Iron deficiency anaemia (IDA) is the commonest cause of anaemia worldwide. Automated blood counts are helpful in indicating the presence of hypochromic and microcytic anaemia, yet iron deficiency is not the sole cause of this blood picture. It may be seen in anaemia of chronic disorders (ACD), which is the commonest cause of anaemia in hospitalized population. In contrast to IDA, bone marrow storage iron is adequate but erythroblast iron is reduced. Despite low serum iron, it does not respond to iron therapy. It is only corrected by successful treatment of the underlying cause. Hence it is important to differentiate between these two types of anaemias.1

The conventional laboratory tests of iron status include serum iron, total iron binding capacity (TIBC) and serum ferritin. Each one of these investigations, have its own advantages and diagnostic pitfalls. Serum iron and TIBC are useful in diagnosing advanced iron deficiency anaemia, but their sensitivity for early iron deficiency is limited. By contrast, serum ferritin is very useful in diagnosis of early iron deficiency, yet its advantage is limited by its non-specific increase in inflammatory diseases and hepatocellular damage.2,3

In recent years, remarkable insight into the control of iron homeostasis, has been accomplished at the molecular level. In the blood, iron is transported as a complex with transferrin. Transferrin delivers its iron to target cells by binding to specific cell surface transferrin receptors (TfR), followed by endocytosis of the complex. The metabolic need of an iron deficient cell requires increased production of TfR to facilitate iron uptake and suppression of ferritin synthesis. Conversely, in iron excess, ferritin synthesis needs to be enhanced and TfR is suppressed.4,5

Soluble TfR present in serum comprise the truncated
form of the tissue receptors, originating mainly from the developing erythrocytes, which shed their receptors during maturation. Concentration of these serum receptors rises steeply in the presence of IDA, whereas in ACD, it decreases.6

This study was carried out to evaluate the efficacy of serum concentration of TfR in diagnosing and differentiating IDA from ACD. The results of biochemical measurements were compared with the bone marrow slides stained for iron, which served as standard in the diagnosis of IDA and ACD.

Materials and Methods

This prospective comparison study was conducted in the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi. Adult anaemic patients both males and females, outpatient/inpatient in various military and civil hospitals of Rawalpindi/Islamabad, reporting for bone marrow examination were included in the study.

Patients with haemoglobin <12 g/dl in males and <11 g/dl in females and having low indices (MCV <75 fl, MCH <25 pg and MCHC <30 g/dl) were assessed for marrow iron stores by Perl's stain (Prussian blue). Absence of marrow iron stores denoted IDA whereas abundance of these stores, with decreased siderocytes and sideroblasts denoted ACD. Other causes of hypochromic microcytic anaemia such as thalassaemia trait and sideroblastic anaemia were excluded from the study.

Five ml of blood in plain tube was collected from each patient for the estimation of serum iron, TIBC, serum ferritin and sTfR concentration. Estimation of serum iron and TIBC was done by calorimetric method, whereas estimation of serum ferritin was done by Chemiluminescence method. These tests were carried out in Chemical Pathology Department, AFIP, Rawalpindi.

Estimation of sTfR was carried out using sTfR immuno-enzymometric assay test (IEMA). The commercial kit used was supplied by Orion Diagnostica, Turku, Finland (IDEA sTfR IEMA test). In this study two such kits were utilized, each with 96 tests (including 6 standards and 2 controls). So the total number of tests that could be carried out on patient's sample with each kit was eighty eight.

The principle of this test was a non-competitive sandwich type assay technique using a monoclonal antibody Anti-CD71 (Anti-TfR antibody).

The procedure of the test was strictly followed as per literature supplied by the manufacturer. All laboratory measures and precautions were strictly adhered to for ensuring precision and accuracy of the test results. The findings were meticulously recorded. Absorbance values of the standards were plotted on a Linear-Linear graph paper with the absorbance values on the ordinate and the known sTfR concentrations (mg/l) on the abscissa. A standard curve was obtained.

Results

From the graph, the sTfR concentration of the unknown samples were read out (Table 1). The mean ± SD of the results of other biochemical parameters and haematologic indices in patients of IDA and ACD are shown in table 2 and 3 respectively.

A total of 176 cases were studied; 94 (53.4%) males and 82 (46.5%) females with male to female ratio of 1.14:1. Out of these, 90 (51.1%) cases were diagnosed as ACD and 86 (48.8%) cases as IDA. Hundred percent cases of IDA (n= 86) had raised sTfR levels above the reference range (1.3-3.3 mg/l), with a mean + SD sTfR level of 9.68 ± 2.48 mg/l. The mean + SD sTfR levels in ACD patients was 2.96±1.28 mg/l. 66.4% of ACD patients (n=60) had sTfR levels lower than the reference range (1.3-3.3 mg/l). However, 33.3% of

Table 1. Summary of haematologic and other biochemical parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Iron deficiency anaemia (n=86)</th>
<th>Anaemia of chronic disorder (n=90)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>7.26</td>
<td>1.93</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>68.7</td>
<td>6.0</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.3</td>
<td>2.84</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>27.1</td>
<td>1.91</td>
</tr>
<tr>
<td>Serum iron (µmol/l)</td>
<td>10.5</td>
<td>3.84</td>
</tr>
<tr>
<td>Serum TIBC (µmol/l)</td>
<td>93.7</td>
<td>8.0</td>
</tr>
<tr>
<td>Serum ferritin (ng/ml)</td>
<td>18.4</td>
<td>13.8</td>
</tr>
<tr>
<td>sTfR concentration (mg/l)</td>
<td>9.68</td>
<td>2.48</td>
</tr>
</tbody>
</table>
ACD patients (n=30) had sTfR levels >3.3 mg/l.

Table 2. Statistics by t-tests (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>STD</th>
<th>STD error mean 95% CI Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDA</td>
<td>86</td>
<td>9.68</td>
<td>2.48</td>
<td>0.267 9.15</td>
<td>10.21</td>
</tr>
<tr>
<td>ACD</td>
<td>90</td>
<td>2.96</td>
<td>1.28</td>
<td>0.13 2.69</td>
<td>3.23</td>
</tr>
</tbody>
</table>

Table 3. Diagnostic sensitivity and specificity.

<table>
<thead>
<tr>
<th></th>
<th>True positive</th>
<th>True negative</th>
<th>False positive</th>
<th>False negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDA</td>
<td>66</td>
<td>90</td>
<td>0</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>ACD</td>
<td>60</td>
<td>86</td>
<td>0</td>
<td>30</td>
<td>66.6%</td>
<td>100%</td>
<td>100%</td>
<td>74.1%</td>
</tr>
</tbody>
</table>

Data was stored and maintained in a stat programme, SPSS (Statistical Programme for Social Sciences). One sample t-test was employed to compare the standard error of mean of sTfR concentration of two groups. The 95% confidence interval (CI) of IDA was 9.15 to 10.21, while that of ACD was 2.69 to 3.23. The visual inspection of 95% CI of two groups showed significant difference, statistically (p<0.05). Therefore, it is inferred that sTfR can clearly differentiate between IDA and ACD in our set up.

The diagnostic sensitivity and specificity of the test was 100% in case of IDA, with 100% PPV (positive predictive value) and NPV (negative predictive value). However in case of ACD the diagnostic sensitivity was found to be 66.6% and diagnostic specificity 100%. In this case the PPV was found to be 100% whereas NPV was 74.1%.

Discussion

Although iron deficiency is the commonest cause of hypochromic microcytic anaemia, yet it is not the sole cause of this blood picture. It is frequently observed in anaemia of chronic disorders. Since the etiology, pathogenesis, treatment and outcome of the two conditions differ greatly, hence it is of paramount importance to distinguish between the two types of anaemia. Therefore, high sensitivity and specificity are particularly important in a test of iron status. A suggestion of depleted iron stores calls for tedious clinical and laboratory investigations to identify the underlying cause. Early detection of unexplained iron deficiency warrants extensive investigations of the GIT, as ulcers and malignant tumours are not uncommonly associated with unexplained anaemia, and potentially curable if diagnosed at a resectable stage.

Apparently it seems logical to measure serum iron and TIBC for study of iron status. The principle limitation of the usefulness of serum iron is considerable variability in values, which may result from both technical and physiological factors. Moreover it is useful only in advanced IDA, and not in early diagnosis of IDA. Presently serum ferritin is considered the best marker of iron status, the values closely correlate with marrow iron stores. It is very useful in detection of early iron deficiency in uncomplicated cases. But its nature as an acute phase reactant protein, limits its usefulness, especially in chronically ill hospitalized patients.

One of the major difficulties encountered in clinical medicine is to distinguish between IDA and ACD. Unfortunately the clinical presentations of the patients are often not typical and the results of the above assays not uncommonly appear conflicting, especially if the patient of ACD has co-existing IDA. Although serum ferritin <15 ng/ml is virtually diagnostic of iron deficiency, its sensitivity is grossly compromised in the presence of inflammation. Thus making the diagnosis further difficult and often requiring a bone marrow aspirate, stained for iron, for accurate assessment of iron status. This procedure is invasive, unpleasant and unacceptable to most of the patients.

In the light of an obvious need for improved laboratory test, determination of the soluble TfR in serum has been introduced. It is a recent discovery and a useful addition to the list of investigations of anaemia. Iron deficiency results in prompt upregulation of TfR on the individual cells. So this analyte is regarded as the first to signal iron deficient erythropoiesis, even before a reduction in MCV is observed. Since receptor synthesis is up-regulated in iron deprived tissues, it can be argued that the assessment of iron status at the tissue level is of more functional importance,
assessment of iron status at the tissue level is of more functional importance, especially when examining the effects of iron depletion on the body, than an assessment of stores. Conversely, inflammatory/autoimmune diseases do not produce any significant change in serum TfR concentrations. This is one of the major advantages of sTfR estimation. Unlike serum iron, TIBC and serum ferritin, the sTfR levels are not influenced by the acute phase and other responses and are therefore more reliable in the differential diagnosis of iron status.

In this study, the results of serum iron, TIBC and serum ferritin, correlated well in established IDA, as confirmed by absence of stainable iron in the marrow, with a fall in serum iron, raised TIBC and decreased serum ferritin. However, there was not any definite correlation between these values in ACD. On the other hand, sTfR level was significantly raised in IDA (100% sensitivity and specificity), whereas values remained low in majority of patients with ACD (diagnostic sensitivity 66.6% and specificity 100%). Thus clearly separating the two conditions. The results of this study are also in accordance with the previous studies on the subject, reported in western literature according to which this assay is as reliable as bone marrow aspirate in large majority of patients with IDA and is of value in diagnosing and distinguishing IDA from ACD.

References