Soluble Transferrin Receptor (sTfR) Identifies Iron Deficiency Anemia (IDA) in Pulmonary Tuberculosis Patients

Tri Ratnaningsih[1], Novi W. Sukirto[2], Arum T. Wahyuningsih[1]

[1] Department of Clinical Pathology and Laboratory Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.
[2] Department of Internal Medicine, Faculty of Medicine, Ahmad Dahlan University, Yogyakarta, Indonesia.

Corresponding Author:
Tri Ratnaningsih, MD. Department of Clinical Pathology and Laboratory Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jalan Farmako, Sekip Utara, Yogyakarta 55281, Indonesia. email: triratnaningsih@ugm.ac.id; arumtriw@ugm.ac.id.

ABSTRAK

Latar belakang: *kekurangan zat besi pada penderita tuberkulosis (TB) paru akan melemahkan kekebalannya sehingga menyebabkan kesulitan dalam mengatasi infeksi. Diagnosis yang akurat dari anemia defisiensi besi (IDA) pada pasien TB paru sangat penting. Untuk membuktikan keadaan defisiensi zat besi, diagnosis harus fokus pada faktor inflamasi, yang sangat mempengaruhi hasil status zat besi, seperti pengukuran serum ferritin (SF). Soluble Transferrin Receptor (sTfR) adalah parameter terbaik untuk mendiagnosis defisiensi zat besi pada kondisi inflamasi. Penelitian ini bertujuan untuk memahami peran sTfR dalam mengidentifikasi IDA pada pasien TB.* ***Metode:*** *studi potong lintang diterapkan pada 3 kelompok penelitian yaitu TB paru anemia (68 subyek), IDA (7 subyek), dan TB paru non anemia (15 subyek). Rata-rata uji dan korelasi antara sTfR, SF, dan parameter hematologi lainnya diukur dan dianalisis.* ***Hasil:*** *perbedaan signifikan sTfR ditemukan pada kelompok TB anemia dibandingkan dengan kelompok IDA dan juga antara kelompok IDA dan kelompok TB non-anemia (p <0,0001). Namun, tidak ada perbedaan bermakna (p> 0,05) antara kelompok anemia TB dan kelompok non anemia. Kami juga tidak menemukan perbedaan bermakna antara sub kelompok anemia TB dengan kadar sTfR normal dibandingkan dengan kelompok non anemia. Tidak ada perbedaan signifikan kadar sTfR antara sub kelompok peningkatan sTfR dan kelompok IDA (p> 0,05). Namun, terdapat korelasi yang kuat antara sTfR dan SF pada kelompok IDA (r = -0,89; p = 0,007).* ***Kesimpulan:*** *penemuan ini menyarankan untuk memverifikasi jumlah sTfR pada pasien anemia dengan TB paru yang menderita peradangan, sehingga kekurangan zat besi dapat diperiksa dengan lebih akurat dan diperbaiki dengan benar.*

Kata kunci: *soluble transferrin receptor (sTfR), anemia, defisiensi zat besi, tuberkulosis paru, penyakit kronis.*

ABSTRACT

Background: *iron deficiency in pulmonary tuberculosis (TB) patients may weaken their immune system, causing difficulty in overcoming the infection. Accurate diagnosis of iron deficiency anemia (IDA) in pulmonary TB patients is essential. In order to prove the iron deficient state, diagnosis should focus on inflammatory factors, which could highly affect the outcome of iron status, such as measurement of serum ferritin (SF). Soluble Transferrin Receptor (sTfR) is the best parameter to diagnose iron deficiency in the inflammatory condition. This study aimed to understand the role of sTfR to identify IDA in TB patients.* ***Methods:*** *cross-sectional study were applied to 3 study groups: anemic pulmonary TB (68 subjects), IDA (7 subjects), and non-anemic pulmonary TB (15 subjects). The test averages and correlations between sTfR, SF, and other hematological parameters were measured and analyzed.* ***Results:*** *significant differences of sTfR were found in the anemic TB group compared*

to the IDA group and also between the IDA and non-anemic TB groups (p<0.0001). However, there was no significant difference (p>0.05) between TB anemic and non-anemic groups. We also found no significant difference between the TB anemic sub-group with normal levels of sTfR compared with the non-anemic group. There was no significant difference of sTfR levels between sub-group of increasing sTfR and group IDA (p>0.05). However, there was strong correlation between sTfR and SF in the IDA group (r=-0.89; p=0.007). ***Conclusion:*** *the findings suggest verifying the sTfR amount in anemic patients with pulmonary TB suffering from inflammation, so that the iron deficiency could be more accurately identified and properly treated.*

Keywords: *soluble transferrin receptor (sTfR), anemia, iron deficiency, pulmonary tuberculosis, chronic disease.*

INTRODUCTION

Tuberculosis (TB) has been one of the main infectious diseases in the world with incidence of 3 million death cases and 8 million new cases annually. The World Health Organization (WHO) predicts an increase of TB cases and has been estimating the increased incidence of this disease since approaching 12 million cases in 2005. This disease caused by Mycobacterium tuberculosis has also been the center of attention in health disaster programs in Indonesia. The WHO estimates approximately 170,000 death cases happen every year in Indonesia.[1,2] Anemia as a hematologic manifestation is commonly found among TB patients with 60% to 80% of all cases. Anemia associated with TB is typically anemia of chronic disease (ACD) and iron deficiency anemia (IDA), while other types of anemia have been rarely reported.[3,4] Iron deficiency condition, in line with other inflammatory diseases or chronic infections in TB patients, is very important to be determined because iron deficiency inside the body could weaken some immune functions, which would decrease the ability of patients to recovery.[4,5] In order to prove IDA, routine hematologic tests and iron status need to be confirmed. The IDA in patients with inflammation or chronic infections who also suffer from iron deficiency as in TB patients could bring difficulties in interpretation of laboratory test results. The considerable similarities between results from routine hematologic tests and that of conventional iron status in evaluating iron deficient state as well as ACD has brought up many diagnostic complications.[6,7] Iron deficient states are sometimes detected as a rise in ferritin value and are related to inflammatory conditions.[8,9] Serum ferritin (SF) level below 12 µg/l reflects a drop in suggestive iron in the bone marrow. However, SF is an acute phase protein, which increases in inflammatory conditions and infection until its value may potentially become disproportionate to suggestive iron. Bone marrow examination to determine the suggestive iron status is the gold standard in diagnosis of iron deficiency in the complicated conditions mentioned above.[5] However, this method is uncomfortable for patients, and is also invasive and expensive.

Inflammation and age have significant influence on the widely used iron parameters; SF, serum iron (SI) and total iron binding capacity (TIBC). Therefore, accurate diagnosis requires a parameter, which is able to better evaluate iron status without the complicating influence of the above conditions and is expected to give benefits in diagnosis of IDA with inflammatory conditions. Soluble transferrin receptor (sTfR) recently is known as a new diagnostic parameter to assess iron deficiency. This parameter is not influenced by chronic disease nor inflammation and the result can be compared to bone marrow examination.[10,11]

Transferrin receptor (TfR) is a trans membrane protein with two identical components, which can bind two molecules of transferrin respectively. Transferrin receptor segments, which become detached from the cell surface are of cone-shaped fragments. These molecules, which are soluble in serum and measured as sTfR are also found to have proportionate value with the transferrin receptors in tissues. Since erythropoietic cells have the highest receptor concentration, these cells are also the largest contributor for sTfR. The sTfR values in circulation are equivalent with

membrane-associated TfR cellular expression and increase according to iron requirements and cell proliferation.[8,12] Considerable studies have been done internationally to support sTfR examination in various clinical conditions. It is found in IDA that an increase of sTfR concentration is parallel to iron deficient state.[13] The sTfR examination is reported capable as a diagnostic parameter for detection of iron deficient state in RA (Rheumatoid Arthritis) patients and in other varieties of chronic diseases.[14,15] Studies suggested that sTfR may replace bone marrow examination in the important diagnosis of knowing suggestive iron in the body have been well-established.[6,14,16] The use of sTfR examination methods have been rapidly increasing, initiated in early 1987 with radioimmunoassay and followed with the development of the enzyme-linked immunosorbent assay (ELISA) method. This method is currently implemented in immunologic automatic instruments as prepared in most clinical settings.[17,18]

Diagnosis of iron deficiency in inflammatory or chronic disease has been a routine problem nowadays, especially for inpatients. The ACD in patients with TB as the underlying disease are also found to be associated with iron deficiency. This condition is related with malnutrition and chronic bleeding from the respiratory tract.[19] This iron deficient state can be corrected by giving iron supplements to improve anemia as well as improve the clinical condition. There have been many studies of the sTfR parameter overseas, but in Indonesia studies are still lacking. Since a number of parameters to measure iron status ranging from direct (hematology) to indirect (biochemistry) tests are known, the increased use of examinations involving sTfR as a new parameter should be arranged and confirmed since it is considered more accurate, prompt, less expensive and of course less invasive. This research was aimed to demonstrate that sTfR could identify IDA in pulmonary tuberculosis patients in Indonesia.

METHODS

The design of this research was observational with cross-sectional method. The subjects were divided into 3 groups: anemic pulmonary TB patients, IDA patients, and non-anemic pulmonary TB patients. The Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Gadjah Mada University – Dr. Sardjito General Hospital states that the protocol meets the ethical principle outlined in the Declaration of Helsinki 2013 (reference number: KE/FK/1246/EC/2018).

Subjects

Subjects were TB patients who came to BP4 Yogyakarta or RSUP Dr. Sardjito Yogyakarta. Participants were selected consecutively and fulfilled the inclusion criteria (patients diagnosed with TB and positive acid fast basil (AFB) on therapy, age of more than 18 years old, agreed to participate in this research) and exclusion criteria (pregnant women, patients with therapy and other hematinic drugs, hemolytic anemia, vitamin B12 and folic acid deficiency anemia and hematologic malignancies). The IDA group was enlisted from medical checkups of employees of an organization and from patients with complaint of menorrhagia who fulfill the inclusion criteria of hemoglobin (Hb) < 12 g/L and SF < 12 ng/mL and similar exclusion criteria as mentioned above. Laboratory data evaluated included level/value of Hb, MCV, MCH, RDW, SF, and sTfR.

The Department of Pulmonology BP4 Yogyakarta or Internal Ward RSUP Dr. Sardjito Hospital according to Medical Service Standards determined the pulmonary TB diagnosis. We obtained the data from outpatient medical records. History taking was performed by medical doctors in the respiratory clinic or in-wards doctors and then completed by the researchers. Information on any given iron tablet or other hematinic drugs, transfusion history, and hematologic malignancies were obtained from anamnesis and medical records. Anamnesis of pregnancy status was conducted on productive aged female patients. Peripheral blood smear morphology was examined to determine the presence of hemolytic process and vitamin B12 and folic acid deficiency anemia.

Complete Hematologic Examination

Hemoglobin assessment was simultaneously performed with other routine hematologic tests, which included erythrocyte index (MCV and MCH) and RDW also known as CBC

(complete blood count) using the automatic hematologic machine Sysmex 9500.[20] Peripheral blood morphology was examined by observing the Wright stained blood smear. Anemia was classified based on WHO criteria, which is Hb value < 13 gr/dL and < 12 gr/dL, for males and females, respectively.

Assessment of Serum Ferritin and sTfR

Blood samples were obtained by using phlebotomy techniques for evaluation of SF and sTfR taken within 1 week after receiving CBC data. The sample was directly processed into serum by centrifuge at velocity 3000 rpm for 10 minutes. Serum was obtained and immediately stored inside an Eppendorf tube 2 ml at temperature -200C until it was time to perform SF and sTfR examinations. The SF measurement was done by the Elecsys 1010 (Roche Diagnostic) using the electro-chemiluminescence method, while sTfR measurement was performed by the machine Hitachi 902 (Roche Diagnostic).[21] All research samples were taken before any transfusion was conducted.

Data Analysis

Characteristics data of research subjects are shown by descriptive analysis. Proportionality tests were conducted together with average test amongst subject characteristics based on each studied groups. Average tests of Hb and RDW between studied groups of more than 2 populations were completed by a one-way ANOVA (analysis of variance) test and t-test independent for 2 populations. Average tests of MCH, SF, and sTfR in studied groups of more than 2 populations were performed using the Kruskal-Wallis test and the Mann-Whitney test was used for 2 non-homogenous populations. Correlations between analytical variables (Hb, MCV, MCH, RDW, SF, and sTfR) were tested using the Spearman correlation test because the data were not normally distributed. Statistical analyses used significance level $p<0.05$ and the confidence interval of 95%. All statistical calculations were generated with SPSS for Windows version 11.

RESULTS

Pulmonary TB patients with history of AFB positive under therapy included 97 people, of which 64 were males and 33 females. Out of that number, 29 patients were with normal Hb value while another 68 patients suffer from anemia. All TB patients with anemia were included as research subjects, while from the 29 TB patients without anemia, 15 patients were randomly selected to be grouped as subjects in the TB group without anemia. The group of IDA patients who were selected from medical checkups of the employees and patients with complaint menorrhagia consisted of 7 people.

Research subjects in the anemic pulmonary TB group consisted of 48 (70.59%) males and 20 (29.41%) females. Subjects' age range was 19-85 years old, with average 42.09 (SD 14.72) years old. The SF levels in all three groups differ significantly ($p<0.05$) whereas values of Hb, MCV, MCH, RDW, and sTfR concentration exhibit a very significant difference ($p<0.001$). Amongst the other groups, the TB patients with anemia group showed the widest range of serum ferritin values (0.5 - 1825 ng/mL). Minimal value in this group was found to be lower than that of the lowest value within the normal range, and maximum value was much higher than that of the upper limit value within the normal range

Table 1. Hematologic Examination and Iron Parameter in All Research Groups

Parameter	Groups		
	Anemic Pulmonary TB (n= 68)	IDA (n=7)	Non anemic Pulmonary TB (n=15)
Hb (g/dL), mean (SD)	10.7 (1.62)	10.34 (1.19)	13.79 (0.85)
MCV (fL), median (range)	82 (57.9 – 92.3)	71.4 (68.5 – 75.7)	83.2 (81.2 – 88.7)
MCH (pg), mean (SD)	26.01 (2.41)	22.81 (1.26)	28.53 (1.00)
RDW (cv), median (range)	14.95 (12.4 –23.9)	17.10 (16.3– 19.2)	13.20 (12.5 – 14.3)
SF (ng/mL), median (range)	182.25 (0.5-1825)	6.31 (3.91 – 9.32)	211.20 (38.99265.4)
sTfR (mg/L), median (range)	3.15 (1.2 – 12.9)	7.10 (2.8 – 10.7)	2.90 (1.6 – 3.8)

Table 2. Significance Test of Hematologic Parameter and Iron Parameter Between Research Groups.

Parameter	A vs. B	A vs. C	B vs. C
Hb	10.7 vs. 10.34	10.7 vs. 13.79*	10.7 vs. 13.79*
MCV	82 vs. 71.4*	82 vs. 83.2	71.4 vs. 83.2*
MCH	26.01 vs. 22.81 **	26.01 vs. 28.53**	22.81 vs. 28.53**
RDW	14.95 vs. 17.10 **	14.95 vs. 13.20 **	17.10 vs. 13.20*
SF	182.25 vs. 6.31**	182.25 vs. 211.20	6.31 vs. 211.20*
sTfR	3.15 vs. 7.10*	3.15 vs. 2.90	7.10 vs. 2.90**

Results in average value. *Significance test of p< 0.0001. **Significance test of p< 0.001. A: Group anemic pulmonary TB; B: Group IDA; C: Group non-anemic pulmonary TB.

Table 3. Correlation Between sTfR Levels and Other Hematologic Parameter in IDA Group.

	r	p
Hemoglobin (g/dL)	-0.32	0.48
MCV (fL)	-0.43	0.34
MCH (pg)	-0.75	0.05
RDW (cv)	-0.21	0.64
SF (μg/mL)	-0.89	0.006

(30-400 ng/mL for males and 13-150 ng/mL for females).[21]

In the group of non-anemic TB patients, all average values from the hematologic examination lie within the normal range. Even though this group consists of TB patients, the SF levels were still within the normal range, which was 38.99 – 265.4 ng/mL; also, there is no increase of sTfR concentration found in this group. Results from Mann-Whitney U test show that in the anemic pulmonary TB patients' group, the ferritin level does not differ significantly from the group of non-anemic TB patients (**Table 2**). Correlation test was performed between sTfR as a new parameter for evaluation, and hematologic parameter and SF in IDA group, it is found that there is a strong negative correlation and very significant difference between sTfR and SF (r = -0.89; p<0.01). This result means the lower of SF level, the higher concentration of sTfR (**Table 3**).

In **Figure 1** the SF and sTfR distribution values of the 3 studied groups are shown. All IDA patients have ferritin value of less than the lower border of normal range. Outcome range obtained in TB patients without anemia is narrower than that of TB patients with anemia. Three participants in this group have values above normal and none lie below the normal range. The TB patients with anemia group have a wide range of ferritin values. The number of patients with value of below normal, normal and above normal is 4, 38, and 26 people,

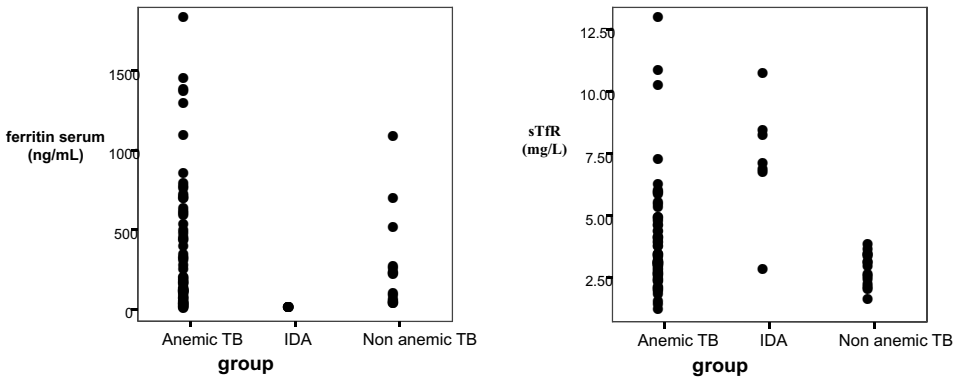

Figure 1. Distribution level of (A) ferritin level and (B) sTfR in 3 studied groups.

Table 4. Result of Hematologic Parameter and Iron Parameter Examination on Studied Groups (Divided Based on sTfR Levels).

Variables	Groups				p*
	Anemic pulmonary TB		IDA (n=7)	Non-anemic pulmonary TB (n=15)	
	normal sTfR (n= 53)	Increased sTfR (n=15)			
Hb* (g/dL)	11.50 (11.03 ± 1.50)	9.90 (9.57 ± 1.56)	10.70 (10.34 ± 1.19)	13.70 13.79 ± 0.85	0.0001
MCV** (fL)	83.10 (81.97 ± 6.21)	80.60 (78.55 ± 6.46)	71.40 (71.51 ± 2.38)	83.20 84.15 ± 2.27	0.0001
MCH* (pg)	26.80 (26.47 ± 2.18)	24.39 (24.39 ± 2.57)	22.90 (22.81 ± 1.26)	28.30 28.53 ± 1.00	0.0001
RDW** (cv)	14.60 (15.05 ± 2.03)	25.00 (16.81 ± 2.12)	17.10 (17.59 ± 1.1)	13.25 (13.25 ± 0.52)	0.0001
SF** (μg/mL)	275.50 (420.74 ± 42.82)	65.28 (186.06 ± 260.3)	6.31 (6.32 ± 2.04)	211.2 (140.43±94.6)	0.0001
sTfR** (mg/L)	3 (3.03 ± 0.85)	5.9 (6.75 ± 2.50)	7.1 (7.24 ± 2.40)	2.9 (2.80 ± 0.66)	0.0001

Result in median (mean ± standard deviation). * One-way ANOVA significance test, there is a very significant difference.
** Kruskall-Wallis significance test, there is a very significant difference

Table 5. Significance Test of Hematology Parameter and Iron Parameter Between Two Studied Groups.

Variables	A1 vs. A2	A1 vs. B	A1 vs. C	A2 vs. B	A2 vs. C	B vs. C
Hb (g/dL)	11.03 vs. 9.57 **	11.03 vs. 10.34	11.03 vs. 13.79**	9.57 vs. 9.90	9.57 vs. 13.79**	10.34 vs. 13.79**
MCVa (fL)	83.10 vs. 80.60*	83.10 vs. 71.40**	83.10 vs. 83.20	80.60 vs. 71.40**	80.60 vs. 83.20**	71.40 vs. 83.20**
MCH (pg)	26.47 vs. 24.39**	26.47 vs. 22.81**	26.47 vs. 28.53**	24.39 vs. 22.81	24.39 vs. 28.53**	22.81 vs. 28.53**
RDW a (cv)	14.60 vs. 25**	14.60 vs. 17.10**	14.60 vs. 13.25**	25 vs. 17.10	25 vs. 13.25**	17.10 vs. 13.25**
SF a (μg/mL)	275.50 vs. 65.28**	275.50 vs. 6.31**	275.50 vs. 211.2	65.28 vs. 6.31**	65.28 vs. 211.2	6.31 vs. 211.2**
sTfR a (mg/L)	3 vs. 5.9**	3 vs. 7.1**	3 vs. 2.9	5.9 vs. 7.1	5.9 vs. 2.9**	7.1 vs. 2.9**

A1: Group anemic pulmonary TB with normal sTfR level; A2: Group anemic pulmonary TB with increased sTfR level; B : Group IDA; C : Group non anemic pulmonary TB.
[a] Shown as median value. Significance test by Kruskall Wallis test. * Significant test result ($p < 0.05$). ** Very significant test result ($p < 0.001$).

respectively. Only 1 IDA patient appears to show sTfR concentration within normal range, while the other 6 are above normal value. The entire sTfR examination result of TB patients without anemia lies within normal range. Likewise, for SF outcome, the TB patients with anemia group consists of a very wide range of sTfR value; the result turned out to be 53 patients with normal level of sTfR and 15 patients of higher value based on the normal range set by the test manufacturer. When this group is divided into 2 based on level of sTfR, the results obtained are displayed in **Table 4**.

Median value and average SF value in groups with a rise in sTfR concentration is still in normal range, similar to a study by Chua et al.[22] which found a rise in sTfR in patient with normal ferritin level which was mean 45 ng/mL. A very significant difference for all outcomes of parameters being measured was acquired when the ANOVA significance test was performed between groups. The results of further analysis with the Kruskal-Wallis significance test between the 2 groups are displayed in **Table 5**. Results from Hb measurement between groups A1 and A2 exhibit a significant difference, which means in a group with an increase of sTfR concentration, the level of Hb is lower. In accordance to the role of sTfR as iron deficiency indicator, in subject groups with an increase of sTfR, patients are diagnosed with iron deficiency. This finding supports the statement that a severe degree of anemia in patients with inflammatory condition or chronic infections as in TB is normally accompanied by other causal factors beside the chronic disease mechanism. In the group A2

there were about 5 patients with Hb below 8 g/L. Anemia associated with TB usually is slowly progressive and improving with anti-tuberculosis drugs. Another causal factor for anemia other than TB must be considered whenever anemia worsens.[23]

Test results show that there is a significant difference of MCV measurement outcome between groups A1 and A2 which means in patients with an increase in sTfR concentration, there is a smaller erythrocyte morphology although it cannot be classified as microcytic since the MCV is still within normal range (83.10 vs. 80.60 fL), but when evaluated from the value of MCH, then the erythrocyte cell appears more hypochromic (26.47 vs. 24.39 pg). Group A1 has the profile as normocytic and little hypochromic. MCV value in this group does not differ much from group of TB patients without anemia (83.10 vs. 83.20). The hematologic parameter of the TB patients group with anemia in which there appears to be a significant rise of the sTfR does not necessarily suggest the presence of IDA. However, it is still unwise to rule out IDA based on the erythrocyte morphology only. This careful approach to diagnosis is due to the fact that only 30% of ACD patients demonstrate the hypochromic microcytic appearance with different spectrum of RDW values.[24]

DISCUSSION

Based on the Mann-Whitney U test, the group of TB patients with anemia demonstrates ferritin values of no significant difference compared to the group of TB patients without anemia (**Table 2**). This result implies that the status of iron deficiency cannot rely only on ferritin value as a parameter. Ferritin is an acute phase protein, which will increase in times of infection and inflammation. Iron deficient state in patients with chronic infection or inflammation cannot be detected because the inflammation process considerably influences the increment of ferritin value.[8,9]

All hematology variables of the IDA group show results below normal and in comparison with the anemic TB group, they have the lowest Hb, MCV, and MCH values with the highest RDW value. This result indicates microcytic hypochromic anemia with anisocytosis which is specific for IDA.[25,26] Ferritin is the most accurate and sensitive iron parameter to diagnose IDA. This IDA group gives the lowest SF value (6.32 (SD 2.04) ng/ml) and the highest sTfR (7.24 (SD 2.40) mg/L). To diagnose IDA, SF value should be <12/ml. The sTfR level of IDA group was the highest compared to the other groups. This parameter is expected to rise in IDA and the increase in concentration is proportionate with the level of deficiency iron.[13] This ratio is due to the fact that sTfR value in circulation is corresponding to the membrane associated TfR cellular expression and increases equivalent to the need of iron and cell proliferation.[8,12]

The IDA control group consists of productive-age women (20-23 years old) who are in active menstruation. This group is taken from the worker candidates of a factory and patients with the complaint of menorrhagia. All these subjects in this group have regular menstruation cycles but they never consumed hematinic supplements. Individuals such as these are very susceptible to get IDA. Enough iron consumption is really crucial to meet the body's demand. The requirement for iron can be increased up to 18 mg per day at the age of 11 and above for the increased body's need due to rapid growth during adolescence especially for girls who need more iron to replace the iron lost by menstruation.[26] IDA is the world's most common nutritional deficiency and the prevalence is high in children under 5 years old and women in reproductive age.[27]

Comparison of all parameters between the TB patients with anemia group and the iron deficiency group shows significantly difference except for Hb. The MCV and MCH values in the first group are significantly larger compared to the second group, while sTfR concentrations are much lower (**Table 3**). This implies that the anemia suffered in most of the TB patients is not the IDA type. Based on morphology, 72% of anemia in TB is normocytic normochromic anemia while microcytic hypochromic anemia is only 9%.[23] Dosumu 28 in Nigeria conducted research on 500 new TB patients and from them, 95% (475 patients) were normocytic normochromic anemia and 5% cases with anisocytosis, poikilocytosis, and polychromasia.

This finding is different from the research done in Russia in which the results showed from 129 cases of milliary TB, 74% of whom were diagnosed as IDA, which indicates the morphology is microcytic hypochromic.[29]

One of the patients in the study group has SF value 9.32 ng/ml, but normal sTfR concentration, which is 2.80 mg/L (**Table 4**). This finding is different from the research by Mast et al.[30] in which the anemia screening was conducted on a population of 112 healthy students. The subjects with low Hb were diagnosed to suffer from IDA because the incidence of this type of anemia which is still high. Subjects with ferritin level below 12 ng/ml, only 2 had an increase in sTfR. The results support a conclusion that when screening is being done for populations where the highest incidence is due to the IDA in the healthy population, the SF parameter is more sensitive for screening. Serum ferritin level is still the most sensitive and specific biochemical parameter for iron deficiency condition without inflammation (simple iron deficiency). The specificity is 100% when compared to bone marrow biopsy as a gold standard in iron examination.[31]

As indicated above in **Table 5**, when one group of TB patients with anemia is divided into 2 subgroups, in the subgroup where sTfR is normal or raised, they showed consistent results where there were no significant differences between both subgroups and the TB group without anemia (275.50 vs. 211.2 and 65.28 vs. 211.2). The SF value in group A2 was significantly lower compared to group A1 (275.50 vs. 65.28). Even though it was not statistically significant, the ferritin in the TB with anemia group had sTfR which was normally higher than the TB without anemia group, while the ferritin in TB with anemia group, which had the sTfR increased, was lower than the TB without anemia group.

The highest sTfR was found in the IDA group (median 7.1), while the lowest was in the TB without anemia group. There was little to no difference in concentrations of sTfR between the subgroup sTfR normal and the TB without anemia group (3 vs. 2.9). There was also only a small difference in the subgroup where sTfR was raised compared with the patients in the IDA group (5.9 vs. 7.1). This comparison clearly explains that low Hb and high sTfR in subgroup A2 compared with A1 indicate that there is anemia mechanism besides inflammation. In corresponding to the Hb value and sTfR concentrations between A2 and IDA patient groups, the result implies that TB patients with anemia that have elevation in sTfR can be proved to be iron deficient. This finding is in accordance to the research by Abedin et al.[32] which found that there was increase in sTfR in the ACD patient's group. The researchers are convinced that there is IDA coexisting in that group. There was no significant difference between the IDA and ACD groups. The patients in the ACD group consist of those who have RA who received the NSAID therapy, which has the potential to cause GI bleeding in chronic users. Different factors also play a role in the pathogenesis of IDA in patients with RA. In this research, the patient's characteristics are difficult to access due to incomplete information about the history of disease and treatment. TB patients, which are included in this research, are acid-bacilli fast positive and thus are assumed to have the risk of suffering from iron deficiency. The risk factor of iron deficiency in TB patients is hemoptysis or coughing blood. The expulsion of blood which is only in small amounts but chronic will result in the loss of iron from the body and if not balanced by sufficient iron intake could cause iron deficiency. This will be marked by the decrease in ferritin. Other than that, the level of SF in inflammatory conditions usually is not proportional to the actual iron storage. This is because as an acute phase protein, the level will increase in inflammation, infection and malignancy.

Taking into account that sTfR has no history of being influenced by inflammation, thus this parameter is considered to be able to differentiate between anemic patients with normal ferritin level or increased. This application of sTfR is supported by many researches, which propose that sTfR is the best iron deficiency indicator in patients associated with infection or inflammation. sTfR concentration increases in IDA case and is not influenced by acute or chronic inflammatory diseases.

CONCLUSION

The results of sTfR examination in three groups show significant differences ($p<0.01$). If the TB with-anemia group is divided into 2 sub-groups based on sTfR content, then there is no difference in sTfR concentration between the sub-group anemic pulmonary TB with normal sTfR and the non-anemic pulmonary TB group. Similar sTfR results are also found in the sub-group anemic pulmonary TB with increasing sTfR and the IDA group ($p>0.05$). This finding means that increasing sTfR levels in patients with anemic pulmonary TB indicate concomitant condition with iron deficiency. Strong negative and very significant correlations between sTfR and SF ($r=-0.89$, $p=0.007$) are found in the IDA group. Based on these confirmatory finding, it is advisable to examine the sTfR concentration in anemic patients who are suffering from infection or inflammation. If there is an increase in the sTfR concentration, iron deficiency is suspected.

FUNDING

The authors declare that this research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

AUTHORS' CONTRIBUTIONS

TR performed the experiments, concept and idea and was a major contributor. NWS reviewed the clinical aspects of the manuscript. ATW was contributor in writing the manuscript. All authors read and approved the final manuscripts.

ACKNOWLEDGMENTS

Deepest gratitude to Head and staff of Internal Ward Sardjito Hospital and Head and staff of Balai Pengobatan Paru-Paru DI Yogyakarta, Head of Clinical Pathology Sardjito Hospital and staff, Head of Permata Hati Laboratory and staff, and Head of Prodia Laboratory Yogyakarta and staff for them kind help. Thanks also to Roche Diagnostica Indonesia for sTfR reagent contribution. A big thank you to Dr. Setyawati and Dr. Usi Sukorini who gave many suggestions on the course of the research.

REFERENCES

1. World Health Organisation. Global tuberculosis report 2018. 2018. Available from: http://www.who.int/tb/publications/global_report/en/.
2. Departemen Kesehatan Republik Indonesia. Available from: www.depkes.go.id. Accessed: June 18th 2017.
3. Abay F, Yalew A, Shibabaw A, Enawgaw B. Hematological abnormalities of pulmonary tuberculosis patients with and without HIV at the University of Gondar Hospital, Northwest Ethiopia: A comparative cross-sectional study. Hindawi Tuberculosis Res Treat. 2018;2018:6. Article ID 5740951. doi: 10.1155/2018/5740951.
4. Barzegari S, Afshari M, Movahednia M, Moosazadeh M. Prevalence of anemia among patients with tuberculosis: A systematic review and meta-analysis. Indian J Tuberculosis. 2019;66:299-307.
5. Lee GR. Anemia: a diagnostic strategy. In: Lee GR, Foerster J, Lukens J, et al, editors. Wintrobe's clinical hematology. 13th ed. Baltimore: Williams and Wilkins; 2015.
6. Suega K, Kandarini Y, Tubung J. Role of soluble transferrin receptor and transferrin receptor- ferritin index to detect iron deficiency anemia in regular hemodialysis patients. Open Access Maced J Med Sci. 2019;7(1):97-102. https://doi.org/10.3889/oamjms.2019.012.
7. Hoffbrand AV, Pettit JE, editors. Essential hematology. 7th edition. London: Blackwell Science Ltd; 2015.
8. Vázquez-López MA., López-Ruzafa E., Ibáñez-Alcalde M., et al. The usefulness of reticulocyte haemoglobin content, serum transferrin receptor and the sTfR-ferritin index to identify iron deficiency in healthy children aged 1–16 years. Eur J Pediatr. 2019;178(1):41-9. doi: 10.1007/s00431-018-3257-0.
9. Rodak BF. Hematology clinical principles and application, 5th edition. WB Saunders Company; 2016.
10. Suominen P, Mottonen T, Rajamaki A, Irjala K. Single values of serum transferrin receptor and transferrin receptor ferritin index can be used to detect true and functional iron deficiency in rheumatoid arthritis patients with anemia. Arthritis Rheum. 2000;43:1016-20.
11. Gangat N, Wolanskyj AP. Anemia of Chronic Disease. Seminars in Hematology. 2013;50(3):232–238. doi: 10.1053/j.seminhematol.2013.06.006.
12. Kim A, Nemeth E. New insights into iron regulation and erythropoiesis. Curr Opin Hematol. 2015;22(3):199–205. doi:10.1097/MOH.0000000000000132.
13. Shin DH., Kim HS., Park MJ., et al. Utility of access soluble transferrin receptor (stfr) and stfr/log ferritin index in diagnosing iron deficiency anemia. Annals of Clinical & Laboratory Science. 2015;45(4):396-402.
14. F. Alam, S. S. Fatima, S. Noor, et al. Stages of chronic kidney disease and soluble Transferrin Receptor (sTfR), Ferritin, ratio. J Pak Med Assoc. 2017;67(6):848-51.

15. Suominen P, Mottonen T, Rajamaki A, Irjala K. Single values of serum transferrin receptor and transferrin receptor ferritin index can be used to detect true and functional iron deficiency in rheumatoid arthritis patients with anemia. Arthritis Rheum. 2000;43:1016-20.
16. Krawiec P, Pac-Koz˙uchowska E. Soluble transferrin receptor and soluble transferrin receptor/log ferritin index in diagnosis of iron deficiency anaemia in paediatric inflammatory bowel disease. Dig Liver Dis. 2018. doi: 10.1016/j.dld.2018.11.012.
17. Roque ME, Sandoval MJ, Aggio MC. Serum erythropoietin and its relation with soluble transferrin receptor in patients with different types of anemia in a locally defined reference population. Clin Lab Haem. 2001;23:291-5.
18. Pfeiffer CM, Looker AC. Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. Am J Clin Nutr 2017;106(Suppl):1606S–14S.
19. Sebastiani G, Pantopoulos K. Disorders associated with systemic or local iron overload: from pathophysiology to clinical practice. Metallomics. 2011;3:971–986. doi: 10.1039/c1mt00082a.
20. Sysmex SE-9500 Operator's manual. Sysmex Corporation, Japan. 1999.
21. Ferritin, Tina-quant. Soluble transferrin receptor (sTfR). Roche Diagnostics GmbH. Germany. 2010.
22. Chua E, Clague JE, Sharma AK, Horan MA, Lombard M. Serum transferrin receptor assay in iron deficiency anemia and of anemia of chronic disease in the elderly. Q J Med. 1999;92:587-94.
23. Lee SW, Kim DK, Ko DS, Yoo CG, Han SK, Shim YS, Yim JJ, Kim YW. Prevalence and evolution of anemia in patients with tuberculosis. Chest Journal. 2004.
24. Higgins C. Science review: anemia of chronic disease. Accessed: June 18th 2017.
25. Hoffbrand AV, Pettit JE. Essential hematology, 6th ed. Oxford: Blackwell Science; 2011.
26. Muñoz M, Gómez-Ramírez S, Besser M, et al. Current misconceptions in diagnosis and management of iron deficiency. Blood Transfus. 2017;15:422-37. doi: 10.2450/2017.0113-17.
27. Camaschella C, Longo DL (editor). Iron-deficiency anemia. N Engl J Med. 2015;372:1832-43. doi: 10.1056/NEJMra1401038.
28. Dosumu EA. Pattern of some hematological indices in newly diagnosed pulmonary tuberculosis cases in Iwo, Nigeria: diagnostic and therapeutic implications. Niger J Med. 2001;10:18-20.
29. Voloshina VV, Fomicheva NI. Efficiency treatment of new cases of destructive pulmonary tuberculosis in the presence of concurrent iron deficiency anemia. Probl Tuberk. 2002;10-2.
30. Mast AE, Blinder MA, Gronowski AM, Chumley C. Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. Clinical Chemistry. 1998;44:145–51.
31. Ong KH, Tan HL, Lai HC, Kuperan P. Accuracy of various iron parameters in the prediction of iron deficiency in an acute care hospital. Ann Acad Med Singapore. 2005;34:437-40.
32. Abedin MY, Saleh MT, El Wakkad AS, Fathy GA, Awadallah ZE. Serum transferrin receptor and transferrin receptor/ferritin ratio distinguish iron deficiency in anemia of chronic disorders in children. Egypt Med J. N RC. 2003;2(2):75-92.