N-acetylcysteine vs placebo as adjunctive treatment in paediatric leukaemia: A single blind, randomized controlled trial

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Abstract

Background: Acute lymphoblastic leukaemia (ALL) accounts for about 25% of all cancer types in children. Many chemotherapeutic agents have been shown to be related to increased oxidative stress and hepatotoxicity which affect the survival rate. N-acetylcysteine (NAC) has been used as adjunctive treatment in malignancy.

Objectives: To analyse the effect of NAC administration towards blood oxidant, transaminase enzyme, and bilirubin level in ALL children undergoing induction phase of chemotherapy.

Method: This is a single-blind placebo-controlled randomized clinical trial carried out from August to December 2020 in Cipto Mangunkusumo National Hospital, Jakarta, Indonesia. Subjects were randomized consecutively into 2 groups treated with NAC or placebo. Inclusion criteria were newly diagnosed ALL-standard risk (SR) children already diagnosed with liver dysfunction, and already taken antioxidants. Data were analysed using SPSS 21. Normality test was conducted followed by data analysis with unpaired t-test.

Results: A total of 18 subjects were included in study and 2 of them were excluded. Malondialdehyde levels showed an increase of 0.17nmoL in the NAC group and a decrease of 0.19nmoL in the placebo group. Aspartate transaminase (AST) level increased in mean of 11.40 U/L in the NAC group compared to placebo group 5.67 U/L. Alanine transaminase (ALT) level in the NAC group showed an increase in mean of 23.24 U/L compared to placebo group which showed a decrease in mean of 3.5 U/L. Bilirubin level in the NAC group showed an increase of 0.7 mg/dL compared to placebo group 0.17 mg/dL. There was no significant difference in MDA levels, AST, ALT, and bilirubin levels in the 2 groups (p=0.186; p=0.638; p=0.164; p=0.352, respectively).

Conclusions: Higher malondialdehyde level was shown as a trend in subjects in both groups before chemotherapy was done. N-acetyl cysteine administration showed no significant difference in reducing MDA levels, transaminase enzymes, and bilirubin levels in ALL-SR patients compared to placebo.

(Key words: Acute lymphoblastic leukaemia, Liver dysfunction, Malondialdehyde, N-acetylcysteine, Oxidative stress, Randomized clinical trial)

Background

Acute lymphoblastic leukaemia (ALL) accounts for 25% of cancer types in children⁴. ALL has an incidence rate of 10-45 per 1,000,000 children per year and a cumulative risk of 1 in 2000 up to the age of 15 years⁵. Multidrug regimen chemotherapy improved long-term outcome of children with ALL with survival rates above 90%; 5-year survival rate of ALL has reached 88.6% with 10-year survival rate 85.5% in developed countries⁶. Oxidative stress (OS) results when there is an imbalance between the generation of reactive oxygen species (ROS) and response from the antioxidant defence system. Many chemotherapeutic agents have been shown to exert their biologic activity through induction of OS in affected cells during cancer therapy⁷. Decomposition of lipid peroxidation product, including malondialdehyde (MDA) is considered as a marker for oxidative stress in the body⁸.

Hepatotoxic complications such as increased transaminases and cholestasis were commonly found at the beginning of the induction phase of chemotherapy and when administering drugs such as 6-mercaptopurine (6-MP), methotrexate (MTX), cytarabine, and L-asparaginase⁹,¹⁰. Ariawati K, et al² study of 41 ALL patients who underwent chemotherapy were found to have highest elevated transaminase enzyme, 7-12 times above normal range in induction phase when MTX, cytarabine, vincristine, and steroid were administered. Joshi M,
et al study found 1-2% patients had elevated bilirubin level caused by anthracycline administration. These complications can affect chemotherapy and cause stoppage of treatment, prolonged length of stay, and affect outcome and prognosis.

Mortality rate of leukaemia patients after 5 weeks of therapy were higher in patients with high MDA level. Administration of antioxidants such as vitamin E and N-acetylcysteine (NAC) in ALL children who underwent chemotherapy were related to decrease in hepatotoxicity, haematology complication, need for blood transfusions, and decrease in MDA levels. NAC was used as mucolytic agent in respiratory disease and also as an antidote for hepatotoxicity caused by acetaminophen administration; however, lately NAC has been used as adjunctive treatment in malignancy. Eroglu N, et al study showed significant decreased of ALT and gamma glutamyl-transferase (GGT) values in subjects who received NAC on the 1st, 3rd, 5th, and 7th day of chemotherapy compared to those who did not received NAC (p <0.05).

Objectives
The aim of the study was to analyse the effect of NAC administration on oxidant level, transaminase level, and bilirubin level in ALL patients undergoing induction phase of chemotherapy compared to placebo group.

Method
The current study was a randomized single-blind placebo-controlled clinical trial carried out from August to December 2020 in Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.

Sample size: This was 18 newly diagnosed ALL-standard risk (ALL-SR) children in each group using the numerical unpaired two group data analysis formula. Sample size was calculated using the formula of numerical analytic for 2 unpaired group shown below.

\[ n1 = n2 = \frac{(Z_\alpha + Z_\beta)S^2}{X_1 - X_2} \]

Calculation showed the sample size of each group was 16. A 10% drop-out rate was added to the sample size, resulting in a sample size of 18 for each group.

Newly diagnosed children with ALL-SR who will start the induction phase of chemotherapy in Cipto Mangunkusumo Hospital from August to December 2020 were recruited. Figure 1 is a flowchart of the study.

![Flowchart of study protocol](image)
Inclusion criteria: Newly diagnosed ALL-SR children undergoing the induction phase of chemotherapy from 1st week until 6th week, and whose parents agreed to participate in the study and signed the informed consent.

Exclusion criteria: Subjects with allergy or contraindicated to consuming NAC, subjects with liver dysfunction based on clinical and laboratory tests diagnosed before joining the study, and subjects who already consume other antioxidants (vitamin A, vitamin C, and vitamin E).

Subjects were randomized consecutively and distributed into two groups. Randomization was done by a third person to decide the distribution of the subjects based on the serial randomization number. Subjects in treatment group were given capsules containing 600 mg of NAC consumed once a day and subjects in the control group were given capsules containing placebo (600mg of lactose) consumed once a day as the adjunctive treatment during the 6-week induction phase of chemotherapy. Both medicines were prepared by the Installation of Pharmacy in Cipto Mangunkusumo National Hospital with the same type and same colour of capsules. NAC was administered during the induction phase of chemotherapy starting in the first week until 6 weeks. This study analysed the change of MDA levels, transaminase levels and bilirubin levels before and after the 6th week of the induction phase of chemotherapy.

MDA levels were examined in the biochemistry laboratory of the Faculty of Medicine, Universitas Indonesia using Wills method/thiobarbiturate acid reaction substance (TBARS). Transaminase enzymes and bilirubin tests were evaluated in the pathology clinic laboratory in Cipto Mangunkusumo Hospital. ALT levels exceeding three times the upper limit of normal value and total bilirubin concentrations greater than twice the upper limit, were defined as clinically significant abnormalities on liver tests.

Ethical issues: Approval for the study was obtained from the Research Ethic Committee of Faculty of Medicine Universitas Indonesia with protocol number KET-793/UN2.F1/ETIK/PPM.00.02/2019 in 15th July, 2019. Clinical trial registration number is NCT05611086. Written informed consent was obtained from the parents of the study participants.

Statistical analysis: Data were analysed with IBM Statistical Package for the Social Sciences (SPSS) Statistics 21.00 (IBM Corporation; Armonk USA). Normality test was conducted to analyse data distribution. Normally distributed data was analysed using unpaired t-test. Data not normally distributed were analysed using Mann-Whitney test. The expected result is the mean difference and a p value <0.05 was considered significant.

Results
A total of 18 subjects was included during the study period. Two subjects were excluded from the study (one subject from the NAC group was excluded because chemotherapy protocol was switched from ALL-SR to ALL-high risk (HR) protocol and one subject from the placebo group was excluded because the chemotherapy was done after the subject had remission in the first week of therapy). The study was stopped before reaching the target sample size because of the limitation in time period and placebo-making regulation in the Cipto Mangunkusumo Hospital Pharmacy Installation.

Table 1 shows the baseline characteristics of the subjects. The male: female ratio was 2.2:1. The youngest subject was 1 year and 3 months old and the oldest was 7 years 10 months old with a median age of 3 years 6 months.

Table 2 shows the laboratory test results before and after the induction phase of chemotherapy. The MDA level before the chemotherapy was started showed a significant difference between the NAC group and placebo group.

Table 1: Baseline characteristics of the subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N-acetylcysteine (n=8)</th>
<th>Placebo (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months): Mean ± SD</td>
<td>44.25 ± 28.26</td>
<td>48.63 ± 18.85</td>
</tr>
<tr>
<td>Sex: n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>06</td>
<td>05</td>
</tr>
<tr>
<td>Female</td>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td>Haemoglobin (g/dl): n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤7</td>
<td>04</td>
<td>05</td>
</tr>
<tr>
<td>&gt;7</td>
<td>04</td>
<td>03</td>
</tr>
<tr>
<td>Total leucocyte count/cu mm: n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10,000</td>
<td>06</td>
<td>03</td>
</tr>
<tr>
<td>10,000-50,000</td>
<td>02</td>
<td>05</td>
</tr>
</tbody>
</table>

The MDA level was found to be high before the induction phase of chemotherapy in both groups. MDA levels evaluated after chemotherapy showed an increase of 0.17nmol/L in the NAC group and a decrease of 0.19nmol/L in the placebo group. The
difference of MDA levels before and after the induction phase of chemotherapy was found to be higher in the placebo group compared to the NAC group but had no statistically significant difference.

**Table 2: Laboratory tests results before and after the induction phase of chemotherapy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>p (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (MDA)</td>
<td>NAC mean (SD)</td>
<td>0.001 (-0.67 – -0.23)</td>
</tr>
<tr>
<td></td>
<td>Placebo mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.98 (SD 0.21)</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>1.21 (SD 0.38)</td>
<td>0.944 (-0.39–0.37)</td>
</tr>
<tr>
<td>Delta</td>
<td>0.17 (SD 0.52)</td>
<td>0.186 (-0.20–0.93)</td>
</tr>
<tr>
<td>Aspartate transaminase (AST)</td>
<td>Before</td>
<td>0.355 (-15.03–5.79)</td>
</tr>
<tr>
<td></td>
<td>24.53 (SD 7.28)</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>35.43 (SD 15.57)</td>
<td>0.693 (-13.13–19.13)</td>
</tr>
<tr>
<td>Delta</td>
<td>11.40 (SD 21.42)</td>
<td>0.638 (-20.38–31.84)</td>
</tr>
<tr>
<td>Alanine transaminase (ALT)</td>
<td>Before</td>
<td>0.266 (-45.42–13.63)</td>
</tr>
<tr>
<td></td>
<td>20.67 (SD 13.95)</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>46.29 (SD 26.17)</td>
<td>0.744 (-22.89–31.18)</td>
</tr>
<tr>
<td>Delta</td>
<td>24.23 (SD 27.23)</td>
<td>0.164 (-13.16–68.62)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Before</td>
<td>0.388 (-0.33–0.14)</td>
</tr>
<tr>
<td></td>
<td>0.22 (SD 0.18)</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>0.28 (SD 0.17)</td>
<td>0.169 (-0.42–0.08)</td>
</tr>
<tr>
<td>Delta</td>
<td>0.07 (SD 0.11)</td>
<td>0.352 (-0.33–0.13)</td>
</tr>
</tbody>
</table>

Three among 7 subjects in the NAC group had a decreased MDA levels after the induction phase of chemotherapy compared to 4 among 7 subjects in the placebo group as shown in Figure 2.

AST level in the NAC group showed an increase in mean of 11.40 U/L and placebo group showed an increase in mean of 5.67 U/L (p = 0.638; 95% CI -20.38–31.84). After the 5th week of induction phase, there was an increase in mean level of AST 1.4 times. AST levels in both groups were still in normal range as shown in Figure 3.

ALT level in the NAC group showed an increase in mean of 23.24 U/L compared to placebo group which showed a decrease in mean of 3.5 U/L (p = 0.164; 95% CI -13.16–68.62). There was a 2-fold
increase of mean ALT level in subjects after chemotherapy induction. Increased mean ALT level in the 5th week of chemotherapy induction was found in the placebo group and increased in the NAC group seen after the induction phase of chemotherapy was done, as shown in Figure 4. ALT levels in both groups were still in the normal range after the induction of chemotherapy. However, there was no statistically significant difference between the two groups.

![Graph of ALT level before, in the 3rd week, 5th week, and after chemotherapy induction](image)

Bilirubin level in the NAC group showed an increase of 0.7 mg/dL compared to placebo group 0.17 mg/dL (p = 0.352; 95% CI -0.33–0.13). There was a 1.3-times increase in means of bilirubin before chemotherapy and after the induction phase of chemotherapy. Higher increase of means in bilirubin level was found in the placebo group compared to NAC group, shown in Figure 5, though there was no statistically significant difference between the two groups.

![Graph of bilirubin level before, in the 3rd week, 5th week, and after chemotherapy induction](image)

**Discussion**

This is the first study in Indonesia which analysed the effect of N-acetylcysteine administration on the MDA level, transaminase level and bilirubin level compared to placebo administration in children with ALL-SR who underwent the induction phase of chemotherapy. These children were given prednisolone, methotrexate, vincristine, daunorubicin, and L-asparaginase as the chemotherapy protocol.

Studies by Almeida MB, et al\(^1\), Al-Tonbary Y, et al\(^2\) and Pujari KN, et al\(^3\) showed that the MDA level was higher in ALL patients compared to normal subjects. This indicated that the increased lipid peroxides in ALL patients was the result of oxidative stress produced by free radicals.\(^5\) Our study found significantly high MDA levels in both the NAC and placebo groups (p=0.001; 95% CI -0.67 – -0.23). This may have resulted from oxidative stress produced in the body as the previous studies mentioned. Oxidative stress in our study tended to decrease after the induction phase of chemotherapy in comparison to the MDA level before chemotherapy, the placebo group showing a higher rate of decrease compared to the NAC group. This
was probably caused by the limited number of participants in the study. A study by Al-Tonbary Y, et al\textsuperscript{16} showed a significant decrease in MDA level after the induction phase of chemotherapy; however, in comparison between the treatment group (vitamin E and NAC) and the placebo group, the decrease of MDA level was not significant. A study by Singh V, et al\textsuperscript{17} reported significant decrease of MDA level between newly-diagnosed ALL patients and after the patients finished the chemotherapy. Increase of oxidative stress (increased MDA level) in ALL patients after ALL diagnosed positively correlated with increase in anti-apoptosis factors sFas-L and Bcl-2 which can initiate and promote ALL\textsuperscript{13}.

MDA levels in our study were higher before chemotherapy compared to after chemotherapy. This showed that oxidative stress already occurred before chemotherapy was given and was still produced during chemotherapy. A study by Battisti V, et al\textsuperscript{18} reported that MDA levels in subjects in remission before chemotherapy and during induction phase of chemotherapy were higher compared to subjects in control group and subjects in remission during the maintenance phase (p<0.05). The difference between our study and previous studies is that our study only evaluated MDA levels before and after chemotherapy and did not evaluate them in serial examinations.

Hepatotoxicity in chemotherapy is caused by methotrexate, cytosine arabinoside, L-asparaginase and vincristine. Evaluation the risk of hepatotoxicity in the patients can be done by liver function examination, such as AST/ALT and bilirubin examination. In some instances, liver function tests may not indicate the severity of liver cell damage\textsuperscript{8}. Increases in AST/ALT levels after the induction phase of chemotherapy were seen in both groups; however, NAC group showed an increase in transaminases which was higher compared to placebo group even though those results were still in the normal range. Al-Tonbary Y, et al\textsuperscript{16} study showed increased AST/ALT enzyme level in subjects after chemotherapy, but hepatotoxicity incidence was lower in the treatment (NAC and vitamin E) group. Our study showed different results from other studies. This difference may have resulted from the limited participants and probably because the chemotherapy protocol used in this study was already proved to be safe for liver function. In our study, the AST level during chemotherapy showed similar results compared to AST level before chemotherapy. A study by Ariawati K, et al\textsuperscript{8} reported similar results in AST level before and after chemotherapy. In our study, during chemotherapy, 3 (27.3%) subjects had increased AST levels (≥2 times increased); this result was similar to Denton CC, et al\textsuperscript{19} who reported hepatotoxicity in 25% of the patients during the induction phase of chemotherapy. This was also similar to the study by Ariawati K, et al\textsuperscript{8} which reported 2 times increase in AST levels during chemotherapy compared to before chemotherapy. Ariawati K, et al\textsuperscript{8} study reported AST/ALT level was highest in the induction phase after the second time of chemotherapy administration. In our study, highest ALT/AST level was found in the third and fifth week of the induction phase of chemotherapy. This difference may be due to different chemotherapy protocols used. Our study was using Indonesian Protocol ALL Standard Risk 2013 (year 2015) as chemotherapy protocol. In the third week of the induction phase, daunorubicin was administered and in the fifth week L-asparaginase was administered. Both chemotherapy regimens increased the risk of hepatotoxicity. L-asparaginase, vincristine, and daunorubicin needs to metabolically detoxified in the liver, therefore liver dysfunction will affect the risk of hepatotoxicity\textsuperscript{20}. Increase in mean AST/ALT level in both groups in our study was not clinically significant since the results were still in the normal range. Our study showed an increase in bilirubin levels in both groups, 0.07 mg/dL in the NAC group and 0.17 mg/dL in the placebo group. There was no cholestasis found in the participants. Ariawati K, et al\textsuperscript{8} study reported similar results with no significant difference between bilirubin level before and during chemotherapy. Hashmi SK, et al\textsuperscript{21} reported conjugated hyperbilirubinaemia found in patients with older age, Hispanic, and received 4 types of medicines given during the induction phase of chemotherapy, including anthracyclines. The study also showed a predictor factor of increased bilirubin level was related to IMT ≥95\textsuperscript{th} percentile. Our result in this study was different than previous studies because of different characteristics, younger age and Asian.

One limitation of this study was that most of the subjects were recruited in outpatient clinics and therefore the compliance of taking medicines could not be evaluated. We did remind the parents to administer the medicine in the correct dose. Parents were also given the chance to consult or report their child’s condition by phone or personal messages. Further investigations with larger sample size are needed including serial MDA level evaluation during chemotherapy.

Conclusions

Higher MDA level was shown as a trend in subjects in both groups before chemotherapy. Decreases of MDA level before and after chemotherapy did not significantly differ in the 2 groups. Liver transaminase and bilirubin levels in both groups were still in the normal range; however, this study found ≥2 times increased in ALT levels after chemotherapy induction in one of the subjects.
There was no significant benefit of N-acetyl cysteine administration found in this study.

References


