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Original Article

Variables associated with malondialdehyde level in thalassemia major patients

Arum Gunarsih, Pustika Amalia, Imam Boediman

Abstract

Background Thalassemia is the most common hereditary haemolytic anaemia in the world, including in Indonesia. The main treatment for thalassemia is regular transfusions, but these are known to cause iron overload. Moreover, iron overload in β-thalassemia patients generates oxygen free radicals and peroxidative lipid injury. Ferritin serum concentration is used as indirect measurement of iron overload. Malondialdehyde (MDA), a terminal compound of lipid peroxidation, is used as an index of oxidative stress status.

Objective To assess the correlation between iron overload (serum ferritin level) and MDA as a marker of oxidative stress in thalassemia major patients.

Methods This cross-sectional study was conducted at Cipto Mangunkusumo Hospital, Jakarta, from May-June 2009. Subjects were thalassemia major patients (homozygous β-thalassemia or β-thalassemia/HbE) who received regular blood transfusions, iron-chelation, and vitamin E as an antioxidant. Data was collected by history-taking, physical examination, medical records, and questionnaires. Blood specimens were drawn from the thalassemia major subjects before transfusion and examined for serum ferritin and MDA levels.

Results Fifty-five subjects with thalassemia major (34 homozygous β-thalassemia and 21 β-thalassemia/HbE) were included in our study. Mean serum ferritin level was 3693.2 (SD 2142.3) μg/L and mean MDA level was 0.641 (SD 0.283) nmol/mL. No correlation was found between serum ferritin and MDA levels in thalassemia major subjects (r=0.147, P=0.285). As additional results, this study also showed no correlation between MDA to reguler vitamin E consumption (r=0.277, P=0.028) as well as MDA and nutritional status (r=0.371, P=0.004).

Conclusion There was no correlation between serum ferritin level and plasma MDA level in thalassemia major subjects, no correlations between MDA and regular vitamin E consumption, as well as MDA and nutritional status. **[Paediatr Indones. 2012;52:125-31]**.

Keywords*: thalassemia, oxidative stress, ferritin, malondialdehyde*

Thalassemia is the most common hereditary chronic hemolytic anaemia in the world, including in Indonesia. Thalassemia and hemoglobinopathies are caused by impaired synthesis of α- or β-globin chains.[1-3] The number of thalassemia patients in Indonesia has increased.[4] As of December 2008, there were 1,435 patients registered at the Thalassemia Centre, Department of Child Health, Cipto Mangunkusumo Hospital (DCH-CMH), Jakarta.[5]

Patients with β-thalassemia major often receive regular blood transfusions, leading to iron overload. Excess iron deposits in tissues and organs are known to generate oxygen free radicals, which then react with cellular phospholipid membranes. Peroxidative tissue injury is detectable in these patients, and results in damage and failure of organs.[6-12] An abundance of free radicals requires an optimal antioxidant system to persist in thalassemia patients.[7,8] The level of cellular antioxidant vitamins, such as vitamin A, C, and E, were found to be considerably lower in thalassemic patients compared to normal subjects.[11-13] These results suggest major antioxidant consumption in thalassemic

From the Department of Child Health, Medical School, University of Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Reprint requests to: Arum Gunarsih, Department of Child Health, Medical School, University of Indonesia, Cipto Mangunkusumo Hospital, Jl. Diponegoro No. 71, Jakarta 10430, Indonesia. Tel +62-21-70052266/+62-8161676615. E-mail: *gunarsih_arum@yahoo.com.sg*

patients with iron overload from continuous blood transfusions or oxidative stress.[14-16]

Serum ferritin is a common indicator of iron storage.[17] MDA, a terminal compound of lipid peroxidation, is used widely as an index of oxidative status. Increased plasma MDA levels have been observed in patients affected by β-thalassemia major.[7,18-23] This substrate also causes cell damage and death, or genetic mutations.[24] This study was aimed to assess the correlation between iron overload and MDA as a marker of oxidative stress in thalasemia major patients.

Methods

This cross-sectional study was performed in the outpatient clinic at the Thalassemia Centre, DCH-CMH, Jakarta in May-June 2009. Subjects were selected by consecutive sampling of thalassemia major patients. The inclusion criteria comprised of children diagnosed with thalassemia major who had received transfusions more than 10 times or had been given iron-chelation treatment. Thalassemia major patients with acute or chronic infection, including hepatitis B or C, or hypersplenism were excluded. Based on the calculated required sample size, our study included 55 subjects ranging from 2-18 years in age. Informed consent was obtained from all of the participants/parents and the study was approved by the Ethics Committee of the University of Indonesia Medical School. Data on adherence to vitamin E and iron chelating agent consumption was collected by questionnaire and medical records.[25] Blood sample was analyzed for serum lipid peroxide by quantitative assay of MDA using thiobarbituric acid reactive substances and a UV spectrometer (Shimadzu Corporation, Japan), at the Biochemistry Laboratory of the CMH, Jakarta.[26] The serum ferritin quantitative test was based on a solid phase enzyme-linked immunosorbent assay, using a Cobas Kit, at the Clinical Pathology Laboratory of the CMH, Jakarta.[27] Patients' blood was drawn just before blood transfusion. Study results were analyzed by SPSS version 15. Differences were considered significant for $P < 0.05$. All results are expressed as mean $\pm$ standard deviation (SD). The relation between serum ferritin concentrations and MDA level was determined with Pearson's coefficient correlation. Linear regression analysis was used to determine a correlation between MDA and other variables.

Results

Fifty-five subjects diagnosed with β-thalassemia major were consecutively recruited for this study. Characteristics of the subjects are presented in **Table 1**.

The mean MDA level of all subjects in this study was 0.641 nmol/mL (SD 0.283) with a range of 0.204-1.722 nmol/mL. Mean ferritin serum level was 3693.2 μg/L (SD 2142.3) with a range of 659.5-9129 μg/L. Ferritin serum and MDA levels in this study were not correlated (r=0.147, P=0.285), as shown in **Figure 1.**

Older subjects had higher MDA levels than younger subjects as shown in **Table 2**. Homozygous ß-thalassemia subjects had higher MDA levels than ß-thalassemia/HbE subjects. Subjects who used deferasirox as an iron-chelating agent, had a length of illness ranged between 25-120 months, and were malnourished also had higher levels of MDA than subjects in other categories. **Table 4** shows the bivariate analysis of the above variables with MDA level.

Table 1. Characteristics of subjects

Characteristics	Number (%) n=55
Sex	
Male	36 (65.4)
Female	19 (34.6)
Thalassemia type	
Homozygous β-thalassemia	34 (61.8)
β-thalassemia/HbE	21 (38.2)
Age, years	
< 5	7 (12.7)
5-10	27 (49.1)
>10	21 (38.2)
Age at diagnosis, years	
< 1	24 (43.6)
1-2	19 (34.6)
> 2	12 (21.8)
Nutritional status based on MUAC	
Well-nourished	18 (32.7)
Undernourished	25 (45.5)
Malnourished	12 (21.8)
Iron-chelation type	
Desferoxamine	12 (21.8)
Deferiprone	40 (72.7)
Deferasirox	2 (3.6)
No iron-chelation	1 (1.8)
Transfusion frequency, times per year	
< 10	23 (41.8)
10-15	29 (52.7)
> 15	3 (5.5)
Pre-transfusion hemoglobin, g/dL	
< 6	8 (14.5)
6-8	45 (81.8)
>8	2 (3.6)

MUAC: mid-upper arm circumference

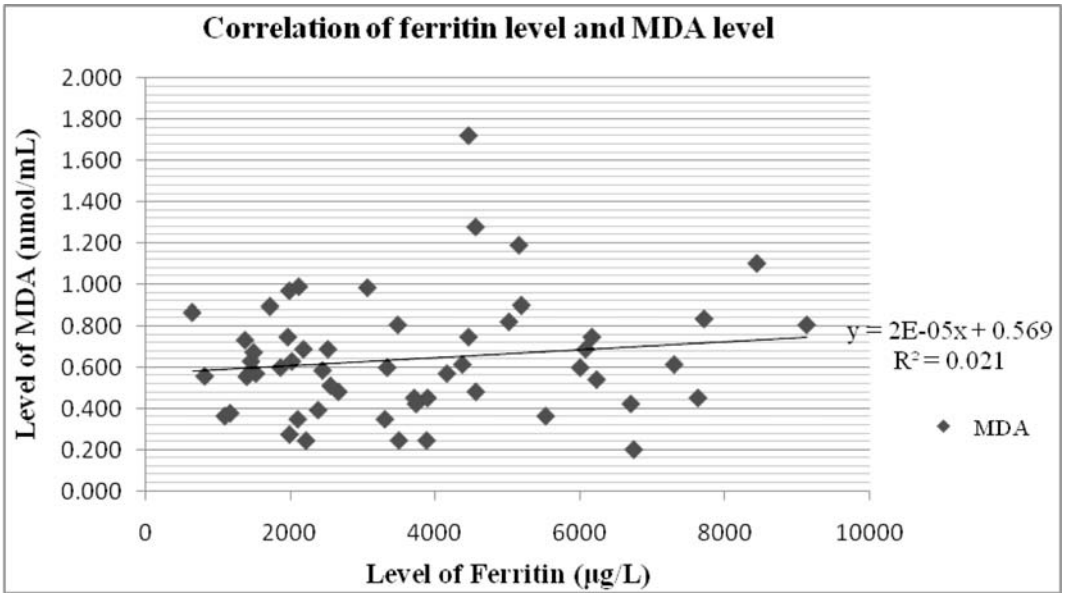

Figure 1. Correlation of ferritin and MDA levels (r=0.147, P= 0.285)
Notes: MDA = 2x105 ferritin + 0.569. R^2 = 0.021.

Table 2. Distribution of mean ferritin and MDA levels

Characteristics	n	Mean ferritin level (SD) µg/L	Mean MDA level (SD) nmol/mL
Age (in study period)			
< 5 years	7	2853 (803.9)	0.52 (0.166)
5-10 years	27	5909 (1547.1)	0.59 (0.213)
≥10 years	21	4749 (2113.4)	0.75 (0.343)
Type of thalassemia			
Homozygous β-thalassemia	34	3950.0 (2308)	0.68 (0.32)
β-thalassemia/HbE	21	6256.9 (1817)	0.59 (0.21)
Iron-chelation type			
Desferoxamine	12	4711.8 (2627.0)	0.74 (0.36)
Deferiprone	40	3318.6 (1771.1)	0.58 (0.23)
Deferasirox	2	6504.5 (2741.0)	1.19 (0.12)
Duration of disease			
< 24 months	10	1752.0 (878.5)	0.63 (0.14)
25-120 months	27	4983.8 (2171.0)	0.76 (0.35)
> 120 months	18	3551.7 (1958.0)	0.58 (0.26)
Nutritional status (based on MUAC)			
Well-nourished	18	3413.4 (2306.6)	0.62 (0.21)
Undernourished	25	3681.4 (1789.4)	0.58 (0.23)
Severely malnourished	12	4137.0 (2529.2)	0.84 (0.36)

MUAC: mid-upper arm circumference

Nutritional status was divided into 2 categories, normal (well-nourished) and abnormal (malnourished and undernourished), so mean of the MDA level became linear. Bivariate analysis **(Table 4)** revealed three statistically significant variables: gender, adherence to vitamin E consumption, and nutritional status.

Moreover, adherence to vitamin E consumption (r=0.277, P=0.028) and nutritional status (r=0.371, P=0.004) had no correlation to MDA level.

Discussion

The mean of age at first transfusion of our subjects was 19.8 months (SD 20.8). Modell *et al.* reported the age

Table 3. Distribution of characteristics according to thalassemia type

Characteristics	Homozygous β-thalassemia n = 34	β-thalassemia/HbE n = 21
Mean ferritin, µg/mL (SD)	3950 (2308)	6256.9 (1817)
Mean MDA, nmol/mL (SD)	0.68 (0.32)	0.59 (0.21)
Mean age, years	8.5	11.4
Mean duration of disease, months	89.8	10.8
Iron-chelation type, n		
Desferoxamine	9	18
Deferiprone	22	3
Deferasirox	2	0
None	1	0
Nutritional status, n		
Well-nourished	14	4
Undernourished	13	12
Severely malnourished	7	5
Adhered to iron-chelating agent consumption, n	15	13
Adhered to vitamin E consumption, n	7	7

Table 4. Correlation of various variables to mean MDA level

Variables	n		P
		Correlation coefficient to MDA level, r	
Ferritin level	55	0.147	0.285
Age in study period	55	0.233	0.087
Age at diagnosis	55	-0.137	0.319
Duration of disease	55	0.251	0.064
		Mean MDA (SD)	
Sex			
Male	36	0.57 (0.22)	0.034
Female	19	0.77 (0.35)	
Type of thalassemia			
Homozygous β-thalassemia	34	0.68 (0.32)	0.220
β-thalassemia/Hb E	21	0.59 (0.21)	
Iron-chelation type			
Deferiprone	40	0.58 (0.23)	0.191
Desferoxamine	12	0.74 (0.36)	
Adhered to iron-chelating agent consumption			
Poor adherence	27	0.70 (0.33)	0.161
Good adherence	28	0.59 (0.23)	
Adhered to vitamin E consumption			
Poor adherence	41	0.68(0.29)	0.012
Good adherence	14	0.51(0.19)	
Nutritional status			
Severely malnourished	12	0.84 (0.36)	0.046
Undernourished	25	0.58 (0.23)	
Well-nourished	18	0.62 (0.21)	

at first transfusion to be 62% for < 1 year olds, 29% for 1-2 year-olds, and as high as 9% for > 2 year-olds.[28] In contrast, most of our subjects were diagnosed later, since thalassemia screening is not routinely conducted in Indonesia. Consequently, blood transfusions would be received later.

In our study, 25 (45.5%) subjects were undernourished and 12 (21.8%) were malnourished, based on MUAC. Constantoulakis *et al.* reported that low body weight and growth impairment in ß-thalassemia major patients can be caused by various factors, such as chronic and severe anemia, enlarged spleen, and coexistence of other diseases, especially infections.[29]

The most common type of iron-chelating agent used was deferiprone (72.7%), an agent in which consumption adherence is typically better than others. Only 50.9% of thalassemia major patients in our study used iron-chelating agents regularly. This figure was higher than that from the Thalassemia Center, DCH-CMH in 2005. However, this improved compliance

may reflect that oral iron-chelating agents were not available in Indonesia in 2005.[27]

Malondialdehyde is a marker to determine the existence of oxidative stress.[24] We did not compare MDA levels of thalassemia patients to normal controls. A study on MDA levels in the normal Jakarta population in 2005 reported the mean level was 0.086 (SD 0.016) nmol/mL.[30] In comparison, the mean MDA level in this study was 7 times higher. This result was similar to a previous study that showed increased MDA in thalassemia major subjects compared to normal subjects.[14] This situation can be explained by there being lipid peroxidation processing by auto-oxidation of unstable hemoglobin in thalassemia patients, with α-chain producing radical peroxide (O_2^-), erythrocyte-rich iron, catalyst-like copper, and iron overload that make an oxidative stress condition.[15,17,19]

Mean MDA level in thalassemia major subjects in this study was lower than in previous studies. This lower mean MDA may have been due to our use of homozygous ß-thalassemia and ß-thalassemia/HbE subjects, while other studies used normal control subjects.[7-9,18,20] We found the mean MDA level in ß-thalassemia/HbE subjects to be 0.587 (SD 0.206) nmol/mL, lower than that of homozygous ß-thalassemia subjects 0.688 (SD 0.316) nmol/mL, (P>0.05). This result differs from a study by Goswami *et al* that showed MDA levels in homozygous ß-thalassemia subjects were lower [(mean 2.05; SD 1.17) nmol/mL] than in ß-thalassemia/HbE patients [(mean 2.46; SD 1.03) nmol/mL].[12] This condition was thought to be due to iron-binding by protein, thus free radical products were not increased.

There was no correlation between MDA level and serum ferritin level in all subjects in this study (r=0.147; P=0.285). This result differs from studies performed by Livrea *et al*,[7] Cighetti *et al*,[23] and Naithani *et al*,[19] possibly due to different characteristics of the subjects. Their studies all used only homozygous ß-thalassemia subjects. Furthermore, existence of other factors such as age, type of iron-chelating agent, adherence to iron-chelating agent consumption, adherence to vitamin E consumption, and nutritional status, could influence the results. As presented in **Table 3**, homozygous ß-thalassemia subjects had a higher level of MDA than ß-thalassemia/HbE subjects, but also had lower level of serum ferritin, probably because homozygous ß-thalassemia subjects had a younger mean age, shorter length of disease, and better nutritional status than thalassemia-ß/HbE subjects. In addition, the majority of the homozygous ß-thalassemia subjects used deferiprone.

Free iron may act as a catalyst to form reactive oxygen species by Fenton reaction.[31] However, free iron-measurement (non-transferrin-bound iron) is not a routine examination in thalassemia patients. Serum ferritin level shows the amount of reserved body iron, and is not a measurement of free iron. This examination is relatively easy to do and inexpensive. So it has been routinely performed to measure body iron-content, although its value can be influenced by infection, inflammation, diet, nutritional status, liver disease, and diurnal variation.

The regularity of vitamin E consumption showed no correlation with level of MDA (r=0.277; P=0.028). Vitamin E deficiency in thalassemia subjects might be caused by increased consumption as a consequence of oxidative stress and pressure on erythrocyte and other organs due to hemochromatosis. Liver damage induced by iron is related to the lack of lipid antioxidants.[7,8,16,33] Mahjoub *et al*[13] and Das *et al*[8] reported that MDA level decreased in thalassemia subjects who received vitamin E.

There was also no correlation between nutritional status and MDA level in all subjects (r=0.371; P=0.004). No prior study has reported a correlation between thalassemia patients' nutritional status and MDA level, but MDA level has been correlated to malnourishment. Khaled *et al* stated that insufficient nutritional substances increased oxidative stress.[34] Nutritional substances that play a part in the free radical reaction process include vitamin E, vitamin A, vitamin C, selenium, zinc, manganese, copper, and ß-carotene. These substances act in enzymatic reactions. Malnourished children usually lack those nutritional substances.[35] Arijanty, in 2005, found plasma zinc levels in thalassemia subjects to be low, although there was only a weak correlation between plasma zinc level and nutritional status.[35]

There were several limitations in this study. Subjects were collected by consecutive sampling. As such, it was difficult to recruit subjects based on age group, gender, and other variables in balanced proportion to each other. Data on adherence to iron-chelating agent and vitamin E consumption was collected by history-taking, and relied on parents'

memories, which may be subjective and prone to recall bias.

We conclude that the mean MDA level was 0.641 (SD 0.283) nmol/mL, and the mean serum ferritin level was 3693.2 (SD 2142.3) μg/L. There was no correlation between MDA and ferritin serum levels (r=0.147; P=0.285). There was also no correlation between consumption of iron-chelating agent and MDA level (P=0.07). This study also revealed no correlation between adherence to vitamin E consumption and MDA level (r=0.277; P=0.028), as well as no correlation between nutritional status and MDA level (r=0.371; P=0.004).

References

1. Rund D, Rachmilewitz E. β-thalassemia. N Engl J Med. 2005;353:1135-46.
2. Olivieri NF. The β-thalassemias. N Engl J Med. 1999;341:99-110.
3. Advani R, Sorenson S, Shinar E, Lande W, Rachmilewitz E, Schrier SL. Characterization and comparison of the red blood cell membrane damage in severe human α- and β-thalassemia. Blood. 1992;79:1058-63.
4. Prevalensi talasemia terus naik [homepage on the Internet]. c2008 [cited 2009 January 20]. Available from: *http://www.mediaindonesia.com/print.php?ar_id=19143*.
5. Data of Thalassemia Center Cipto Mangunkusumo Hospital, Jakarta, December 2008.
6. Higgs DR, Thein SL, Woods WG. The molecular pathology of the thalassemias. In: Weatherall DJ, Clegg B, editors. The thalassemia syndromes. 4th edition. London: Blackwell Science; 2001. p. 133-91.
7. Livrea MA, Tesoriere L, Pintaudi AM, Calabrese A, Maggio A, Freisleben HJ, et al. Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. Blood. 1996;88:3608-14.
8. Das N, Das CT, Chattopadhyay A, Datta AG. Attenuation of oxidative stress-induced changes in thalassemic erythrocytes by vitamin E. Pol J Pharmacol. 2004;56:85-96.
9. Kassab-Chekir AK, Laradi S, Ferchicli S, Khelil AH, Feki M, Amri F, et al. Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. Clin Chim Act. 2003;338:79-86.
10. Kattamis A, Papassotiriou I, Palaiologou D, Apostolakou F, Galani A, Ladis V, et al. The effects of erythropoetic activity and iron burden on hepcidin expression in patients with thalassemia major. Haematologica. 2006;91:809-12.
11. Laksmitawati DR, Handayani S, Udyaningsih SK, Kurniati V, Adhiyanto C, Hidayat J, et al. Iron status and oxidative stress in β-thalassemia patients in Jakarta. Bio Fac. 2003;19:53-62.
12. Goswami K, Ghosh S, Bandyopadhyay, Mukherjee KL. Iron store and free radicals in thalassemia. Indian J Clin Biochem. 2005;20:192-4.
13. Ghone RA, Kumbar KM, Suryakar AN, Katkam RV, Joshi NG. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. Indian J Clin Biochem. 2008;23:337-40.
14. Simsek F, Ozturk G, Kemahli S, Erbas D, Hasanoglu A. Oxidant and antioxidant status in beta thalassemia major patients. Ankara Universitesi Tip Fakultesi Mecmuasi. 2005;58:34-8.
15. Pavlova LE, Savov VM, Petkov HG, Charova IP. Oxidative stress in patients with β-thalassemia major. Sec Biol Med Sci. 2007;28:145-54.
16. Meral A, Tuncel P, Surmen E, Ozbek R, Ozturk E, Gunay U. Lipid peroxidation and antioxidant status in β-thalassemia . Ped Hem Onc. 2001;17:687-93.
17. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardiani C, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med. 2000;343:327-31.
18. Chigetti G, Duca L, Bortone L, Sala S, Nava L, Fiorelli G, et al. Oxidative status and malondialdehyde in β-thalassemia patients. Eur J Clin Inves. 2002;32:55-60.
19. Eposito BP, Breuer W, Sirankaprancha P, Pootrako C, Cabantchik ZI. Labile plasma iron in iron overload: redox activity and susceptibility to chelation. Blood. 2003;102:2670-7.
20. Naithani R, Chandra J, Bhattacharjee J, Verma P, Narayan S. Peroxidative stress and antioxidant enzymes in children with β-thalassemia major. Pediatr Blood Cancer. 2006;46:780-5.
21. Walter PB, Fung EB, Killilea DW, Jiang Q, Hudes M, Madden J, et al. Oxidative stress and inflammation in iron overloaded patients with β-thalassemia or sickle cell disease. Br J Haematol. 2006;135:254-63.
22. Widad NM, Al-Naama LM, Hassan MK. Lipid peroxidation in beta-thalassemia. Haema. 2006;9:374-9.
23. Nourooz-Zadeh J, Chiani M, Khadam MHA, Hejazi S. Plasma measures of oxidative stress in β-thalassemia. J Urmia Univ Med Sci. 2005;16:118.
24. Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clin Chem. 1997;43:1209-14.

25. Data of Thalassemia Center Cipto Mangunkusumo Hospital, Jakarta, December 2005.
26. Wills, ED. Evaluation of lipid peroxidation in lipids and biological membranes. In: Snell, K. and Mullock, B, editors. Biochemical toxicology. A practical approach: practical approach series. London: IRL press, Oxford;1987. p.76-84.
27. Englebienne P, Hoonacker AV, Valsamis J. Rapid homogeneous immunoassay for human ferritin in the Cobas Mira using colloidal gold as the reporter reagent. Clinical Chemistry. 2000;46:2000-3.
28. Modell B, Berdoukas V. Transfusion-dependent thalassemia: a new era. Med J Aust. 2008;188:68-9.
29. Constantoulakis M, Logothetis J, Loewenson RB, Augoustaki O, Economidov J. Body growth in Cooley's anemia (homozygous beta thalassemia) with a correlative study as to other aspects of illness in 138 cases. Pediatrics. 1972;50:92-9.
30. Puspasari M. Kadar glutation and malondyaldehide plasma darah mahasiswa Universitas Negeri Jakarta. [bachelor's thesis]. [Jakarta]: Universitas Negeri Jakarta; 2005.
31. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur MJ, Telser J. Free radicals and antioxidant in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44-84.
32. Herbert V, Jayatilleke E, Shaw S, Rosman AS, Giardina P, Grady RW, et al. Serum ferritin iron, a new test, measures human body iron stores unconfounded by inflammation. Stem Cells. 1997;15:291-6.
33. Dhawan V, Kumar K, Marwaha RK, Ganguly NK. Antioxidant status in children with homozygous thalassemia. Indian Pediatr. 2005;42:1141-5.
34. Khaled MA. Oxidative stress in childhood malnutrition and diarrhoeal diseases. J Diarrhoeal Dis Res. 1994;12:165-72.
35. Arijanty L, Nasar SS, Madiyono B, Gatot D. Relationships between plasma zinc and ferritin with nutritional status in thalassemic children. Paediatr Indones. 2006;46:220-4.