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Original Article

Using iron profiles to identify anemia of chronic disease in anemic children with tuberculosis

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Abstract

Background Anemia of chronic disease (ACD) is commonly found in patients with chronic inflammation or infection. By examining soluble transferrin receptor or bone marrow iron, ACD was found in 80% of anemic adult tuberculosis (TB) patients. Iron profile, another tool to differentiate ACD from iron deficiency anemia (IDA), is both less expensive and less invasive. Few studies have been reported on iron profiles of anemic children with TB in Indonesia.

Objective We aimed to use iron profiles to determine the proportion of ACD in anemic children with tuberculosis.

Methods A cross-sectional study on anemic children with TB who came to Cipto Mangunkusumo Hospital and Tebet *Puskesmas* (community health center) was performed in September-November 2010. Iron profiles included the measurements of serum iron (SI), total iron binding capacity (TIBC), transferrin saturation (TF), and serum ferritin (SF).

Results Our study comprised of 66 subjects, with a median age of 3.8 years (6 months–18 years). Most subjects had normal SI (85%), normal TIBC (71%), low transferrin saturation (51%), and normal SF (71%). Only 10 children had iron homeostasis disorder and 6 of these were diagnosed as having ACD. Thus, iron profiles failed to prove that iron metabolism was disturbed. The profile of children with organ-specific TB was more consistent with ACD compared to the profile of childhood TB. [SI 29.1 (11-83) vs 44 (10-151) μg/dL; TIBC 239.3 (100.80) vs 299.0 (58.51) μg/dL; TF 18.3 (4-100) vs 15 (1-53) %; and SF 154 (34.9-655) vs 36.1 (2.5-213.4) μg/L].

Conclusion The proportion of ACD (9%) diagnosed by using iron profiles was not as high as previously reported. Further research using newer techniques is needed to detect ACD in anemic children with TB. **[Paediatr Indones. 2011;51:217-22]**.

Keywords: *anemia of chronic disease, iron profile, tuberculosis, children*

Anemia of chronic disease (ACD) is commonly found in patients with chronic inflammation or infection.[1-3] Nowadays, prominent involvement of inflammatory factors in ACD pathogenesis make it more commonly referred to as anemia of inflammation. It is important to distinguish ACD from iron deficiency anemia (IDA) because treatments for these ailments typically differ. Pathogenesis of ACD is associated with iron metabolism disturbance causing iron reserves to be stored in the cell body and unable to be used for red blood cell production.[4-8]

Assessment of iron stores is typically done by iron profile measurement, which is less invasive and available in most laboratories. Evaluation of bone marrow iron actually is the most sensitive and reliable method for assessing iron pools and is considered the gold standard. However, it is invasive and impractical for routine use. Other tests, such as soluble transferrin receptor (sTR) and erythrocyte zinc protoporphyrin, are two innovative methods to differentiate ACD from IDA, but these tests are expensive and not yet applicable to daily practice in Indonesia.[9]

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Tuberculosis (TB) is a chronic infectious disease in developed and developing countries. Almost 80% of TB cases occur in children in the 22 countries having the highest percentage of TB patients. Indonesia is a country with the third highest percentage of TB patients.[10,11] Traditionally, the cause of anemia in TB was thought to be IDA, as malnutrition that commonly occurs in these patients can lead to limited iron stores. However, recent research shows that ACD is more common in TB patients, consistent with the fact that TB is a chronic disease associated with inflammation.[12-14] Research on ACD in TB cases has not been widely reported and is generally performed on adult TB patients rather than children. A study on a TB patient population including children aged > 10 years found only 5 of 33 subjects had iron deficiency. Other research on adult TB patients in Jogjakarta indicated that most subjects (78%) had ACD.[15,16]

This study was designed to use iron profiles of children with TB in order to determine the etiology of anemia and the prevalence of ACD. It is important to identify ACD, since IDA remains high in Indonesia and is the main differential diagnosis of ACD.[17]

Methods

A cross-sectional study was done in Cipto Mangunkusumo Hospital (CMH) and Tebet *Puskesmas* in September-November 2010. We included children with TB, based on the Paediatric TB scoring system and anemia, defined as having a hemoglobin value in the less than normal range. Subjects were excluded if they were treated with antituberculosis drugs for more than two months, or had received anti-inflammatory drugs or iron supplementation. Those with other conditions causing anemia (bleeding, thalassemia, hemoglobinopathy, and bone marrow myelosuppression), inflammation (infection, sepsis, hypersensitivity reaction, burn wounds, and Kawasaki disease), or other chronic diseases were also excluded. Informed consent was obtained from parents, and this study was approved by Ethics Committee of the Faculty Medicine, University of Indonesia.

Subjects' iron profiles were measured at the Clinical Pathology Laboratory, CMH. Parameters tested were serum iron, total iron binding capacity, transferrin saturation, and serum ferritin. C-reactive protein (CRP) was also measured as an inflammation marker. The diagnosis of childhood TB was made based on a scoring system, while organ-specific TB was confirmed by specialist consultants. Type of anemia was distinguished by iron profiles as described in **Table 1.**[1,18]

Statistical analysis was performed by using SPSS 17.0 (SPSS, Chicago, IL). Normally distributed data was expressed as mean (SD) and non-normally distributed data was expressed as median (range).

Results

From September to November 2010, 42 children with TB visited CMH and 50 visited Tebet *Puskesmas*. Eleven children were excluded, and hemoglobin (Hb) level exams were conducted on the remaining 81 children. Anemia was found in 66 children. These 66 underwent iron profile and CRP measurements.

Distribution of boys and girls were similar. The highest age group was 6 months to < 5 years. Median age of subjects was 3.8 years with a range of 6 months - 18 years. There were more subjects with childhood TB than with organ-specific TB. **(Table 2)**

Median (range) of Hb level, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin

Table 1. Iron profiles in ACD and IDA

Variable	ACD	IDA	Combination
Serum iron	Reduced	Reduced	Reduced
TIBC	Reduced	Elevated	Normal/reduced
Transferrin	Reduced/normal	Elevated	Reduced
Transferrin saturation	Reduced	Reduced/normal	Reduced
Ferritin	Normal/elevated	Reduced	Reduced/normal
Soluble transferrin receptor (sTR)	Normal	Elevated	Normal/elevated
sTR and log ferritin ratio	Low (<1)	High (>2)	High (>2)
Cytokine	Elevated	Normal	Elevated

Table 2. Characteristics of the subjects

Characteristic	N	%
Sex		
- Boys	35	53
- Girls	31	47
Age		
- 6 months - <5 years old	36	54
- 5 - <14 years old	21	32
- 14-18 years old	9	14
Nutritional status		
- Severely malnourished	9	14
- Undernourished	30	45
- Well-nourished	22	33
- Overweight	5	8
Type of tuberculosis		
- Childhood TB	50	76
- Organ-specific TB	16	24

concentration (MCHC) were 10.6 (6.4 to 12.8) g / dL; 73.3 (54.0 to 84.4) pg/cell; 23.1 (14 0.8 to 31) fl/ cell; and 31.3 (27-51) g / dL, respectively. We found that the average serum iron levels, TIBC, transferrin saturation and serum ferritin levels were 36.5 (10-151) ug / dL, 284.5 (74.76) ug / dL, 13.5 (1-100)%, and 55.4 (2.5 to 655) ug / L, respectively. Subject groupings based on below normal, normal, and above normal ranges for each test are shown in **Table 3.**

Table 4 shows iron reserve levels in childhood TB and organ-specific TB. The median serum ferritin level was higher in the organ-specific TB group than in the childhood TB group. The median (range) CRP level was also higher in organ-specific TB patients [12.4 (3.5 - 96) mg / L] compared to the childhood

Table 3. Iron profile of anemic children with TB

	Below normal limits		Normal limits		Above normal limits	
Parameter	n (%)	Average	n (%)	Average	n (%)	Average
Serum iron, µg/dL	10 (15)	15.3 (3.65)*	56 (85)	43 (22-151)**	0	-
TIBC, µg/dL	16 (24)	216 (70-248)**	47 (71)	307.4 (41.23)*	3 (5)	432 (420-437)**
Transferrin saturation, %	34 (51)	8.6 (3.61)*	29 (44)	26.1 (8.7)*	3 (5)	53 (50-100)**
Serum ferritin, µg/L						
5 mo-15 yrs	8 (12)	5.1 (1.34)*	40 (60)	45.8 (8.4-133.7)**	11 (17)	213.4 (150-655)**
>15 yrs	0	-	7 (11)	111.8 (75.20)*	0	-

* Mean (SD)
** Median (range)

Table 4. Iron profiles in anemic children with childhood TB and specific-organ TB

Parameter	Childhood TB	Organ-specific TB
Serum iron, µg/dL	44 (10-151)*	29.1 (11-83)*
TIBC, µg/dL	299.0 (58.51)**	239.3 (100.80)**
Transferrin saturation, %	15 (1-53)*	18.3 (4-100)*
Serum ferritin, µg/L	36.1 (2.5-213.4)*	154 (34.9-655)*
CRP, mg/L	1.8 (0.2-59.3) *	12.4 (3.5-96) *

* Median (range)
** Mean (SD)

Table 5. Iron profiles and CRP values in anemic children with TB

Diagnosis	Serum iron (µg/dL)	TIBC (µg/dL)	Transferrin saturation (%)	Serum ferritin (µg/L)	CRP (mg/L)	Type of anemia
Childhood TB	10 (↓)	262 (N)	4 (↓)	61 (N)	58.9 (↑)	Combination
TB meningitis	11 (↓)	290 (N)	4 (↓)	66.7 (N)	8 (↑)	Combination
Miliary TB	12 (↓)	81 (↓)	15 (N)	619.3 (↑)	86.9 (↑)	ACD
Childhood TB	13 (↓)	420 (↑)	3 (↓)	2.5 (↓)	0.2 (N)	IDA
TB spondylitis	15 (↓)	375 (N)	4 (↓)	88 (N)	7.7 (↑)	Combination
Childhood TB	16 (↓)	390 (N)	4 (↓)	5.9 (↓)	0.4 (N)	IDA
TB meningitis	18 (↓)	313 (N)	6 (↓)	160 (N)	18.8 (↑)	Combination
Childhood TB	19 (↓)	437 (↑)	4 (↓)	4.5 (↓)	0.2 (N)	IDA
Childhood TB	19 (↓)	432 (↑)	4 (↓)	4 (↓)	0.5 (N)	IDA
Childhood TB	20 (↓)	294 (N)	7 (↓)	51.8 (N)	0.7 (N)	Combination

TB group [1.8 (0.2 - 59.3) mg / L].

We found only 10 children with below normal serum iron levels. Furthermore, only 1 child was diagnosed with pure ACD, while 5 children were diagnosed with combination anemia (ACD and IDA) as shown in **Table 5.** Thus, we found 9% of subjects with ACD, either pure or in combination with IDA.

Discussion

This cross-sectional study of iron profiles in anemic children with TB was done to determine the proportion of ACD in these subjects. However, profiles of iron reserves obtained from subjects were not compared with actual levels of iron in the body. Additionally, anemia was defined by laboratory parameters, hence, the actual situation may be unknown. It was also difficult to ascertain if ACD and IDA occurred simultaneously. Bone marrow iron examination as the gold standard for assessing body iron reserves was not done because of its invasive nature. Soluble transferrin receptor (sTR) examination, the latest technique to assess iron storage, was also not performed because it is expensive and not done routinely in our laboratory.

Average Hb levels and erythrocyte indices for our subjects was consistent with mild anemia, with below normal values of MCV, MCH, and MCHC. Anemia of chronic disease is usually mild normocytic, normochromic anemia, but in severe circumstances or in combination with IDA it may be microcytic, hypochromic anemia.[5,20]

Serum iron levels in our study cannot help distinguish between ACD and IDA, both conditions in which serum iron levels are low. Most subjects in our study (85%) had normal serum iron levels. Serum iron levels may be influenced by other factors, including diurnal variation, with highest levels during the mid-afternoon, or rising shortly after consumption of iron-containing nutrients.[21]

Normal TIBC levels were observed in 71% of subjects. Normal TIBC levels may be due to few subjects' having ACD or combination ACD with IDA. Another possibility is that subjects with IDA had poor nutritional status, so whole body protein content was also low. Albumin examination could be used to test this hypothesis, but was not performed in our study.[22]

Low serum ferritin levels were found in 12% of subjects. Diagnosis of IDA should be considered in this subject group. Other subjects had normal or high serum ferritin levels, which could be more consistent with ACD or combination ACD with IDA.[1,18]

Two studies using bone marrow iron examination for definitive diagnosis of IDA in TB patients have been published. One of these was research done on 33 TB patients, aged > 10 years, with microcytic hypochromic anemia in Nepal. This study identified five subjects with iron deficiency based on this technique. The mean serum ferritin was higher in the iron-repleted group compared with the iron-deficient group, 349 ug/L and 104 ug/L ($p < 0.05$), respectively, though both were still within the range of normal values. The authors suggested that a threshold value of serum ferritin of lower than 15 ug / L be used to determine IDA diagnosis in TB patients.[23] Kotru *et al.*[16] determined a ferritin value of less than 30 ug/L as an effective threshold for determining the presence of iron deficiency in 55 adult patients with TB, particularly in areas with high prevalence of IDA. This study also used bone marrow iron examination as the gold standard for diagnosing IDA. They found no increase in predictive value of iron deficiency when ferritin values were combined with MCV, erythrocyte sedimentation rate, and TIBC. However, 89.5% of cases could be classified correctly using logistic regression equation between ferritin, erythrocyte sedimentation rate and CRP.

Iron profiles were quite different when subjects were grouped by TB type. Organ-specific TB patients had lower serum iron levels, lower TIBC, similar transferrin saturation, and higher serum ferritin levels than childhood TB patients. The description of organ-specific TB patients seemed more similar to the findings of ACD.[1,18] Consistent with this finding, higher CRP levels were observed in the organ-specific TB group, suggesting that inflammatory factors play a role, as in ACD pathogenesis.

We found ACD in 9% of subjects, most of which had combination ACD with IDA. This result was much smaller than reported in adult TB patients. Ratnaningsih[15] found 78% of 68 adult TB patients had ACD and the remainder (22%) experienced IDA with ACD. However, that study used only sTR values to determine ACD, without comparing to other iron profiles.

Another possible cause of the small number of ACD subjects in our study may be the definition of ACD. We assumed that anemia was the result of iron metabolism disorders if serum iron levels were low.[1,18] Further assessments were conducted only on the 10 subjects with below normal serum iron levels, while the etiology of anemia in the 56 remaining subjects was not explored. The exclusion criteria for anemia due to other reasons was based simply on history and physical examination, which limited the findings of this study. In addition, nutritional intake before blood sampling was not known. So subjects may have consumed iron-containing nutrients prior to the test. Serum iron levels in a study that examined bone marrow iron in 55 adults with TB appeared lower in the group of patients who proved to have adequate iron reserves. The iron deficient group had a mean serum iron (SD) of 54.4 (20.1) ug/dL, while the iron sufficient group had 47.9 (14.6) ug/dL. Meanwhile, the mean (SD) serum ferritin level was lower in the iron deficient group compared to the iron sufficient group [17.7 (16.7) and 91.9 (104.1) ug/L, respectively]. The study also found that raising the threshold value of ferritin from 10 ug/L to 30 ug/L improved the predictive ability of iron deficiency from 61% to 88%.[16]

Another of our exclusion criteria of patients' taking more than 2 months of anti-TB drugs, could also have affected the results of our study. Although patients were still in the intensive treatment phase within the first two months of treatment, the possibility that inflammation has subsided could not be ruled out. This was also supported by the normal levels of CRP found in most patients. Subsequent improvement of anemia or elevated Hb level also was not known, as the Hb examination was performed only once.

This is the first study in Indonesia on iron profiles in anemic children with chronic infectious disease. Research has commonly highlighted ACD in autoimmune diseases, such as lupus erythematosus or juvenile rheumatoid arthritis. Publications on iron profiles in anemic children with TB in Indonesia have not been found. The results of this research should be followed up because a correct diagnosis is important for patient management. Iron has an important role in TB infection. It is a required component of the immune system, but it is also used in bacterial replication. Excessive iron intake may increase the risk of active TB disease.[24-26] We showed that only 14% of subjects suffered from iron deficiency. Thus, iron deficiency in an anemic child with TB should be proven before iron supplementation is given, although the incidence of ADB is high in Indonesia.

We conclude that most subjects had normal serum iron levels (85%), normal TIBC (71%), low transferrin saturation (51%), and normal serum ferritin levels (71%). The proportion of ACD (9%) diagnosed by using iron profile was not as high as previous findings in adult TB patients. Further research using newer techniques, such as sTR, is needed to detect ACD in anemic children with TB.

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