Soluble Transferrin Receptor, Ferritin and Soluble Transferrin Receptor – Ferritin Index in Assessment of Anaemia in Rhaeumatoid Arthritis

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SUMMARY

Anaemia of chronic disease (ACD) is a frequent complication of rheumatoid arthritis (RA). A diagnostic difficulty in RA is the distinction between iron deficiency anaemia (IDA) and ACD. The aim of our study was to evaluate the usefulness of serum soluble transferrin receptor (sTfR) and sTfR/log ferritin (TfR-F) index to diagnose iron deficiency in RA patients with anaemia. Routine laboratory indices of anaemia and sTfR were measured in 20 healthy persons to form the control group, 30 patients with iron deficiency anaemia and 28 RA patients with anaemia. Serum sTfR levels were significantly elevated above the cut-off value in patients with IDA and those in the iron depleted RA subgroup (ferritin <60μg/L) compared with those in the control and iron repleted RA subgroup (ferritin >60μg/L). The same was observed for TfR-F index. However, five patients in the iron repleted RA sub group had an elevated sTfR level, of which two had increased TfR-F index. Serum sTfR correlated well with the markers of anaemia and not with ESR. Ferritin had no correlation with markers of anaemia but correlated well with ESR. Measurement of sTfR and TfR-F index are good indicators of iron deficiency in RA patients with anaemia. To be cost effective, sTfR can be estimated in RA patients with anaemia when the ferritin level is more than 60 μg/L.

KEY WORDS:
Rheumatoid arthritis, Serum ferritin, Serum soluble transferrin receptor, sTfR/log ferritin index

INTRODUCTION

Anaemia is a common extra-articular manifestation of rheumatoid arthritis (RA). A Dutch study has found that about 60% of patients with rheumatoid arthritis are anaemic[1, 2]. The anaemia in RA may be due to anaemia of chronic disease (ACD), iron deficiency, vitamin B12 or folate deficiency. ACD owes its name to the fact that it accompanies a very broad spectrum of diseases most commonly represented by cancer, chronic infections and inflammatory disorders. The underlying mechanisms of ACD are not fully understood but a variety of processes have been shown to be involved in the pathogenesis of ACD including a diversion of iron traffic from the serum to stores within the (RE)system, diminished erythropoiesis, a blunted response to erythropoietin, and decreased red cell survival[3]. There is growing evidence that inflammation-mediated cytokines particularly tumour necrosis factor (TNF-α) and interleukin 1 (IL-1) and IL-6 contribute to ACD in RA[4].

In ACD, functional iron deficiency is the limiting factor of erythrocyte haemoglobinisation. Functional iron deficiency is defined as an imbalance between the iron needs of the erythroid marrow and iron supply, which is not maintained at a rate sufficient to allow haemoglobinisation of the erythrocytes. In iron deficiency anaemia (IDA) the iron supply depends on the amount of iron stores, whereas in ACD, the supply depends on the rate of mobilisation of iron from the RE system. In ACD, functional iron deficiency may occur even in the presence of large iron stores when release is impaired[5].

The prevalence of iron deficiency is up to 50-70% in RA[6]. Serum iron, transferrin and ferritin are widely used to assess iron status. Serum iron shows diurnal variation and may transiently reach reference values after ingestion of meat or oral iron supplements. The synthesis of hepatic transferrin is down-regulated in chronic disease[7]. Ferritin is an acute phase reactant and its synthesis is increased independently of iron status in patients with acute or chronic inflammation, malignancy, or liver disease[4].

In ACD, serum iron levels and TIBC are low and serum ferritin levels are normal or increased. In contrast, serum iron and serum ferritin levels are decreased and TIBC is elevated in IDA. In the general population, serum ferritin has been used as the most reliable marker of iron deficiency, but as an indicator of iron deficiency in RA it is of limited value. In evaluating accurate body iron stores in patients with rheumatoid arthritis, the examination of stainable iron in bone marrow aspirate is the gold standard. However, marrow examinations should not be performed routinely in clinical practice for the sole purpose of diagnosing IDA because this procedure is invasive, expensive and time consuming. Moreover, the quantitation of stainable iron is dependent on technique and interpretation. Therefore, there is a need for non-invasive and sensitive means of detecting iron deficiency.

Studies have shown that serum transferrin receptor (sTfR) is a sensitive, quantitative measurement of tissue iron

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deficiency[8,9]. Unlike conventional laboratory investigations, sTfR is not affected by inflammation. Transferrin receptors mediate cellular uptake of iron and their expression and the concentration in serum are increased when the intracellular iron level is decreased. Several studies have indicated that sTfR level is significantly increased in patients with IDA[8, 9, 10]. The sTfR is not an acute phase reactant and remains normal in patients with inflammatory conditions. As a result, measurement of sTfR may be particularly helpful in differentiating between IDA and ACD[11].

Serum ferritin reflects the storage iron compartment and sTfR is proportional to the cellular iron needs. The ratio of sTfR/log ferritin (TfR-F index) has been suggested as a good estimate of body iron stores[12]. Suominen et al found TfR-F index useful in detecting iron deficiency in anaemic RA patients[13].

The role of sTfR and TfR-F index in anaemic RA patients has been studied in the Caucasian population. This study was undertaken to evaluate the clinical usefulness of sTfR and TfR-F index to identify the iron deficiency in rheumatoid arthritis patients with anaemia in the local population.

MATERIALS AND METHODS

Full blood counts were determined using Cell Dyn 4000 analyser (Abbott Laboratories, Abbott Park, Illinois, USA). Serum sTfR was measured by particle enhanced immunoturbidimetric method on COBAS INTEGRA 800 (Roche Diagnostics, Manheim, Germany). The reference range of the kit as mentioned by the manufacturer was 2.2-5.0 mg/L for males and 1.9-4.4 mg/L for females. Ferritin, which had a reference range of 22-322.0 μg/L, was measured using ADVIA Centaur Immunoanalyser (Bayer Healthcare, New York, USA). Serum iron (reference range 9.5-29.9 μmol/L) and transferrin (reference range 2.0-3.6g/L) were analysed on COBAS INTEGRA 800 (Roche Diagnostics, Manheim, Germany).

Blood samples were obtained from 20 healthy female donors, twenty eight female RA patients who met the criteria set by The American College of Rheumatology[14] and presented with anaemia and thirty patients who had only iron deficiency.

None of these patients had abnormal renal function, haematological disorders or recent blood transfusion. Serum TIBC was calculated using the formula which is TIBC (μmol/l) = TF (g/l) x 25.2[15]

Statistical evaluations were performed by analysis of variance and linear regression analysis.

RESULTS

A summary of the results of the control and study populations is presented in Table I. Serum sTfR levels in the 20 control subjects were 3.37 ± 1.82 mg/L (mean ± 2 SD), which agreed with manufacturer's stated reference range. Patients with IDA had a mean sTfR level of 17.16 mg/L (range 4.52 – 43.41 mg/L) and statistically significant increase ($p<0.0001$) was observed compared with that of control group (Figure 1). In RA patients, sTfR level (7.94 ± 8.48 mg/L) was not significantly different from that of control ($p>0.05$). However, a significant difference was noted between RA and IDA patients ($p<0.0001$). TfR-F Index was significantly higher (31.03 ± 48.2) in the IDA population ($p<0.001$) whereas in RA patients (7.81 ± 18.22) it was not significantly different from that of the control group (2.45 ± 1.47) ($p>0.05$) as shown in Figure 3.

Correlation between serum transferrin receptor levels and indicators of anaemia in RA patients:

Serum sTfR correlated inversely with haemoglobin ($r = -0.808$, $p<0.0001$) and ferritin ($r = - 0.408$, $p=0.03$). It correlated positively with transferrin ($r = 0.389$, $p = 0.04$) and TIBC ($r = 0.389$, $p =0.04$). There was no correlation between sTfR level and ESR ($p>0.05$)

Table I

	Control (n=20)	IDA (n=30)	RA(n=28)
HGB g/L	132.5 ± 14.31	75.87 ± 40.92	98.06 ± 27.83
MCV fl	89.95 ± 9.30	65.03 ± 15.95	71.43 ± 9.74
Iron μmol/L	15.81 ± 10.73	2.88 ± 4.86	5.20 ± 7.44
Ferritin μg/L	27.90 ± 27.32	5.9 ± 12.78	60.89 ± 133.46
Transferrin g/L	2.78 ± 0.65	3.7 ± 1.13	2.77 ± 1.50
TIBC μmol/L	70.13 ± 16.43	93.9 ± 28.39	69.91 ± 39.74
sTfR mg/L	3.37 ± 1.82	17.16 ± 19.20	7.94 ± 8.48
sTfR – F index	2.45 ± 1.47	31.03 ± 48.2	7.81 ± 18.22

Values are expressed as mean ± 2 SD

Table II

	Iron depleted Ferritin <60 μg/L	Iron repleted Ferritin >60 μg/l
HGB g/L	93.76 ± 28.12	104.71 ±22.50
MCV fl	71.47 ± 10.4	71.36 ± 9.13
Iron μmol/L	4.42 ± 7.07	6.42 ± 7.66
Transferrin g/L	3.25 ± 0.98	2.05 ± 0.83
TIBC μmol/L	81.80 ± 24.78	51.15 ±20.97
sTfR mg/L	9.13 ± 7.98	6.10 ± 8.23
sTfR – F index	10.9 ± 21.06	2.98 ± 4.19

Values are expressed as mean ± 2 SD

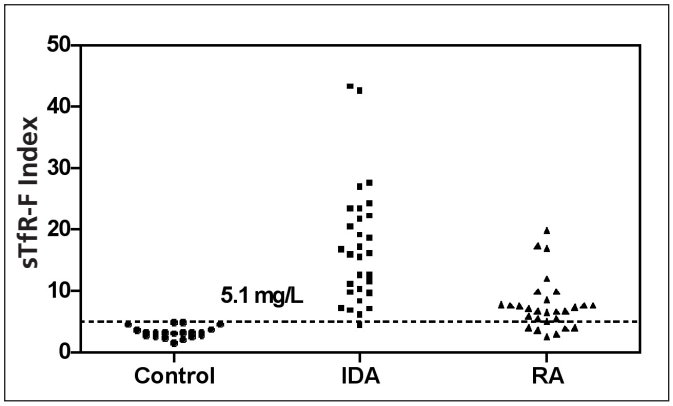

Fig. 1: Serum level of sTfR in different study groups. Abbreviation: IDA -iron deficiency anaemia, RA-rheumatoid arthritis. Dotted line indicates the cut-off value for sTfR obtained from the control (mean+ 2SD) =5.1mg/L

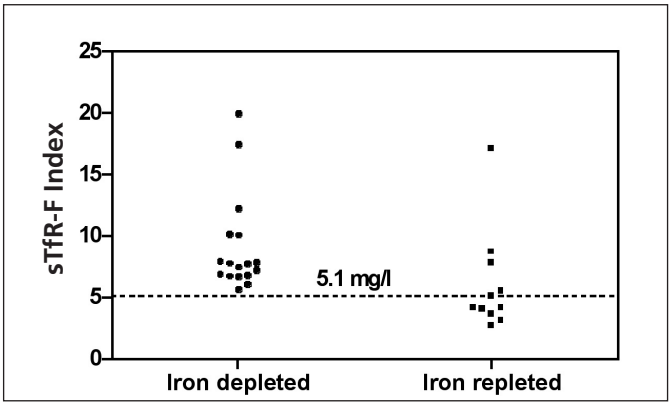

Fig. 2: sTfR level in iron depleted and iron repleted rheumatoid arthritis patients. Dotted line indicates the cut-off value for sTfR obtained from the control (mean + 2SD) = 5.1mg/L

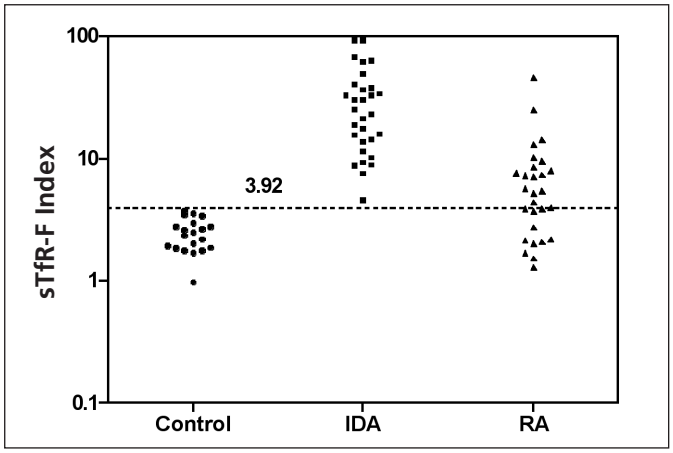

Fig. 3: TfR-F Index in the study population. Abbreviation: IDA - iron deficiency anaemia, RA-rheumatoid arthritis. Dotted line indicates the cut-off value for TfR-F index obtained from the control (mean+ 2SD) = 3.92

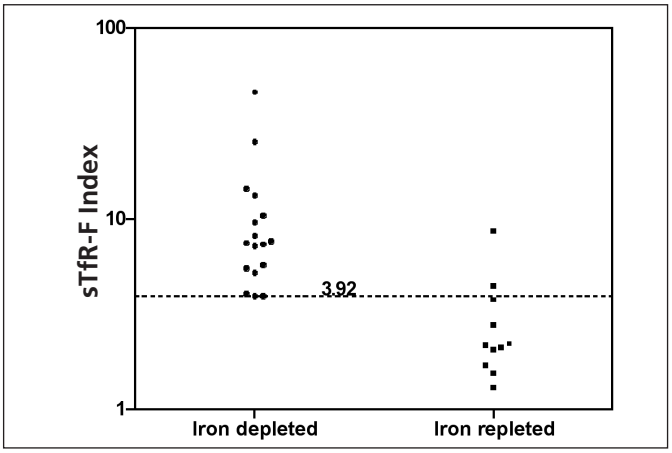

Fig. 4: TfR-F index in iron depleted and iron repleted rheumatoid arthritis patients. Dotted line indicates the cut-off value for TfR-F index obtained from the control (mean + 2SD) =3.92

Correlation of ferritin with other indicators in RA:
Serum ferritin levels had no correlation with haemoglobin and MCV. An inverse correlation was noted with sTfR (r = -0.408, p=0.03), transferrin (r=-0.733, p<0.0001) and TIBC (r=-0.733, p<0.0001) and a positive correlation was seen with ESR (r=0.39, p<0.05).

Using serum ferritin level of 60 µg/L as a cut off value, RA patients were divided into two sub groups i.e., iron depleted and iron repleted respectively. The results are shown in Table II. The values of sTfR in iron repleted group (6.10 ± 8.23 mg/L) were significantly lower than the iron depleted group (9.13 ± 7.98 mg/L) (p <0.05). TfR-F index was significantly higher in iron depleted group (10.9 ± 21.06) compared with that of iron repleted group (2.98 ± 4.19) (p<0.05) as in Figure 4. However, we observed the sTfR level was more than 4.4 mg/L in five patients in iron repleted group (Figure 2) of which two had raised TfR-F index.

DISCUSSION

One of the major difficulties encountered in clinical medicine is to distinguish between IDA and ACD, especially when they occur simultaneously. A hypochromic microcytic blood picture is more frequently observed in patients with RA without iron deficiency than those with chronic infection or malignancy[16]. A serum ferritin of <12 µg/L is generally diagnostic of iron deficiency. Patients with inflammatory disorders however will usually have a serum ferritin of >12 µg/L even when they have iron deficiency. Serum ferritin is therefore not a reliable measure of iron deficiency in patients with inflammation.

Serum iron and serum transferrin are also not reliable indicators of iron deficiency in patients with inflammation. Serum iron may be reduced in patients with inflammatory diseases irrespective of their iron status[16]. Serum transferrin concentration may not increase in the presence of inflammation as it would in uncomplicated IDA[17]. Since transferrin is a negative acute phase reactant, it may not be

increased in iron deficiency when associated with inflammation.

The purpose of our study was to assess the clinical usefulness of the measurement of sTfR and TfR-F index to distinguish between patients with IDA and those with ACD in the Malaysian population. For this we used three cohorts - the control consisting of healthy persons, patients with only iron deficiency anaemia, and RA patients with anaemia.

In our study all RA patients had hypochromic microcytic anaemia. The mean serum iron level in these patients with RA was 5.20 μmol/L, (range 1.33 to 17.51 μmol/L) which is significantly lower than that of those in the control group ($p<0.0001$). We also noted in our study that there was no significant difference in the transferrin level between those in the control group and RA patients ($p>0.05$). Serum ferritin levels in the RA patients were higher than that of those in the control group ($p<0.01$). These findings indicate that the conventional laboratory measurements of iron status (serum iron, transferrin and ferritin) are unable to differentiate between patients with iron deficiency from those with ACD.

Serum sTfR levels are raised in IDA and in conditions with erythroid hyperplasia whereas it is decreased in erythroid hypoplasia. Unlike ferritin, sTfR concentrations are not influenced by chronic inflammatory disease and therefore would be a better indicator of iron deficiency when associated with inflammation[3]. In our study, the sTfR level was increased in IDA patients (mean 17.16 mg/L) concurring with the findings of Petterson *et al*[11]. The mean sTfR level in patients with RA was 7.94 mg/L which is not statistically different from that of the control group ($p>0.05$). However, 17 of 28 RA patients had increased sTfR levels (>4.4mg/L). As the sTfR level should not be elevated in uncomplicated ACD, the increased value is suggestive of the presence of concurrent iron deficiency. Serum sTfR levels correlated well with the markers of anaemia and showed no correlation with ESR in patients with RA. In contrast, ferritin had no correlation with markers of anaemia but correlated well with ESR suggesting it was influenced by the disease activity. Similar findings have been reported by J S Song *et al* and Chijiwa *et al*[18, 19].

Serum ferritin level less than 60 mg/L was chosen as an indicator of iron deficiency in patients with RA[20, 21]. This is five times higher than the usual limit for diagnosing iron deficiency (12 μg/L)[22]. Based on this cut-off value, the patients with RA were divided into two sub groups - iron depleted and iron repleted. The sTfR levels and TfR-F index were significantly higher in the iron depleted group compared with that of control and iron repleted group. These two values were, however, not significantly different between iron repleted and control groups ($p>0.05$). The raised sTfR level and TfR-F index suggest iron deficiency in the iron depleted group of patients. These finding concur with those of Suominen *et al.* In their study they also observed a significant decrease in sTfR as well as TfR-F index values in the iron depleted group after iron supplementation[13].

In our study, five of the 11 patients in the iron-repleted group had increased sTfR levels of which two had elevated TfR-F index. According to Günter Weiss, a low TfR-F index is characteristic of functional iron deficiency while a higher ratio may point to true iron deficiency in ACD patients[23]. This suggests that three of the five patients probably had functional iron deficiency while the remaining two had true iron deficiency. We suggest that sTfR and TfR-F index be estimated in RA patients with serum ferritin levels of more than 60 μg/L to exclude IDA, whereas in patients with serum ferritin level less than 60 μg/L a course of iron therapy could be given.

The anaemia of chronic disease in RA may mimic iron deficiency as we have observed in our study. ACD will improve with the adequate treatment of underlying disease. IDA in patients with rheumatoid arthritis can be caused by dietary iron deficiency or iron malabsorption, but most often it is caused by chronic blood loss from gastritis induced by prednisolone [24] and/or by non steroidal anti-inflammatory drugs (NSAIDs). It is important in clinical practice to differentiate between IDA and ACD in patients with RA. Iron therapy in patients with ACD on the assumption that low haemoglobin (Hb) is caused by IDA will not only fail to increase Hb levels but will also add unnecessary medication load to these patients. Recognising IDA helps detect chronic gastro-intestinal blood loss due to steroids or NSAIDs induced gastritis. Such patients require endoscopy and further treatment options.

Differentiation of RA patients into those with ACD only and those that have both ACD and IDA is not possible with the use of routine laboratory indices. However, such distinction is crucial to institute proper treatment to achieve the required therapeutic outcomes for both ACD and IDA patients. Serum soluble transferrin receptor (sTfR) and sTfR/log ferritin (TfR-F) index permit such a distinction.

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