td>
<td>0.180</td>
<td>2.710</td>
</tr>
<tr>
<td>age</td>
<td>0.014</td>
<td>0.038</td>
<td>0.144</td>
<td>1</td>
<td>0.705</td>
<td>1.014</td>
</tr>
</tbody>
</table>

*Table 4: Logistic regression analysis of anemic patients based on ferritin with probability of exposure to low hemoglobin, gender groups and age.*

We also categorized the patients in age categories (20 - 30 years, 30 - 40 years and more than 40 years) and tried to observe their relation and exposure. No statistically significant relation or exposure was noted in all age categories with cat1 of ferritin (less than 15 mg/ml ferritin).

To strengthen the observations noted in the logistic regression analysis in table 4, we tried the apply correlation test (Spearman ranked correlation) on gender vs ferritin categories prepared as per WHO recommendations¹. We observed that there was a statistically significant correlation between female gender and cat 1 (patients with ferritin less than 15ng/ml) with ($p = 0.015$, $\rho = 0.5$) (Table 5).

### Spearman Correlation Table

<table>
<thead>
<tr>
<th>Gender</th>
<th>Ferritin Categories</th>
<th>Spearman Correlation rho</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
<th>149</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>.498*</td>
<td>.015</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>.498*</td>
<td>1</td>
<td>149</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed).

*Table 5: Spearman correlation statistics of gender like hood for low ferritin levels.*
Simultaneously, the Positive Predictive Value (PPV) of Hemoglobin as predictors of IDA was 39.1%, while the Positive Predictive Value of serum ferritin as predictors of IDA was 60.4%.

**Positive predictive value of ferritin**

\[
\frac{a}{a \pm b} = \frac{90}{149} = 60.4.
\]

**Positive predictive value of hemoglobin**

\[
\frac{a}{a \pm b} = \frac{149}{381} = 39.1.
\]

**Discussion and Conclusion**

Iron deficiency anemia in pregnancy is due to depleted iron stores, similarly the growing fetus’s increased demands for iron can contribute to ID. IDA is reported with a decreased working capacity, increased maternal morbidity and mortality especially in third trimester of pregnancy and with poor infant and maternal outcomes at the end of gestational period [13]. Serum ferritin level estimation is a cost effective and indirect measurement of iron stores in the body while the hemoglobin levels give an initial intimation of anemia that could be of any type.

Similarly, we observed that the difference in Mean ± SD hemoglobin of the male gender (8.5 g/dl ± 1.43) was statistically significant with the Mean ± SD hemoglobin female gender (9.14 g/dl ± 1.86) with p-value of 0.053 (Independent Sample Test). The same was reported by another study that the difference in haemoglobin concentration between the gender groups was significant with a p-value of < 0.0001. Their findings matched our study that there is a positive correlation of hemoglobin concentration in gender groups as well as with serum ferritin levels [14]. Another study from India reported the mean value of hemoglobin in girls was (12.57 ± 1.16) with female pre-dominance for acquiring state of anemia. Many things can contribute to this including nutrition, gestational anemia and socioeconomic factors etc [15].

Another study reported ferritin levels below normal level in 242 (82%) children with anemia [17]. Abuaisha M., et al. [18] reported the prevalence of iron deficiency in 57.5% cases based on ferritin levels at 95% confidence interval that closely matching our findings. A study from India also reported that Serum ferritin levels were less than 12 ng/ml in 58% of their study population with subclinical iron deficient anemia [20] that matches with our findings.

There was a statistically moderate to strong uphill positive relation of hemoglobin with Category 1 (serum ferritin less than 15 ng/ml) with p-value 0.01, \( r = 0.6 \). The same was reported by Franchin M., et al [14]. It has been reported in a study published in international study of medical research that there was a statically significant correlation between serum ferritin and Hemoglobin levels [21].

Furthermore it was noted that there was a weak inverse (negative) relation of Hb and ferritin with an increase in age, but not statistically significant (p-value 0.185, \( r = -0.1 \), (p-value = 0.38r = -.07) respectively. Another study revealed that there were statistically significant differences in age (36.3 ± 26.1 years in the group with low ferritin as compared to 51.6 ± 16.4 years in the group with normal ferritin with a p-value of 0.001 and a similar relation of age with low hemoglobin concentration vs normal concentration [22].

We studied through logistic regression analysis and found that probability of low Hb% is 4 times when ferritin decreases to less than 15 ng/ml (p-value 0.01, r = 3.38), however no such relation of low ferritin was found with age of patients. Our further findings through logistic regression showed that probability of female gender was 4.47 times more to have low serum ferritin levels (P-value 0.022, OR = 4.47). Abuiaisha M., et al. [18] reported the prevalence of iron deficiency in 57.5% females and 7.6% in males that closely matching our findings.

We concluded that the Positive Predictive Value (PPV) of Hemoglobin as predictors of IDA was 39.1%, while the Positive Predictive Value of serum ferritin as predictors of IDA was 60.4%. Therefore, serum ferritin is a good indicator of iron deficiency anemia.

Out of 149 patients 60.4% were iron deficient with ferritin < 15 g/ml. Furthermore, there is a statistically significant relation of hemoglobin with gender and ferritin levels. While the serum ferritin relation with gender and age is not statistically significant at 95% confidence level. However, there was a trend of low ferritin less than 15 ng/ml (anemia as per criteria of WHO) for female gender without reaching at statistical significance. Age as variable showed moderate downhill negative linear relationship (inverse relation) with Hemoglobin and ferritin levels but that was not statistically significant with p-value more than 0.05.

Hence it is recommended that as IDA is more common in female gender therefore all the females attending the antenatal care services in tertiary and secondary care hospital with Hb% less than 11 g/dl must be screened for serum ferritin levels and remedial action may be taken well in time to combat iron deficiency in pregnancy specially in third trimester to avoid the fetal thirst for iron and to avoid neonatal morbidities and mortalities associated with IDA.

Bibliography

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