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Status of *Superoxide Dismutase* in Transfusion Dependent Thalassaemia

Lantip Rujito, MD, MSc, Sri Mulatsih,[1] and Abdul Salam M. Sofro[2]

Department of Molecular Biology, Medical Faculty of Jenderal Soedirman University, Purwokerto, Indonesia
[1]*Department of Pediatric, Sardjito National Hospital, Yogyakarta, Indonesia*
[2]*Department of Biochemistry, Medical Faculty of Yayasan Rumah Sakit Islam Indonesia University, Jakarta, Indonesia*

Address for correspondence: Mr. Lantip Rujito, Medical Faculty of Jenderal Soedirman University, Purwokerto, Indonesia. E-mail: l.rujito@unsoed.ac.id

Abstract

Background:

Thalassemia is a collection of genetic impairments in beta and alpha genes causing various states of anemia. Severe types of the disease need lifelong transfusions, leading to oxidant-antioxidant disturbance due to massive iron deposits.

Aims:

The aim of this study was to assess the antioxidant enzyme *Superoxide Dismutase* (SOD) and ferritin levels of thalassemia major patients in a peripheral health facility.

Materials and Methods:

Two hundred and nine probands were recruited and performed laboratory experiments for SOD and Ferritin levels. Chelation administration and clinical score were taken from interviewing the family and from medical report data.

Results:

The study showed that SOD intensity was lower (162.41 u/ml) compared to the normal cutoff point (P = 0.001), while the mean of Ferritin levels was ten times over the normal value (4226,67 ng/dl). Observations also reported that chelation medicine was not administrated properly.

Conclusions:

The data indicates that thalassemic patients have oxidant-antioxidant uproar due to oxidative stress. Monitored chelating administration, selective antioxidant, and a well-balanced diet may prevent oxidative injury.

Keywords: Oxidant-antioxidant, superoxide dismutase, thalassaemia

Introduction

Thalassemia is a clinical hematology problem caused by a collection of genetic abnormalities in the gene cluster-forming β and α chains of proteins carrying oxygen, hemoglobin.[1] Patients with severe cases cannot produce normal hemoglobin, leading to a life-long anemic state. Treatments for the condition depend on continuous blood transfusions to maintain a good quality of life. However, empirical data shows that regular transfusions lead to an iron overload with a massive increase in non-transferrin-bound iron that may cause more tissue damage than conjugated iron. Further results of these processes are the occurrences of excessive oxidative stress and disturbance defense of oxidant-antioxidant mechanisms.[2]

Iron-induced oxidative stress is one of the most important factors determining cell injury in thalassemic patients. It has been reported that reactive oxygen species (ROS) involved in red cells are damaged due to increased membrane rigidity, deformity, and induced hemolysis.[3] In addition, oxidative stress has been recognized as initiating the removal of red cells by the immune system.[4] Endogenous antioxidants, like superoxide dismutase (SOD), Catalase and glutathione peroxidase (GPx) are the first barriers to the change of the internal environment influenced by the increase of free radicals and abundant stress, creating superactiveoxygen. However, much of the data from thalasemic patients state that SOD level can vary from a low level, no different from healthy individuals, up to high level.[5,6,7] We assume that the variation may be caused by different areas and management. The purpose of this study was to assess the status of the SOD enzyme in transfusion-dependent thalassemic subjects in Banyumas, a remote region in Central Java, Indonesia.

Materials and Methods

Research Subjects

Subjects were patients with Thalassaemia major that were diagnosed based on clinical symptoms, blood analysis index, and hemoglobin electrophoresis. Two hundred and nine subjects aged 6 months to 65 years were included in this study, excluding patients with hypothyroidism and hyperthyroidism, diabetes mellitus, and acute inflammation. Subjects read and signed informed consent waivers approved by the Medical Faculty Ethic Committee.

Laboratory Experiments

Ten milliliters of blood were drawn and stored in EDTA tubes to be used in laboratory experiments. Levels of (SOD) activity in red blood cells were assayed by RANSOD Kit (Randox, United Kingdom) and were expressed in U/ml. Excess of ferritin was measured using ELISA Kit for ferritin (Sigma-Aldrich, USA) and counted in ng/dl. To determine clinical scores of thalasemic patients, the Sripichai Score was adopted. [8]

Statistical Analysis

Data was presented in a descriptive manner, including mean and standard deviation. Comparison between the case and normal cut-off point was performed using one sample t test. Person's correlation was used to determine the relationship between SOD and clinical appearances.

Results

During the study period, ferritin value was collected 3-6 times, reflected in serial retrieval. The mean of serum ferritin among subjectswas 4226,67 ng/dl, significantly increased from standard normal value, for both children and adults. This study also reported that the average level of SOD activity was 162.41 u/ml. Compared with the normal population, it was below the standard value (the average varying from 164-240 u/ml) [Table 1 and Figure 1]. Approximately six percent of the patients in our study had serum ferritin exceeding 2500 ng/dl, which is almost ten times higher than the upper limit of normal. Ferritin level of the patients was depicted in Table 2. Figure 1 depicts that in general, red cell SOD activity in thalasemic patients studied were below average, however some individuals expressed high SOD activity. One sample

t-test performed on the mean of SOD activity expressed lower value than the mean of normal individuals ($P = 0.001$). Using Person's corelation statistic, no relationship resulted between SOD and degree of clinical patients ($P = 0.66$).

Discussion

Ferritin distribution was directly proportional to the age of the patients, as depicted in Table 2. Lowest levels were in the age group of <2 years old, followed by 2-10 years. As transfusions became regular treatment, the amountof ferritin increased as manifest of iron from red blood cells accumulation. For subjects >35 years old, low ferritin levels may relate to the onset of transfusion, as they carry a mild form of Thalassaemia. Patients with moderate severity, regardless of genotype, were characterized usually by moderate anemia and required no or only infrequent red blood cell transfusions.[9]

It was already known that ferritin from thalassemia patients could reach >12,000 ng/dl, and the iron chelating program must be a priority when the threshold (1000 ng/dl) has been exceeded.[10] The program has become part of the management of thalassaemiain local hospitals by means of the medicine, deferiprone. However, the study indicates that patient adherence to administrate chelation was not monitored well. It could be characterized by looking at high ferritin levels in the study subjects. Previous data showed that deferiprone was effective only in the initial conditions of high ferritin (>5000 ng/dl), whereas in 2500-5000 ng/dl levels, ferritin tends to be stagnate.[11] Later studies also displayed similar conclusions; decreasing ferritin with deferiprone was significantly less likely to succeed with lower initial values.[12]

The study also found a correlation between ferritin and clinical score appearance. The score indicates that the degree of clinical impairment is in line with higher ferritin levels in patients ($P = 0.04$). As in previous studies, the data reaffirmed that the excessive iron deposits will affect the clinical development, mainly growth and co-morbid complicating factors.[13] Iron deposits in metabolicorgans (such as the liver, pancreas, and spleen) may develop severe complications caused by disturbance mechanisms, including the immune system, oxidant-antioxidant regulation, and metabolism disruption.

Despite the facts, plasma ferritin is also influenced by the state of acute infection, iron metabolism disorders, and acute physical trauma.[14,15] Such condition may increase the levels 2 to 30-fold.[16] In other words, other assumptions about the increase of ferritin aside from over-transfusion cannot be ruled out.

Together with GPx, SOD is an intracellular enzyme that is responsible for changes in the oxidant-antioxidant balance in cells. Enzyme function is to catalyze modifying ion free radicals, especially 0_2- into H_20.[17] In subjects with thalassaemia, enormous free radicals built up due to the state of iron overload (resulting from transfusions and ineffective erythropoiesis). Iron (Fe) is able to accelerate the change of molecular oxygen into reactive oxygen radicals, superoxide, and hydroxyl groups through the Fenton reaction.[18,19]

These low levels of SOD activity were in agreement withpreviously published data.[20] Thisstudy showed that patients with homozygous thalassaemia decreased 1.5 times lower than the normal individual. In line with the studies, Patne *et al.*, in 2012 also present data showing that the levels of erythrocyte antioxidant enzymes, especially SOD and GPx activity, decreased significantly in patients who were transfusion-dependent.[21] Another study also concluded that the degree of pain and clinical appearance correlate with low levels of antioxidants.[7] All of these studies suggestthat monthly transfusion leads to decreased SOD and GPx levels. However, different results were shown by other research centers. Simsek and colleagues found that the levels of SOD and GPx in thalassemic patients were higher than the healthy controls and careers, while vitamin E levels were lower.[22] Other publications mention that SOD did not show significant differences between healthy controls and thalassemic subjects.[2]

Increased levels of antioxidants, including SOD, occur in various circumstances: Including an acute inflammatory phase, a state of trauma, and upon exposure to increased levels of pro-oxidants. The increase was associated with a compensatory mechanism to break down free radicals that had been caused by oxidative stress and lipid peroxidation.[23] In achronic clinical state, decline was associated with the inability of the antioxidant system to compensate excessive originators. Free radicals could not be offset by

the system, which may have caused the degradation of proteins (including enzymes) and cell membranes, which in turn decreased the levels and activity of antioxidant enzymes.[24] This is supported by other publications, which state that chronic stress in diabetes mellitus, metabolic syndrome, chronic liver disease, SLE, and rheumatoid arthritis affect the decrease in antioxidant enzyme capacity.[25,26,27]

In addition, the study of iron overload diseases, such as Hemochromatosis, also found that the total antioxidant capacity will decline.[28] Rat models with Hemochromatosis (HFE gene mutations) showed to have increased iron levels but decreased levels of antioxidant enzymes and non-enzymes.[29] Other findings also stated that Hemochromatosis subjects expressed low levels of the paraoxonase enzyme (one of the peroxidase lipids degradation enzymes) compared to normal individuals.[30]

Variations in findings on the thalassemic subjects may be caused by other factors or mechanisms that play a role, including iron chelation and daily diet of the patients. Research in Jakarta said that the decrease of antioxidant enzymes in patients related to non-chelating subjects[5], while administration of curcuma on a regular basis, could increase the capacity of the enzymes SOD, GPx, and GSH. The discontinuation, however, caused levels of the enzymesto go back to original value.[31] Iron chelation, including Deferoxamin (DFO) and EDTA, will first bind Fe^{2+}, oxidizing reactive Fe^{2+} into Fe^{3+}, which is more stable. This metal oxidation process significantly lowered oxygen oxidation to become reactive oxygen.[3] In many cases with impaired oxidant-antioxidant mechanisms, administrating an iron chelatorwill improve the prognosis of various disorders, including neurodegenerative disease[32], cardiovascular impairment[33], and iron overload.[34,35] Thus, the presence of varying levels of SOD may be interfered with by the effect of iron chelation management and diet, although other inflammatory factors cannot be ruled out.

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Footnotes

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Conflict of Interest: None declared.

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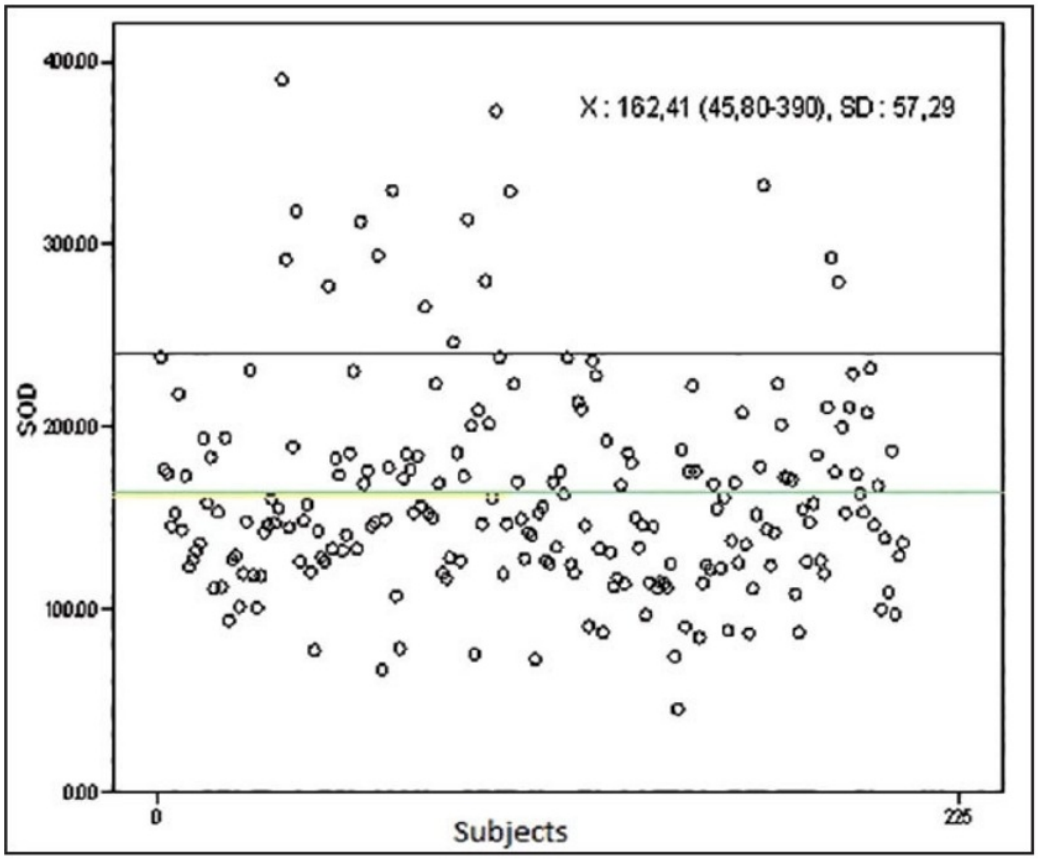
Figures and Tables

Table 1

The mean of ferritin and SOD and their correlation with clinical score

Variable	Mean	SD	Minimum	Maximum	ρ* clinical score
Ferritin (ng/dl)	4226,67	2752,98	20,2	12000	0,04
SOD (U/ml)	162,41	57,29	45,8	390	0,66

Figure 1



SOD Distribution among reaseach subjects. It show that SOD level among thalassaemia patients are under normal value (164-264 u/ml)

Table 2

Ferritin levels according to the mean age of patients

Age (years)	Mean (ng/dl)	SD	Min	Max	F (ρ)
<2	2645, 026	1888, 431	44, 9	7452,6	5,656 (0,001)
2-10	4526, 199	2746, 457	301, 5	11282,8	
10-20	4850, 651	2721, 512	826, 0	12000,0	
20-35	5163, 929	3379, 897	20, 2	11445,3	
>35	2912, 4	2245, 785	102, 6	6452,2	

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