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Original Article

Effect of methotrexate and doxorubicin cumulative doses on superoxide dismutase levels in childhood acute lymphoblastic leukemia

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Abstract

Background Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. Chemotherapeutic drugs for ALL such as methotrexate (Mtx) and doxorubicin produce reactive oxygen species (ROS), a type of free radical. The ROS can reduce levels of antioxidants in the body, including superoxide dismutase (SOD). Decreased SOD levels can cause DNA, lipid, and protein damage, which in turn may lead to adverse effects and treatment failure.

Objective To determine the effect of Mtx and doxorubicin cumulative doses on SOD levels in children with ALL.

Methods We conducted a retrospective cohort study in children with ALL who underwent chemotherapy in Dr. Sardjito Hospital in October 2011 who had completed the induction phase. Risk factors for decreased SOD levels were analyzed by Cox regression and hazard ratio, with a significant level of $P < 0.05$.

Results Of 40 patients enrolled, Mtx $\geq$ 3000 mg/m^2 significantly decreased SOD levels (HR 9.959; 95%CI 2.819 to 35.183; P=0.001). However, doxorubicin $\geq$90 mg/m^2 did not significantly decrease SOD levels (HR 0.59 95%CI 0.194 to 1.765; P=0.34).

Conclusion Methotrexate is associated with decreased SOD levels in children with ALL. However, doxorubicin is not associated with decreased SOD levels in the same patient population. **[Paediatr Indones. 2015;55:239-42]**.

Keywords: *acute lymphoblastic leukemia, methotrexate, doxorubicin, superoxide dismutase*

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children, comprising nearly one-third of childhood malignancies. Every year more children are diagnosed with ALL. In Dr. Sardjito Hospital, Yogyakarta, from 2000 to 2004, 486 children with cancer were treated. Of these children, 35% had ALL and 13% had acute myeloblastic leukemia (AML).[1]

The primary treatment for ALL is chemotherapy. Although chemotherapy has been shown to be successful, the formation of free radicals and their toxic effects has significantly contributed to mortality.[2] In Dr. Sardjito Hospital, mortality due to childhood ALL remains high. The mortality rate was 8.8% for those at standard risk and 17.7% for those at high risk in the induction phase.[1] The detrimental effect of free radicals in the induction phase has been questioned in the past. Chemotherapy such as methotrexate (Mtx) and doxorubicin can produce reactive oxygen species (ROS), a type of free radical.[3] The ROS can be determined by examining superoxide dismutase (SOD) levels.[4] Longer Mtx treatment and greater cumulative doses of this

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drug tends to decrease SOD levels.[5] Similar results were reported for daunorubicin chemotherapy.[6] Patients who received high-dose chemotherapy had a significant decrease in plasma concentrations of antioxidant enzymes including SOD due to the formation of free radicals from chemotherapy.[6,7] Previous studies have examined the relationship between chemotherapy duration in leukemia and SOD levels, without specifying the chemotherapy doses. We aimed to assess the effect of cumulative doses of doxorubicin and Mtx on SOD levels.

Methods

We conducted a retrospective cohort study in October 2011 at the Department of Child Health, Gadjah Mada University Medical School/Dr. Sardjito Hospital, Yogyakarta.

Sampling was conducted consecutively. Inclusion criteria were ALL patients aged 0-18 years who had completed the induction phase of chemotherapy and whose parents provided informed consent. We excluded patients with incomplete medical records. The SOD levels were assessed using the SOD Cayman chemical kit by Elisa method at the Biochemical Laboratory, Gadjah Mada University Medical School. The cut-off points for SOD was 7.3 mg/dL. We used SOD < 7.3 mg/dL as low SOD, dan SOD ≥ 7.3 mg/dL as high SOD. Cumulative doses of Mtx and doxorubicin were calculated from data in the medical records from the initiation of chemotherapy until the examination of SOD levels. This study was approved by Ethics Committee of Gadjah Mada University Medical School.

We analyzed data using Cox regression to calculate hazard ratios for the effects of Mtx and doxorubicin cumulative doses on decreased SOD levels. A P values of <0.05 were considered to be statistically significant. Data analyses were performed using *SPSS version 17.0 for Windows*.

Results

In October 2011 at Dr. Sardjito Hospital, there were 59 ALL patients who had completed the induction phase. We recruited 40 patients into the study, as 17 patients refused participation and 2 patients had

Table 1. Baseline characteristics of subjects

Characteristics	(N = 40)
Mean age (SD) , months	76.9 (44.90)
Maximum	181 months
Minimum	22 months
Gender, n (%)	
Male	22 (55)
Female	18 (45)
Nutritional status, n (%)	
Severely wasted	0
Wasted	4 (10)
Overweight	3 (7.5)
Good	33 (82.5)
Passive smoking, n (%)	
No	17 (42.5)
Yes	23 (57.5)
Cigarette exposure	
Maximum number of cigarettes/day	20
Minimum number of cigarettes/day	5

Table 2. Effect of Mtx and doxorubicin cumulative doses and other variables on SOD levels (univariate analysis)

Variables	SOD Low	SOD High	Hazard ratio (HR)	95% CI	P value
Mtx cumulative dose, n (%)					
≥3000 mg/m^2	20 (64.5)	11 (35.5)	9.96	2.819 to 35.183	<0.0001
<3000 mg/m^2	2 (22.5)	7 (77.8)			
Doxorubicin cumulative dose, n (%)					
≥90 mg/m^2	7 (41.2)	10 (58.8)	0.59	0.194 to 1.765	0.34
<90 mg/m^2	11 (61.1)	7 (38.9)			
Passive smoking, n (%)					
Yes	11 (47.8)	12 (52.2)	0.96	0.370 to 2.496	0.93
No	10 (55.0)	7 (41.2)			
Nutritional status, n (%)					
Malnourished	2 (50.0)	2 (50.0)	1.06	0.094 to 11.864	0.96
Overweight	1 (33.3)	2 (66.7)	0.57	0.123 to 2.645	
Good	15 (45.5)	18 (54.5)			

incomplete data. Characteristics of subjects are shown in **Table 1.**

Chemotherapy cut-off point was determined by Cox regression analysis to determine the cumulative dose with the highest hazard ratios by considering significance. Based on the analysis, the cut-off points for Mtx and doxorubicin were 3000 mg/m^2 and 90 mg/m^2, respectively.

Univariate analysis was conducted to assess the effect of Mtx or doxorubicin cumulative doses and other variables on SOD levels, using Cox regression to obtain hazard ratios (**Table 2**).

Discussion

In our study, the mean age of subjects was 76.95 (SD 44.90) months. This result was consistent with a previous study that showed the mean age of childhood leukemia to be 2 to 15 years with a peak at 2 to 4 years.[2] Males comprised 55% and females 45% of our subjects. Similarly, a previous study reported 55% of their subjects to be male and 45% to be female.[1] Most subjects had good nutritional status (82.5%), followed by wasted (10%), and overweight (7.5%). None of the 40 subjects were severely wasted. In contrast, another study reported 62.5% of their subjects to have malnutrition (wasted and severely wasted) and 31% to have good nutrition.[8] For smoking status, 57.5% of our subjects were passive smokers, exposed to between 5 and 20 cigarettes per day.

Statistical analysis revealed that Mtx cumulative doses of ≥ 3000 mg/m^2 had a hazard ratio of 9.96 (95%CI 2.819 to 35.183; P<0.0001), suggesting that these Mtx doses decreased SOD levels 9.96 times compared to Mtx doses of <3000 mg/m^2, a statistically significant result. This result was consistent with previous research that longer duration of Mtx treatment decreased the SOD levels in mice, assuming that longer period of Mtx treatment equates to greater cumulative doses (P=0.05).[5] Asci *et al.*[9] also mentioned that Mtx can decrease SOD levels in mice (P=0.05). Methotrexate stimulates polymorphonuclear (PMN) cells to release H_2O_2 and other free radicals,[5] as well as reduces the effectiveness of antioxidants to fight free radicals and reduces the availability of NADPH used by glutathione-reductase to maintain glutathione levels. Decreased glutathione-reductase causes the PMN to be more sensitive to oxidative stress, and ultimately will decrease the body's antioxidant levels, including SOD.[10]

On the other hand, cumulative doxorubicin doses of ≥90 mg/m^2 had a hazard ratio of 0.59 (95%CI 0.194 to 1.765; P=0.34). This finding indicates that these doses led to 0.59 times lower risk of decreased SOD levels compared to cumulative doxorubicin doses of <90 mg/m^2. However, this outcome was not statistically significant, nor was it consistent with a study by Akreathy *et al.*[11] which examined cardiotoxicity caused by doxorubicin in mice. They found that doxorubicin reduced total serum antioxidant levels, increasing cardiotoxicity (P<0.05). Furthermore, breast cancer patients treated with doxorubicin had reduced SOD levels (P<0.01).[12] Decreased antioxidant levels are due to the doxorubicin sugar moiety, which has a ring attached to the tetracycline-containing quinone structure. Doxorubicin may compete with coenzyme Q10, which also has a quinone structure as an electron acceptor, resulting in free radical production. Doxorubicin can form semiquinone radical intermediates, which react with oxygen to produce superoxide anion radicals, leading to the production of hydrogen peroxide and hydroxyl radicals.[13,14] Antioxidant such as SOD is needed to neutralize free radical. The more free radical is formed, the more antioxidant is used, finally can decrease antioxidant level.

Passive smokers had a hazard ratio of 0.96 (95%CI:0.370 to 2.496; P=0.93). This result shows that the risk of decreased SOD levels was 0.9 times lower in passive smokers than in children who were not exposed to passive smoking, but the result was not statistically significant. A study reported increased SOD levels in passive smokers compared to non-smokers (P>0.05).[15] Tobacco smoke contains numerous compounds, many of which are oxidants and prooxidants, capable of producing free radicals and enhancing oxidative stress. Passive smokers have higher levels of oxidant.

Malnutrition had a hazard ratio of 1.06 (95%CI 0.094 to 11.864; P=0.96), suggesting a 1.06 times greater risk of decreased SOD levels compared to those with good nutrition, but the result was not statistically significant. Children with malnutrition have decreased antioxidant levels. Many factors may influence antioxidant levels, such as low serum zinc, vitamins A and C, and other micronutrients in the body, leading

to greater cellular sensitivity to oxidative stress and decreased SOD levels.[16]

Univariate analysis revealed that significantly more subjects who received a cumulative Mtx dose of ≥ 3000 mg/m^2 had low SOD levels, compared to those with high SOD levels.

In conclusion, there is a significant relationship between higher cumulative doses of Mtx and decreased SOD levels. A cumulative Mtx dose of ≥3000 mg/m^2 has a 9.96 times higher risk of decreased SOD levels compared to cumulative doses <3000 mg/m^2. There are no significant associations between higher cumulative doxorubicin dose, nutritional status, or passive smoking and decreased SOD levels.

Conflict of interest

None declared.

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