**ABSTRACT**

**Introduction:** High-dose Methotrexate (HD-MTX), a chemotherapy agent for acute lymphoblastic leukemia, is a cytotoxic agent for some organs, including kidneys. One of the most important toxicities due to HD-MTX is acute kidney injury due to Methotrexate crystallization in renal tubules. The prevalence of Acute kidney injury due to HD-MTX administration in pediatric ALL in dr. Soetomo General Hospital was unknown. The study aims to analyze renal function features in pediatric ALL during HD-MTX chemotherapy.

**Methods:** An analytical observational study with a prospective approach was conducted at Dr. Soetomo General Hospital Surabaya from December 2021 - July 2022. The subjects were ALL children aged 1-18 years who met inclusion and exclusion criteria. High-dose Methotrexate chemotherapy was given 3 times every 2 weeks during the consolidation phase. Laboratory examinations were performed before and after HD-MTX chemotherapy. Laboratory results were recorded to determine the GFR value. The difference test was performed using Wilcoxon signed rank test and the Friedman test with a significance value of p<0.05.

**Results:** A total of 20 subjects, the median age was 78 months old, and boys and girls were equal. Standard Risk of ALL was 2, and 36% stage 3. The prevalence of AKI in ALL children after HD-MTX chemotherapy reached 5% of 180 ALL children in 2010-2014 to 26.5% of 336 ALL children. Research on 78 adults with AKI due to HD-MTX administration consisted of 25% stage 1 AKI, 38% stage 2, and 36% stage 3. The prevalence of AKI due to HD-MTX administration at Dr. Soetomo General Hospital Surabaya was unknown. High-dose Methotrexate-induced nephrotoxicity decreases Methotrexate excretion and increases plasma methotrexate concentrations. This causes toxicity to organs and reduces the effectiveness of leucovorin. Impaired renal function affects morbidity and mortality including due to HD-MTX administration. The prevalence of AKI in ALL children after HD-MTX chemotherapy reached 5% of 180 ALL children in 2010-2014 to 26.5% of 336 ALL children.

**Conclusions:** We concluded there was no AKI due to HD-MTX chemotherapy in pediatric ALL. It may be caused by providing adequate hydration, urine alkalinization, and leucovorin rescue.

**Keywords:** Acute Lymphoblastic Leukemia, High Dose Methotrexate, Acute Kidney Injury.


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**INTRODUCTION**

Acute lymphoblastic leukemia (ALL) occurs in children and adults and requires combination chemotherapy. High-dose Methotrexate (HD-MTX) prevents the spread of cancer cells to the central nervous system. However, high-dose Methotrexate, a dose of 500 mg/m$^2$ or more given intravenously, is a cytotoxic agent to some organs, including kidneys. One of the most important toxicities due to HD-MTX is AKI due to Methotrexate crystallization in renal tubules. High-dose Methotrexate-induced nephrotoxicity causes a decrease in methotrexate excretion and an increase in plasma methotrexate concentrations. This causes toxicity to other organs and reduces the effectiveness of leucovorin. Impaired renal function affects morbidity and mortality including due to HD-MTX administration. The prevalence of AKI in ALL children after HD-MTX chemotherapy reached 5% of 180 ALL children in 2010-2014 to 26.5% of 336 ALL children.

Data collection in Pediatric Hematology and Oncology ward, Dr. Soetomo Hospital Surabaya. The Ethics Committee approved this research of Dr. Soetomo General Hospital Surabaya No. 0322/KEPK/XII/2021 on December 2021. Children with ALL aged 1-18
years who were in consolidation phase chemotherapy according to the 2018 Indonesian Childhood ALL Protocol were included. Exclusion criteria were children with previous or known urogenital system disorders at the time of the study. The sample size formula calculated the sample size with a minimum sample was 17 ALL children. Demographic data and risk stratification were evaluated. ALL diagnosis was established by signs, symptoms, and bone marrow aspiration tests.

**High Dose Methotrexate Regimens**
All patients received 1000 mg/m² Methotrexate in 3 cycles every 2 weeks in the consolidation phase according to the ALL Indonesian Chemotherapy Protocol in 2018. Before HD-MTX had been given, all patients received hydration and urine alkalinization with 1000 ml/m² of D5 ½ saline infusion for 12 hours, added by 25 ml 8.4% sodium bicarbonate diluted into 500 ml of fluid then followed by HD-MTX administration. Methotrexate was dissolved in 0.9% NaCl 500-1500 ml for 24 hours. The patients were then given hydration by 2000 ml/m² of D5 ½ saline infusion for 24 hours after HD-MTX administration. Leucovorin rescue was given at a dose of 10 mg/m² every 6 hours after finished hydration.

**Laboratory examination**
Peripheral blood without anticoagulant as much as 3 ml was performed 24 hours before and 48 hours after HD-MTX administration, especially albumin, urine pH, Blood urea nitrogen (BUN), and creatinine serum. Urine pH was measured using a urine sample examined no more than 2 hours after micturition using the Combur-Test® strip reagent (Roche Diagnostics, Rotkreuz, Switzerland). Serum albumin was examined by the Bromocresol Green method. Blood urea nitrogen examination was performed using the GLDH enzymatic method and Jaffe compensated method for Serum creatinine examination.

**GFR measurement**
The GFR value was obtained by calculating the constant x height/serum creatinine. The constant for 2-12 yo: 0.55, boys aged 13-21 yo: 0.70, and girls aged 13-21: 0.55. Normal GFR values for 1-6 months: 39-114; 6-12 mo: 49-157; 12-19 mo: 62-191; 2-5 yo: 102.5-150.5; 5-12 yo: 96.5-136.9. Acute Kidney Injury occurs when there is a decrease in GFR normal value according to age caused by increased serum creatinine.

**Statistical analysis**
The Shapiro-Wilk test analyzed normality data. The difference in laboratory parameters was performed using Wilcoxon signed rank test and the Friedman test. Software SPSS analyzed data for windows version 18.00.

**RESULTS**
The subjects aged 1-10 years were more than subjects aged >10 years, and boys were equal to girls. The body mass index of the research subjects had a median of 15.4 (13-21.1) kg/m². Based on the prognostic classification, more subjects with standard risk were than high-risk subjects. Data characteristics of research subjects can be seen in table 1.

Table 2 shows serial measurements of albumin level, pH urine, and GFR in each cycle. In each cycle, there was no significant difference in albumin levels before and after HD-MTX chemotherapy. There was no significant change in GFR value before and after HD-MTX chemotherapy in each cycle. Urine pH was measured only before HD-MTX chemotherapy.

Based on table 3 there was no decreased GFR in 3 cycles. Median GFR in each cycle was 257.5; 243.5; 228.5, with a minimum GFR of 119 ml/min/1.73 m² and a maximum GFR was 638 ml/min/1.73 m². The result show median GFR in all cycles was >175 ml/min/m² (hyperfiltration).

Based on table 4, there were no significant differences between normal albumin levels and hypoalbuminemia both before and after chemotherapy in three cycles. All the patients were in normal urine pH. There was no subject with AKI in each chemotherapy cycle. However, all cycles had no significant difference between before and after chemotherapy.

Based on table 5, there was no significant difference between albumin, BUN, creatinine serum, and GFR values before the first cycle of chemotherapy and after the third cycle. Before the first cycle of chemotherapy indicates the start of Methotrexate administration and after the third cycle indicates the end of Methotrexate chemotherapy. The mean of GFR before the first cycle of chemotherapy did not change significantly compared to GFR after the third cycle, with a p-value =0.52.

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<th>Table 1. Subject characteristics.</th>
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DISCUSSION

The study differed from previous studies by Pang and Wang, stating AKI-induced HD-MTX occurred in 5% of 180 ALL children and 26.5% of 336 ALL children. This study reported no children who had AKI after HD-MTX administration. It may be caused by providing adequate hydration, urine alkalinization, and leucovorin rescue.

Hydration and urine alkalinization before and hydration after HD-MTX administration is standard procedure in Pediatric Hematology and Oncology ward dr. Soetomo General Hospital. Hydration and urine alkalinization with sodium bicarbonate can prevent MTX crystallization in the urine. Adequate hydration is highly recommended in children with cancer receiving HD-MTX chemotherapy, especially in developing countries where it is not possible to check serial Methotrexate serum. Leucovorin, an active metabolite of folic acid and an essential coenzyme for nucleic acid synthesis, is to neutralize the side effects of HD-MTX. Methotrexate inhibits nucleic acid synthesis by blocking folic acid activation, whereas leucovorin is folic acid in its active form. Leucovorin was administered 24 hours after methotrexate, so it did not interfere therapeutic effect of methotrexate.

In this study, there was no difference in laboratory parameters before the first cycle of chemotherapy, such as albumin, BUN, and serum creatinine, and after the third cycle of chemotherapy. This indicates that HD-MTX chemotherapy is safe to give when accompanied by adequate hydration, urine alkalinization, and administration of leucovorin. According to the previous study, HD-MTX chemotherapy may be safely administered to the patient when combined with hydration, urine alkalinization, and leucovorin rescue.

The doses of HDMTX are considered to influence renal impairment. Methotrexate doses in the previous study were 2 grams/m² for the standard risk group and 5 grams/m² for the high-risk group, and 3 grams/m² for all risk groups. The doses were 2 times greater than our subjects received. Pang stated children who were given higher doses of Methotrexate were more likely to have AKI.

In this study, the lowest GFR value was 119 ml/min/1.73m², the value was still in the normal range according to the age, and the highest GFR value was 638 ml/min/1.73m². The high GFR values in our subjects could be due to leukemia. Kim et al. said patients with cancer might develop glomerular hyperfiltration due to increased protein load due to the breakdown of tumor cells. According to Hjorth’s study found that 31% of 131 patients with cancer had GFR values >175 ml/min/1.73m². The same with Kwatra’s results that 43.5% of 177 pediatrics with cancer had GFR 160 ml/min/1.73m². Kwarta said hyperfiltration in cancer patients is associated with a large tumor burden and an increased solute load to the kidney due to tumor damage.

CONCLUSION

All children with ALL who received HDMTX chemotherapy had a normal renal function. Most of the children were in hyperfiltration with GFR > 175 ml/min/1.73 m². This study did not find acute kidney injury either before or after chemotherapy in all cycles. Hydration, urinary alkalinization, and leucovorin rescue before HD-MTX chemotherapy may be safely administered to the patient.
rescue play an important role in preventing AKI due to HD-MTX chemotherapy.

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CONFLICT OF INTEREST
The authors declare there is no conflict of interest in this study.

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AUTHOR CONTRIBUTION
All authors contributed equally in conducting the study as well as writing and revising the manuscript.

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