INTRODUCTION
Iron deficiency anemia is a common disorder, affecting about 1.2 billion people worldwide.1 According to WHO global database on anemia 2005, about one quarter of the world’s population had iron deficiency anemia (IDA), being most prevalent among preschool children and women.2 The good standard for determination of depleted iron stores is lack of stainable iron in the bone marrow. However, this is an invasive procedure.3 Patient's history, complete blood count, red cell indices, and examination of peripheral blood smear usually allow the clinician to make a presumptive diagnosis of iron deficiency anemia.4 Other less invasive laboratory tests such as determination of serum iron, transferrin (TIBC), transferrin saturation, ferritin and soluble transferrin receptors (sTfR) are proposed as useful in detection of iron depletion before the onset of anemia.5,6 The diagnostic value of serum iron and TIBC have certain limitations.7 Using these tests, iron deficiency can only be detected when it is already relatively advanced, i.e. when the iron stores of the body are already significantly depleted or even exhausted.8 Moreover, TIBC is reportedly negative acute phase reactant. Diurnal variation in circulating iron, also decreases its diagnostic value.9,10 Serum ferritin concentration has been shown to be an excellent indicator of iron stores in otherwise healthy adults and is used as an alternative to bone marrow aspiration examination to assess the iron store in most patients.11,12 Provided that an anemic patient does not have an accompanying infections or inflammatory disease, serum ferritin at cut-off limit of 41 ng/ml has a sensitivity and specificity of 98% and 98%, respectively.13 In many studies so far, both nationally and internationally, serum iron, TIBC and transferrin saturation are still being carried out with equivocal results. Keeping in view this fact, objective of this study was to determine the diagnostic accuracy of serum iron and TIBC in detection of iron deficiency and to evaluate the importance of serum ferritin in this condition.

METHODOLOGY
This study was conducted at Department of Chemical Pathology and Endocrinology, from January 2013 to October 2015. It was a diagnostic accuracy study with retrospective data collection. Relevant clinical details about the patients including history of infections, any
inflammation, malignancy and time of sample collection were also sought from available data. STARD Guidelines (Updated 2015) were used as the study model. The study was approved by the local Institutional Review Board of the Institute. Patients of all age groups and either gender were included in the study. Patients with history of iron therapy, previous blood transfusions, pregnancy, and those taking oral contraceptives were excluded from the study. Record of parameters like serum iron, transferrin (TIBC), ferritin and CRP from LIMS was retrieved. Serum C Reactive Protein (CRP) was done to cater for the cases with hidden infections or inflammations. Cases with raised serum ferritin levels (male > 336 ng/ml, female > 307 ng/ml suggesting iron overload) and raised CRP (> 6.0 mg/l, cut off=6 mg/l) were excluded from the study. Serum iron was determined by ferrozine calorimetric method, TIBC by colorimetric chromazurol dye binding method using ADVIA 1800 system (Siemens Medical Solutions Diagnostics, USA). Serum ferritin was determined by using quantitative two-site chemiluminescent immuno-metric assay by immulite 2000 system (Siemens Medical Solutions Diagnostics, USA). CRP was analysed by latex method (semi-quantitative- Cortez Diagnostics, USA). LIMS was analysed for retrieval of data.

Performance of serum iron, TIBC, TIBC and transferrin saturation were expressed in sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios - negative and positive. Data was entered and analysed by using Statistical Package for Social Sciences (SPSS) version 19 (SPSS, Inc, Chicago, IL, USA). Descriptive statistics for quantitative variables like age, serum iron, transferrin (TIBC), transferrin saturation and ferritin were described and compared between males and females. Since distribution was non-Gaussian, so median and IQR were calculated. Median and IQR were calculated for age, serum iron, TIBC, transferrin saturation and ferritin. The correlation analysis between ferritin and other parameters – serum iron, TIBC and transferrin saturation – was also carried out by Spearman’s correlation coefficient (r). At 95% confidence interval, p-value of < 0.05 was regarded to indicate statistical significance.

RESULTS
A total of 1,815 cases fulfilling the inclusion criteria were included in this study. Result of these 1,815 cases evaluated for iron deficiency, revealed 931 (51.29%) males and 884 (48.71%) females. Table I describes the baseline characteristics of the cases and stratified by gender. All the parameters of diagnostic accuracy, i.e. sensitivity, specificity, PPV and NPV of serum iron, and TIBC were low (Table II). Spearman’s correlation coefficient (r) between ferritin and other parameters - serum, iron (r=0.58, 95% CI 0.011 - 0.102), transferrin (TIBC) (r=0.099, 95% CI 0.52 - 0.145), and transferrin saturation (r=0.012, 95% CI 0.034 - 0.060) also showed poor correlation (Figure 1).

DISCUSSION
In iron deficiency anemia, hemoglobin, ferritin and transferrin saturation become abnormal, and decrease in iron stores is reflected by falling serum ferritin. Decrease in serum iron and increase in transferrin (TIBC) are difficult to correlate and document. All the above tests, except serum ferritin, lack specificity and are time consuming, expensive and these add no additional help in diagnosis. Serum iron, although routinely used for diagnosis of iron deficiency along with transferrin (TIBC) and transferrin saturation, lacks correlation with the disease. Likewise, transferrin (TIBC) levels display variations in disorders of iron metabolism and are decreased in chronic inflammatory disorders and malignancies. In this study, serum ferritin was taken as the gold standard. Serum iron had sensitivity of 63.5% and specificity of 38.6%, while transferrin (TIBC) had sensitivity of 64.5% with

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall Median (IQR)</th>
<th>Male Median (IQR)</th>
<th>Female Median (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.1 (18.1)</td>
<td>32.0 (20.0)</td>
<td>26.0 (11.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum iron (umol/L)</td>
<td>46.0 (27.0)</td>
<td>42.0 (25.0)</td>
<td>51.0 (27.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum TIBC (umol/L)</td>
<td>88.0 (18.1)</td>
<td>88.0 (19.0)</td>
<td>88.0 (17.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum ferritin (ng/mL)</td>
<td>22.19 (92.8)</td>
<td>28.4 (19.8)</td>
<td>15.2 (99.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% Transferrin saturation</td>
<td>52.8 (54.6)</td>
<td>47.7 (37.1)</td>
<td>56.2 (53.8)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*p-value is calculated using Mann-Whitney U-test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall Sensitivity</th>
<th>Male Sensitivity</th>
<th>Female Sensitivity</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive cases (TP)</td>
<td>840</td>
<td>63.5%</td>
<td>454</td>
<td>64.49%</td>
</tr>
<tr>
<td>False positive cases (FP)</td>
<td>650</td>
<td>36.7%</td>
<td>635</td>
<td>42.84%</td>
</tr>
<tr>
<td>True negative cases (TN)</td>
<td>410</td>
<td>42.47%</td>
<td>476</td>
<td>41.68%</td>
</tr>
<tr>
<td>False negative cases (FN)</td>
<td>275</td>
<td>59.85%</td>
<td>250</td>
<td>55.56%</td>
</tr>
<tr>
<td>Likelihood ratio (Positive)</td>
<td>1.03</td>
<td>Likelihood ratio (Positive)</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>Likelihood ratio (Negative)</td>
<td>0.94</td>
<td>Likelihood ratio (Negative)</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Diagnostic accuracy</td>
<td>49.03%</td>
<td>Diagnostic accuracy</td>
<td>51.23%</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = TP/(TP+FN) Specitivity = TN/(TN+FP) PPV = TP/(TP+FP) NPV = TN/(TN+FN)
Likelihood ratio (positive) = Sensitivity/1-Specificity Likelihood ratio (negative) = 1-Sensitivity/Specificity
Diagnostic accuracy = (TP+TN)/(TP+TN+FP+FN)


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specificity of 42.8%. These results are comparable with another study carried out by Khan et al. with sensitivity of 85% and specificity of 15% for serum iron, and for transferrin (TIBC) with sensitivity of 73% and specificity of 28%. High sensitivity seen in that study compared to the present study may be due to smaller sample size in the former.

Another study carried out in Taiwan, revealed high sensitivity and specificity rate for serum iron (74% and 91%, respectively) and for TIBC (65% and 100%, respectively). Such a large difference in results may be related to less prevalence of the disease in China, less sample size, and different method of analysis of these parameters. Alternatively, they might have selected patients without acute inflammation or infection while the present sample was of mixed patients, inspite of efforts made by using available relevant clinical information and CRP.

In this study, it was found that ferritin had a poor correlation with serum iron (r=0.056), transferrin (TIBC) (r=0.099), and transferrin saturation (r=0.012). This study is consistent with the notion that serum iron and transferrin (TIBC) are not reliable parameters of iron depletion state. Almost similar results were seen in the study by Khan et al. However, another study by Peter et al. in Canada showed a relatively good correlation between ferritin and TIBC (r=0.66), while a weak correlation was seen between ferritin and TIBC in a study by Schuepbach et al.

This study indicated that serum iron, transferrin (TIBC), and transferrin saturation had poor sensitivity and specificity for the diagnosis of iron deficiency state. Moreover, owing to more time on the analysis of these parameters plus expenses incurred on their reagent purchase are strong evidences against their utility for diagnosis of iron deficiency state. Limitations of serum ferritin in chronic inflammation, malignancy or chronic infection can be catered for by combing ferritin with other hematological parameters like MCV, RDW, and other RBC indices or with other markers of inflammation such as CRP, fibrinogen and ESR.

CONCLUSION

This study concluded that iron deficiency could be reliably diagnosed by the measurement of serum ferritin, without the need for any further laboratory parameters. The addition of an array of laboratory parameters such as serum iron, transferrin (TIBC) or transferrin saturation may not add any further information. If serum ferritin is available, in the absence of infections and acute inflammation, these tests are redundant for the diagnosis of iron deficiency state. Moreover, further prospective study should be carried out to validate other markers of iron status, e.g. sTfR.

REFERENCES


11. Kell DB, Pretorius E. Serum ferritin is an important disease marker, and is mainly a leakage product from damaged cells. Metalomics 2014; 6:748-73.


