The impact of homocysteine level on methotrexate induced neurotoxicity in children treated with St. Jude total XV acute lymphoblastic leukemia protocol

Wael Zekri1, Mohamed Sedky Mahmoud Sedky2, Mona Khalifa3, Sanaa Kenawy4

1Department Pediatrics Oncology, National Cancer Institute, Cairo, Egypt
2Department of Pediatrics, National Research Centre, Guiza, Egypt
3Department of Pharmacy, National Cancer Institute Cairo University, Cairo, Egypt
4Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

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Original Article

Abstract

**Purpose:** Methotrexate (MTX) is an antimitabolite that is routinely used in the treatment of hematological malignancies and during its metabolism leads to hyperhomocysteinemia that is associated with neurotoxicity. The purpose of this prospective study is to determine whether the increase in plasma homocysteine (Hcy) concentration is related to MTX-induced neurotoxicity. **Methods:** We investigated these changes for both newly diagnosed acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LL) pediatric patients treated at the National Cancer Institute, Egypt. They were treated according to St. Jude total XV protocol to receive 2.5 or 5 g/m\(^2\) MTX as a phase of consolidation and were selected between October 2009 and January 2010. **Results:** Twenty-nine patients were analyzed, M/F: 20/9, the mean age was 8 +/- 4.4 years. Hcy level above 15 µmol/L was considered positive. Hcy levels mean at diagnosis, pre 1\(^{\text{st}}\) HD MTX, post 1\(^{\text{st}}\) HDMTX, Pre 2\(^{\text{nd}}\) HDMTX, Post 2\(^{\text{nd}}\) HDMTX were 12.10 µmol/L ± 4.17, 6.90 µmol/L ± 3.02, 17.59 µmol/L ± 6.00, 7.21 µmol/L ± 2.73 and 13.74 µmol/L ± 4.75 respectively. Seventeen patients (58%) had features suggestive of neurotoxicity. Positive Hcy levels were associated with neurotoxicity \( p = 0.05 \), higher HDMTX 5 g/m\(^2\) \( P = 0.023 \). A highly significant relation was found between initial Hcy level at diagnosis and final Hcy level \( p = 0.001 \); the same as between Hcy level Post 1\(^{\text{st}}\) HDMTX and that Post 2\(^{\text{nd}}\) HDMTX with \( p = 0.006 \). **Conclusion:** Plasma Hcy concentration was significantly elevated after HDMTX administration and this elevation is associated with the observed neurotoxicity. Whether the elevation in Hcy concentration can prove an informative biomarker for neurotoxicity requires additional testing with other MTX regimens.

Keywords: Homocysteine; High Dose Methotrexate; Neurotoxicity; Acute Lymphoblastic Leukemia; Lymphoblastic Lymphoma

1. Introduction

Methotrexate (MTX) is a folic acid antagonist used since many years in the treatment of hematological malignancies. Its high dose (HD MTX) was prompted for the hematological, central nervous system (CNS) and testicular prophylaxis.\(^{1,6}\)

MTX is an inhibitor of dihydrofolate reductase (DHFR) resulting in a cellular depletion of tetrahydrofolates (THF) that are normally essential for the conversion of Homocysteine (Hcy) to methionine. This results into hyperhomocysteinemia\(^{7-10}\) that is associated with neurotoxicity\(^{11}\) and has been reported for years in the medical literature to present in acute, sub- acute or chronic syndromes\(^{12, 13}\). It may be transient and reversible but severe neurological disorders leading to coma or even death may occur as well.\(^{14-21}\)
In this study, assessment of the changes in Hcy concentration in plasma was done for newly diagnosed acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LL) pediatric patients treated with HDMTX. The purpose of the study is to determine whether the increase in plasma Hcy concentration is related to MTX-induced neurotoxicity.

2. Methods and Materials

Pediatric patients newly diagnosed as ALL or non-Hodgkin’s LL, treated at the National Cancer Institute, Cairo University, Egypt were the subject of this prospective study. They were treated according to St. Jude total XV protocol to receive > 1 g/m² MTX according to hematological and CNS initial status and disease stratification. They were selected between 1st of October 2009 and end of January 2010 to assess the correlation between the Hcy level and MTX induced neurotoxicity. The local institute ethical committee approved the study and an informed consent was obtained from the patients guardians prior to their inclusion in the study.

2.1 Consolidation treatment

After the patient has achieved remission induction following a 6-week chemotherapeutic treatment including triple intrathecal therapy22, consolidation consisted of HDMTX given every 2 weeks. The HD MTX (5 g/m² or 2.5 g/m² over 24 hours whether standard/high-risk or low-risk – LR- arms respectively), was given the 1st day in association with the same triple intrathecal therapy including aracytine (Arac), hydrocortisone (HC) and MTX (Table 1). Folinic acid rescue was given intravenously at a dose of 15 mg/m² for standard/high-risk patients or 10 mg/m² for low-risk patient. It started 42 hours later and was given every 6 hours with modifications according to a specific schedule.22 Oral 6-mercaptopurine (6MP), 50 mg/m²/day was associated for 8 weeks to all risk groups.

### Table 1: Details of consolidation phase of the protocol.

| Agent, dosage, and routes of administration | Schedule day of consolidation |
|--------------------------------------------|-------------------------------|
| HDMTX 5 g/m² for SR or HR patients, 2.5 g/m² for LR patients administered 24 hours IVI | D1,15,29 and 43 |
| 6-Mercaptopurine 50 mg/m²/day PO | D1 to 56 |
| TIT age dependent: MTX 8, 10, 12 mg, HC 16, 20, 24 mg, Arac 24, 30, 36 mg for ages 1 to 1.99, 2 to 2.99, and ≥ 3 year old respectively | D1,15,29,43 |

Arac: Aracytine; D: Day; HDMTX: High Dose Methotrexate; HR: High risk; HC: Hydrocortisone; IVI: IntraVenous Infusion; LR: Low Risk; MTX: Methotrexate; PO: Per Oral; SR: Standard Risk; TIT: Triple intrathecal.

For HDMTX, one tenth of the total HDMTX loading dose was given over one hour infusion and the remaining nine tenth of the dose were given via continuous infusion over 23 hours. Before HDMTX administration, IV prehydration crystalloid infusion (at 100 ml/m²/hour for low risk and 125 ml/m²/hour for standard/high risk) with sodium bicarbonate given over 12 hours. Patients started HD MTX if urine PH was ≥ 6.5.23

Folanic acid rescue was given at hour 42 from the start of HDMTX administration. The low dose group received 10 mg/m² of leucovorin every 6 hours for 5 doses, while the standard and high risk groups received 15 mg/m² every 6 hours for 5 doses. The folinic acid dose was adjusted if the methotrexate plasma concentration was ≥ 1 µM at hour 42 or ≥ 0.1 µM at hour 68.23

2.2 Homocysteine assay

Hcy measures were timed at diagnosis and relative to the HDMTX treatment (Figure 1). Blood (3 to 4 mL) was collected in EDTA tubes at diagnosis, immediately before 1st and 2nd high dose MTX and 42 hours after each of them but before leucovorin treatment. The samples were stored at -20°C till time of analysis. Homocysteine was estimated using the Hcy enzyme immunoassay (EIA) kit (Axis-shield, UK) according to the method described by Frantzen et al. 1998.24

![Figure 1: Timing of homocysteine level evaluation.](https://example.com/fig1)

Any increase in Hcy level above 15 µmol/L following at least one of the two HDMTX, the final Hcy value was considered positive. However, normal levels (5 – 15 µmol/L) were considered negative when Hcy level after both 1st and 2nd HDMTX were negative.

2.3 Clinical neurotoxicity

Neurotoxicity was evaluated from the results of clinical neurological examination. This was carried out initially at diagnosis and every other week coinciding with HDMTX during the consolidation phase. The target was to elicit any abnormal cognitive, motor or sensory findings. Moreover to detect any increase in intracranial tension in the form of headache, vomiting, blurring of
vision or signs of meningeal irritation and finally to find out any cranial nerve affection. These were assessed using a form based on common terminology criteria for adverse events version 4.0 CTCAE, national institutes of health, national cancer institute.\textsuperscript{25}

Neurotoxicity was evaluated to be positive and given a score I when was present within one day of methotrexate infusion and included one of the following: severe intractable vomiting > thrice not gastro intestinal tract (GIT) induced, headache, low activity, speech impairment, memory impairment, disturbed level of consciousness, convulsions, syncopal attack (not cardiovascular induced) and coma. In its absence the clinical score was zero.

2.4 Radiological neurotoxicity
Radiological neurotoxicity was evaluated from the results of brain magnetic resonance imaging (MRI) findings. A base line brain MRI was initially carried out at diagnosis and subsequently at the end of consolidation phase 2 weeks after the last HD MTX and intrathecal therapy. All cases were examined by using 1.5 Tesla superconducting MR imager (magnotom ESPREE 1.5 T, Erlangen, Siemens, Germany).

After intravenous administration of Gadolonium –DTPA 0.3 mg/kg contrasted enhanced T1W1 in axial, sagittal and coronal places were obtained.

Radiological methotrexate toxicity was to be considered positive and given a score I at the presence of one or more of the following: demyelination, necrotizing lesions, mineralizing microangiopathy, vascular complications, cerebral infarcts, leukencephalopathy, white matter lesions or atrophy. In its absence the score was zero.

If both clinical and radiological examination were of score zero, the MTX neurotoxicity net result was considered negative and given a grade of I. If at least one of both was score I, MTX neurotoxicity net result was considered positive and given a grade of II. If both were of score I, the MTX neurotoxicity net result was considered positive and given a grade of III.

2.5 Statistical methods
Data was analyzed using SPSS win statistical package version 20 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation. Comparisons between means where carried out using one-way analysis of variance (ANOVA) test followed by Dunnett test for multiple comparisons.

Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher’s exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using either student t-test or Mann-Whitney test (non-parametric t-test) as appropriate. Values of pre and post assessments were analyzed by paired t-test or wilcoxon signed rank test. \( P\)-value \( \leq 0.05 \) was considered significant.

3. Results
Out of 40 cases, 29 cases were newly diagnosed ALL or LL. Of them, 20 were males and 9 were females. Their age ranged from 2 to 17 with a mean of 8 \( \pm \) 4.4 years. Eleven patients were excluded from analysis as they did not complete the study. The patient’s characteristics are shown in Table 2 and 3.

3.1 Plasma Hcy concentrations at different check points
Regarding the final Hcy value, 18 cases (62\%) were with positive (above 15 \( \mu \)mol/L) values corresponding to increase Hcy levels after at least one of the two HDMTX cycles.

In this study the results of Hcy mean levels at diagnosis, pre first HD MTX, post first HDMTX, Pre second HDMTX, Post second HDMTX were 12.10 \( \mu \)mol/L \( \pm \) 4.17, 6.90 \( \mu \)mol/L \( \pm \) 3.02, 17.59 \( \mu \)mol/L \( \pm \) 6.00, 7.21 \( \mu \)mol/L \( \pm \) 2.73 and 13.74 \( \mu \)mol/L \( \pm \) 4.75 respectively as shown in (Figure 2).

Table 2: Patients demographic and clinical characteristics.

| Patient characteristics              | No. | (%) |
|--------------------------------------|-----|-----|
| Total number of patients             | 29  | 100 |
| Gender                               |     |     |
| Male                                 | 20  | 69  |
| Female                               | 9   | 31  |
| Age (years)                          |     |     |
| \( \leq 10 \)                         | 20  | 69  |
| \( >10 \)                            | 9   | 31  |
| Diagnosis                            |     |     |
| ALL SR                               | 18  | 62  |
| ALL LR                               | 9   | 31  |
| NHL                                  | 2   | 7   |
| MTX dose, mg/m\(^2\)                |     |     |
| 2500                                 | 9   | 31  |
| 5000                                 | 20  | 69  |
| Immunophenotype (Lineage)            |     |     |
| B-cell                               | 21  | 72.4|
| T-cell                               | 8   | 27.6|
| Risk group (Arm)                     |     |     |
| SR (ALL+NHLL)                        | 20  | 69  |
| LR                                   | 9   | 31  |
| CNS Status                           |     |     |
| CNS 1                                | 21  | 72.4|
| CNS 2                                | 6   | 20.7|
| CNS 3                                | 2   | 6.9 |
Table 3: Patients with clinical and radiological neurological assessment.

| UPN | Diagnosis | MTX dose g/m² | Age | CNS C. N. score | R. N. score | NNR grade | Blood sample Hcy in µmol/L | Initial Pre | Post | 1st MTX HD Pre | Post | 2nd MTX HD Pre | Post |
|-----|-----------|---------------|-----|----------------|-------------|-----------|----------------------------|------------|------|----------------|------|----------------|------|
| 1   | ALL SR    | 5             | 10  | 1              | 1           | 0         | II                        | 12.5       | 10.5 | 21.1           | 9.5  | 17.1           | 17.6 |
| 2   | NHL Tcell | 5             | 15  | 1              | 1           | 0         | II                        | 12         | 11   | 22.1           | 10.5 | 19.1           | 19.8 |
| 3   | NHL LL B  | 5             | 3   | 1              | 1           | 0         | II                        | 23.14      | 6    | 19             | 6.5  | 12.5           | 12.5 |
| 4   | ALL LR    | 2.5           | 4   | 1              | 1           | 0         | II                        | 12.5       | 8    | 21.5           | 9    | 15             | 15   |
| 5   | ALL LR    | 2.5           | 3   | II             | 1           | 0         | II                        | 7.5        | 5    | 9.5            | 6.5  | 8.5            | 8.5  |
| 6   | ALL SR    | 5             | 14  | II             | 1           | 0         | II                        | 8          | 5    | 11             | 4    | 7.5            | 7.5  |
| 7   | ALL LR    | 2.5           | 3   | II             | 1           | 0         | II                        | 8.5        | 6    | 16.5           | 7    | 11             | 11   |
| 8   | ALL SR    | 5             | 5   | 1              | 1           | 0         | II                        | 10         | 6    | 14.5           | 8.5  | 13             | 13   |
| 9   | ALL Tcell | 5             | 10  | 1              | 1           | 0         | II                        | 12.5       | 11   | 24             | 11   | 17             | 17   |
| 10  | ALL Tcell | 5             | 13  | 1              | 1           | 0         | II                        | 12.5       | 8    | 20             | 8.5  | 16             | 16   |
| 11  | ALL SR    | 5             | 17  | 1              | 1           | 0         | II                        | 14         | 12   | 34.5,36        | 11   | 22             | 22   |
| 12  | ALL LR    | 2.5           | 5   | 1              | 1           | 0         | II                        | 22.22      | 7    | 14             | 7.5  | 20.25          | 20.25 |
| 13  | ALL SR    | 5             | 9   | 1              | 1           | 1         | III                       | 14         | 12   | 24             | 12.5 | 18             | 18   |
| 14  | ALL SR    | 5             | 11  | II             | 1           | 1         | III                       | 12.5       | 5.5  | 14.5           | 6.5  | 15             | 15   |
| 15  | ALL SR    | 5             | 5   | 1              | 0           | 1         | II                        | 11         | 3.5  | 19             | 4.2  | 10             | 10   |
| 16  | ALL Tcell | 5             | 1   | 0              | 1           | 1         | II                        | 9          | 4    | 12.5           | 6.5  | 13             | 13   |
| 17  | ALL Tcell | 5             | 4   | III            | 0           | 1         | II                        | 12         | 6    | 16             | 7    | 12.5           | 12.5 |
| 18  | ALL Tcell | 5             | 5   | I              | 0           | 0         | I                         | 8          | 3.5  | 12.5           | 6.5  | 10             | 10   |
| 19  | ALL Tcell | 5             | 5   | I              | 0           | 0         | I                         | 9.5        | 4    | 12.5           | 3    | 7              | 7     |
| 20  | ALL LR    | 2.5           | 9   | 1              | 0           | 0         | I                         | 10.95      | 9.5  | 12.5           | 10.8 | 12.5           | 12.5 |
| 21  | ALL LR    | 2.5           | 7   | I              | 0           | 0         | I                         | 6.5        | 2.5  | 7.8            | 3    | 6              | 6     |
| 22  | ALL SR    | 5             | 15  | III            | 0           | 0         | I                         | 10.5       | 5.5  | 22             | 5.5  | 12             | 12   |
| 23  | ALL SR    | 5             | 2   | I              | 0           | 0         | I                         | 14         | 11   | 24             | 11.5 | 19             | 19   |
| 24  | ALL Tcell | 5             | 5   | I              | 0           | 0         | I                         | 11         | 3.5  | 12             | 4    | 8              | 8     |
| 25  | ALL SR    | 5             | 13  | I              | 0           | 0         | I                         | 16         | 11   | 24             | 11   | 18             | 18   |
| 26  | ALL LR    | 2.5           | 4   | I              | 0           | 0         | I                         | 12         | 4    | 21.5           | 5    | 16             | 16   |
| 27  | ALL LR    | 2.5           | 4   | I              | 0           | 0         | I                         | 5.215      | 9.5  | 16             | 7.5  | 18             | 18   |
| 28  | ALL LR    | 2.5           | 9   | I              | 0           | 0         | I                         | 11.5       | 6    | 22.5           | 5    | 13             | 13   |
| 29  | ALL SR    | 5             | 13  | II             | 0           | 0         | I                         | 7          | 4    | 9.5            | 3.5  | 7              | 7     |

MTX: Methotrexate; CNS: Central Nervous System; C.N: Clinical Neurotoxicity; R.N: Radiological Neurotoxicity; NNR: Neurotoxicity Net Result

*Significantly different from diagnosis (initial) value at $P \leq 0.05$, $\alpha$ significantly different from the corresponding pre administration value at $P \leq 0.05$.

3.2 Methotrexate-induced neurotoxicity

Neurotoxicity was assessed qualitatively as the aim was to correlate it with the Hcy level. After HDMTX administration, 17 (58%) patients had features suggestive of neurotoxicity with newly developed changes in either MRI findings or clinical neurological examination or both of them. Clinical neurological manifestations were shown in 14 patients where it was the solitary finding in 12 patients and associated to MRI changes in the remaining 2 patients. On the other hand MRI changes were shown in 5 patients where it was associated to clinical neurological findings in 2 patients and as a solitary finding in the remaining 3 patients Table 3.

3.3 Correlation between Hcy levels and various factors

A. Final positive Hcy: A highly significant correlation was found between final positive Hcy value (>15 µmol/L) whenever detected at least once in relation to HDMTX and the development of neurotoxicity ($p = 0.05$). The same was found between final positive Hcy value and the HD MTX dose whether 5 g/m² or 2.5 g/m² ($P =$...
A highly significant relation was found between final Hcy level and initial Hcy level as in 11 patients, the initial Hcy level was 9 µmol/L +/- 1.82 SD. This corresponded to negative final Hcy level, while in 18 patients; the initial level was 14 µmol/L +/- 4.09 SD. This corresponded to positive final Hcy level. 

Table 4: Correlation between the final Hcy level and various prognostic factors.

| MTX Neurotoxicity | Final Hcy value |
|-------------------|-----------------|
| No neurotoxicity  | Negative (5-15 µmol/L) | Positive >15 µmol/L |
| Presence of neurotoxicity | 9 (31%) | 13 (45%) |
| MTX Dose p=0.023 | 2.5 g/m² | 3 (10%) |
| 5 g/m² | 5 (17%) | 15 (52%) |
| Age p=0.41 | ≤ 10 years | 9 (31%) | 11 (38%) |
| > 10 years | 2 (7%) | 7 (24%) |
| CNS Status p=0.281 | I | 7 (24%) | 14 (48%) |
| II | 2 (7%) | 4 (14%) |
| III | 2 (7%) | 0 (0%) |

Table 5: Correlation between Hcy levels post 2nd and 1st HDMTX.

| MTX Neurotoxicity | Final Hcy value |
|-------------------|-----------------|
| Hcy level post 2nd HDMTX | Negative | Positive |
| Negative | 11 patients (38%) | 7 (24%) |
| Positive | 1 (3%) | 10 (34%) |

3.4 Correlation between neurotoxicity and various factors

No significant relation was found between MTX neurotoxicity and various factors including age, MTX dose, patients CNS status and initial Hcy levels. Table 6

Table 6: Correlation between neurotoxicity and various variables.

| MTX neurotoxicity | Negative | Positive |
|-------------------|----------|----------|
| Age (p=0.427) | ≤ 10 years | 11 (38%) | 9 (31%) |
| > 10 years | 3 (10%) | 6 (21%) |
| MTX Dose (p=0.7) | 2.5 g | 5 (17%) | 4 (14%) |
| 5 g | 9 (31%) | 11 (38%) |
| CNS Status (p=0.092) | I | 11 (38%) | 10 (34%) |
| II | 1 (3%) | 5 (17%) |
| III | 2 (7%) | 0 (0%) |

Moreover, there was no correlation between MTX neurotoxicity and initial Hcy levels, as 14 patients showed a mean of 11 µmol/L +/- 3.69 SD corresponding to negative neurotoxicity oppositely to 13 µmol/L +/- 4.54 SD corresponding to positive neurotoxicity in 15 patients.

4. Discussion

In our study, we found a statistically significant correlation between higher plasma Hcy levels and higher dose of MTX 5 g versus 2.5 g and patients with neurotoxicity either clinically or radiologically or both. Kishi et al. 2003, recorded a significant higher plasma Hcy levels among patients with seizures following HD MTX. The same as for an increased risk for encephalopathy after the administration of HD MTX and coinciding with higher MTX level at hour 42 and a higher Hcy concentration in the 1st HD MTX. Hyperhomocysteinemia is classified as mild (15-30 µmol/L), moderate (31-100 µmol/L) or severe (>100 µmol/L). However, the correlation between neurotoxicity and higher level of Hcy was not proved after the administration of MTX 1 or 3 gm/m².

In the current study, Hcy levels post 1st HDMTX were higher than their levels post 2nd HDMTX and both were higher than their corresponding levels prior to HDMTX. These levels prior to HDMTX were however lesser than those at diagnosis. This goes with the higher baseline Hcy level before therapy among our patients that might have probably reflected their disease burden and occasional folate deficiency. However, such baseline differences between risk groups were shown by the end of a 6-week remission induction therapy in other...
5. Conclusion

In conclusion, the results of the present study showed that plasma Hcy concentration was significantly elevated after HDMTX administration and this elevation is related to the observed neurotoxicity. Whether the elevation in Hcy concentration can prove an informative biomarker for neurotoxicity requires additional testing with alternative regimens of MTX.

Conflict of interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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