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Original Article

Serum ferritin, serum nitric oxide, and cognitive function in pediatric thalassemia major

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Abstract

Background Hemolysis and repeated blood transfusions in children with thalassemia major cause iron overload in various organs, including the brain, and may lead to neurodegeneration. Hemolysis also causes decreased levels of nitric oxide, which serves as a volume transmitter and slow dynamic modulation, leading to cognitive impairment.

Objective To assess for correlations between serum ferritin as well as nitric oxide levels and cognitive function in children with thalassemia major.

Methods This analytical study with cross-sectional design on 40 hemosiderotic thalassemia major patients aged 6-14 years, was done at the Thalassemia Clinic in Dr. Hasan Sadikin Hospital, Bandung, West Java, from May to June 2015. Serum ferritin measurements were performed by an electrochemiluminescence immunoassay; serum nitric oxide was assayed by a colorimetric procedure based on Griess reaction; and cognitive function was assessed by the Wechsler Intelligence Scale for Children test. Statistical analysis was done using Spearman's Rank correlation, with a significance value of 0.05.

Results Abnormal values in verbal, performance, and full scale IQ were found in 35%, 57.5% and 57.5%, respectively. Serum nitric oxide level was significantly correlated with performance IQ (P=0.022), but not with verbal IQ (P=0.359) or full scale IQ (P=0.164). There were also no significant correlations between serum ferritin level and full scale, verbal, or performance IQ (P=0.377, 0.460, and 0.822, respectively).

Conclusion Lower serum nitric oxide level is significantly correlated to lower cognitive function, specifically in the performance IQ category. However, serum ferritin level has no clear correlation with cognitive function. **[Paediatr Indones. 2017;57:148-52 doi: http://dx.doi.org/10.14238/pi57.3.2017.148-52]**.

Keywords: thalassemia major; ferritin; nitric oxide; cognitive function; WISC-R

Thalassemia major is the most severe form of a group of inherited hemoglobin disorders, due to the partial or total absence of hemoglobin.[1, 2] The use of intense therapy increases life expectancy as well as the frequency of complications. Children with thalassemia major have multiple risk factors for developing central nervous system (CNS) complications. Hemolysis and repeated blood transfusions cause decreases in nitric oxide levels and iron overload in various organs, including the brain. Increased iron in the brain leads to oxidative stress and possible irreparable brain tissue damage, causing cognitive impairment.[3-5] Ferritin is a protein that stores iron and exists in all tissues including the brain. Serum ferritin level is a good marker for assessing body iron stores. Nitric oxide (NO), a diffusible intercellular messenger, is produced by most mammalian cells, including neurons.[6] Nitric oxide seems to be involved in several aspects of cognition, among which learning and memory are

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implicated, as nitric oxide can act as a retrograde messenger during long term potentiation.[7] Nitric oxide also serves as a volume transmitter and slow dynamic modulator. As such, the decreased level of NO in children with thalassemia major may lead to cognitive impairment.[8]

In most cases, neurological involvement in thalassemia major does not initially present with relevant signs and symptoms (subclinical), and can only be detected during neurophysiological and neuropsychological evaluation.[9] Neuropsychological tests are safe and reliable for diagnosis of cognitive impairment in ß-thalassemia major patients, and they may even facilitate early diagnosis.[10] The *Wechsler Intelligence Scale for Children* (3rd edition) is the most widely used intelligence test for school aged children and adolescents.[11] Early diagnosis through regular neuropsychological testing and appropriate treatment of CNS complications are essential to improve the quality of life of children with thalassemia major.[10] We aimed to evaluate possible correlations between serum ferritin and nitric oxide levels with cognitive function in children with thalassemia major.

Methods

This cross-sectional study was performed in the Thalassemia Outpatient Clinic at the Department of Child Health, Dr. Hasan Sadikin Hospital, Bandung, West Java, from May to June 2015. Subjects were selected by consecutive sampling of hemosiderotic thalassemia major pediatric patients. The inclusion criteria comprised of children diagnosed with thalassemia major aged between 6-14 years with regular blood transfusions and iron chelation treatment, normal body mass index, formal education, and no fever. The exclusion criteria were as follows: (a) had a history of major mental disorders with delayed milestone development; (b) had physical disabilities that could interfere with performance, such as deafness or blindness; (c) received prior treatment with drugs known to be neurotoxic; or (d) had a history of chronic medical illness other than thalassemia that could affect cognition.

Based on the calculated required sample size, our study included 40 subjects with hemosiderotic thalassemia major. Informed consent was obtained from subjects' parents and the study was approved by the Ethics Committee of the Universitas Padjadjaran Medical School/Dr. Hasan Sadikin General Hospital. Data on adherence to iron chelating agent use, frequency of transfusion, and school attendance were collected from medical records and questionnaires.

The eight parameters of the hematology test were based on flow cytometry. The serum ferritin quantitative test, an electrochemiluminescent immunoassay (ECLIA), was done at the Clinical Pathology Laboratory of the Hasan Sadikin Hospital, Bandung.[12] Serum nitric oxide was assayed using a colorimetric procedure based on Griess reactions at the *Prodia Laboratory Centre*, Jakarta.[13] Cognitive function was assessed with the Indonesian version of the *Wechsler Intelligence Scale for Children-Revised* (WISC-R), which provided outputs of their verbal and performance subtests and a combined full scale IQ test. The evaluation was performed by a qualified clinical child psychologist at Hasan Sadikin Hospital, Bandung. Patients' blood was drawn just before blood transfusion and the WISC-R test was performed after blood transfusion. Study results were analyzed by *SPSS version 20.0* software. Numeric data are presented as means, standard deviations, and medians with range. The correlations between serum ferritin and serum nitric oxide with cognitive function were determined with Spearman's Rho coefficient correlation. Results with P values <0.05 were considered to be statistically significant.

Results

Forty thalassemia major patients were included in this study. The mean age of patients (18 males and 22 females) was 10.03 (SD 1.94) years. The median age of transfusion onset was 7 (range 3-24) months. The mean duration of illness was 102.48 (SD 27.99) months. The characteristics of subjects are summarized in **Table 1**. The median ferritin level was 3,710 (range 1,043−11,200) μg/L, and the mean nitric oxide level was 21.83 (SD 9.7) μM. Other hematological parameters are shown in **Table 2**.

Table 1. Characteristics of subjects

Characteristics	N=40
Gender, n(%)	
Male	18 (45)
Female	22 (55)
Mean age (SD), years	10.03 (1.94)
Median age of onset (range), months	7 (3-24)
Median onset of blood transfusion (range), years	7.5 (3-24)
Median onset of chelation therapy (range), years	36 (12-96)
Mean duration of illness (SD), months	102.48 (27.99)
Frequency of blood transfusion per year, n(%)	
< 15	5 (12)
15-20	23 (58)
20-25	8 (20)
25-30	4 (10)
Chelation type, n(%)	
Desferrioxamine	2 (5)
Deferasirox	11 (28)
Deferiprone	27 (67)
Compliance to chelation, n(%)	
Optimal	30 (75)
Not optimal	10 (25)
School attendance per month, n(%)	
50-75%	4 (10)
75-100%	36 (90)
Education, n(%)	
Elementary school	37 (93)
Junior high school	3 (7)
Mean years of schooling (SD), years	3.85 (1.87)

The abnormal values of verbal IQ, performance IQ, and full scale IQ were 35%, 57.5%, and 57.5%, respectively, as shown in **Table 3**. Serum nitric oxide level was significantly correlated with performance IQ (P=0.022) (**Table 4**), but not with verbal IQ (P=0.359) or full scale IQ (P=0.164). There were no significant correlations between serum ferritin level and full scale IQ (P=0.377), verbal IQ (P=0.460), or performance IQ (P=0.822), as also shown in **Table 4**.

Discussion

Of our 40 subjects, 35% had abnormal verbal IQ, 57.5% had abnormal performance IQ, and 57.5% had abnormal full scale IQ (**Table 3**). These abnormal results were higher than reported by Economou *et al.*, who found that 36.4% of patients with ß-thalassemia major had abnormal full scale IQ.[14] In contrast, we also found two subjects with a high average full scale IQ of 117. Factors underlying the high IQ scores in two subjects were likely due to the subjects' young age (6 years), their good nutritional status, short disease duration (5 years), and relatively high hemoglobin levels compared to the other subjects. Similarly, Raafat et al. reported that 4 (0.04%) patients with ß-thalassemia major had superior IQ scores and 20 (0.2%) patients had high average IQ scores.[15]

We observed a significant correlation between serum nitric oxide levels and performance IQ, but no significant correlation with verbal or full scale IQ (**Table 4**). The pathology of thalassemia disease includes accelerated destruction of nitric oxide and limited compensation processes to increase nitric oxide production. Hence, the deficiency of nitric oxide causes a decrease in cognitive function in children with thalassemia major.[16] The significant correlation between low serum nitric oxide level and abnormal performance IQ might be due to low hemoglobin and low nitric oxide levels caused by hemolysis during the critical stages of early childhood development, affecting parts of the brain associated with performance IQ. Children with thalassemia major have severe anemia at an early age, i.e., before the age of 2 years, which can affect the development of performance IQ. On the other hand, hypoxia and chronic anemia tend to decrease performance

Table 2. Biochemical parameters of subjects

		Reference values
Median hemoglobin (range), g/dL	7.2 (4.1–9.5)	11.5–15.5
Median hematocrit (range), %	21 (12–28)	35–45
Median MCV (range), fl	75.1 (62.4–84.2)	77–95
Median MCH (range), pg	25.8 (20–28.2)	25–33
Median MCHC (range), %	33.9 (31.3–35.5)	31–37
Median erythrocyte (range), x106 microliter	2.85 (1.5–4.0)	4.43–6.02
Median leukocyte (range), /mm3	5,500 (4,000–8,800)	4,500–13,500
Median serum ferritin (range), ng/mL	3,687.5 (1,043–11,200)	14–124
Mean serum nitric oxide (SD), µM	21.83 (9.7)	50.6–121.26

Table 3. The Wechsler Intelligence Scale test interpretations

Variables	n						
	n(%)	Superior	HA	Average	LA	Borderline	MD
Verbal IQ		1	1	24	10	4	-
Normal	26 (65)						
Abnormal	14 (35)						
Performance IQ		1	1	15	18	5	-
Normal	17 (42.5)						
Abnormal	23 (57.5)						
Full scale IQ		-	2	15	18	5	-
Normal	17 (42.5)						
Abnormal	23 (57.5)						

HA=high average, LA=low average, MD=mental deficits

Table 4. Correlation between serum ferritin and serum nitric oxide levels with cognitive function parameters of the WISC test

Variables	Verbal IQ		Performance IQ		Full scale IQ		Interpretation	
	r	P value	r	P value	r	P value	r	P value
Serum ferritin	-1.20	0.460	-0.037	-0.822	-0.144	0.377	-0.080	0.626
Serum nitrit oxide	0.149	0.359	0.360	0.022	0.225	0.164	0.240	0.136

capability to lower than verbal ability, since tests for performance IQ require a larger energy supply. In accordance with this theory, a longitudinal cohort study by Ai *et al.* in 171 children in China showed that children with low hemoglobin levels had a lower performance IQ, but normal verbal IQ.[17] Nitric oxide controls the transmission of information and plays a role in cognitive function, such as in learning and memory, so that NO deficiency may cause cognitive impairment. We found no correlation between verbal IQ or full scale IQ and serum NO levels. Low nitric oxide levels in thalassemia may not influence verbal IQ and full scale IQ, since the verbal IQ test assesses general knowledge, comprehension, and fluency in speaking. To date, no other study has been performed on the possible correlations between serum nitric oxide levels and verbal, performance, and full scale IQs.

We found no correlation between serum ferritin levels and cognitive function in children with thalassemia major, in either full scale, verbal, or performance IQs (**Table 4**). Excess iron causes elevated levels of serum transferrin and iron transport to pass through the blood-brain barrier, causing the accumulation of iron in the brain.[18] Furthermore, the iron can catalyze the formation of free radicals that cause oxidative stress which will accelerate brain tissue degeneration that might lead to cognitive impairment.[5] Our results showed no significant correlation between serum ferritin levels and cognitive function, possibly due to the lack of signs of hemosiderosis in the brain caused by iron overload. A previous study reported that ß-thalassemia major subjects aged >16 years with signs of systemic hemosiderosis had significantly lower neuropsychological test scores.[19] In addition, serum ferritin levels also have low specificity as a marker of hemosiderosis because the levels are very susceptible to infections and inflammatory conditions.[20] The results of this study were similar to those of Monastero *et al.*, which showed no significant association between ferritin levels and cognitive function in ß-thalassemia major.[19] Similarly, Raafat *et al.* showed no correlation between serum ferritin level and full-scale IQ, verbal IQ, or performance IQ.[15] However, Retnani found a correlation between serum ferritin level and performance IQ, but no significant correlation between serum ferritin level and verbal or full scale IQ.[21]

The limitations of this study were the lack of comparative assessment of serum nitric oxide levels in healthy subjects, and that the high levels of serum ferritin cannot be used to evaluate iron deposition in tissue, especially in the brain. A more accurate test is needed to assess the level of iron deposition in the

brain.

In conclusion, lower serum nitric oxide level is significantly correlated with lower cognitive function, specifically in performance IQ. However, serum ferritin levels have no clear correlations with cognitive function.

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Conflict of interest

None declared.

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