Serum Methotrexate Level and Side Effects of High Dose Methotrexate Infusion in Pediatric Patients with Acute Lymphoblastic Leukaemia (ALL)

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Abstract Methotrexate (MTX) is an extensively prescribed antimetabolite especially in the treatment of several pediatric cancers, though managing toxicities associated with methotrexate in high dose continues to be a challenge. A prospective study was carried out from April 2017 to October 2018. Children of either sex below 18 years at the time of enrolment and receiving high dose Methotrexate intravenous infusion over 24 h as a 2 g/m², 3 g/m² and 5 g/m² dose was included in the study. The serum methotrexate level was estimated after the start of 48 h HDMTX infusion by using the ARCHITECT methotrexate assay. Toxicity due to HDMTX was assessed by Common Terminology Criteria for Adverse Events v.5.0. A total of 244 HDMTX infusions were delivered to 62 ALL patients. From the total of 244 cycles, serum methotrexate level in 35 cycles after the start of 48 h HDMTX infusion was found to be ≥ 1.0 $\mu\text{mol/L}$ with reported toxicities among 31 cycles (88.6%). In 209 cycles MTX level was found to be less than 1.0 is statistically significant as compared to other cycles ($p < 0.0001$). Highest toxicities reported were in cycle I (38.8%). The toxicities such as oral mucositis,

neutropenia, the elevation of liver enzymes, dermatological toxicities were found more in cycles whose methotrexate level are greater than 1.0 $\mu\text{mol/L}$. Dose reduction, increased the length of stay and treatment delay occurred in patients with severe toxicities. Severe toxicities of methotrexate can be interrelated with serum methotrexate levels at 48 h after the start of HDMTX infusion.

Keywords High dose methotrexate · Serum methotrexate level · Toxicities · Acute Lymphoblastic Leukaemia

Introduction

High dose Methotrexate (HD-MTX) therapy with Folinic acid rescue has been being used for over a decade for the treatment of an assortment of childhood and adult malignancies. Dosages of 500 mg/m² or higher given intravenously are characterized as HDMTX and are beneficial for CNS prophylaxis in ALL patients [1, 2]. Unfortunately, high dose MTX has several undesirable side effects and rarely may it be fatal. The reported side effects of MTX are oral mucositis, minor myelosuppression, and acute liver toxicity with transiently elevated serum aspartate aminotransferase (ASAT) and alanine aminotransferase (ALT) levels [3]. These side effects may cause increase length of hospital stay, and delay of the scheduled chemotherapy. MTX toxicity varies from patient to patient. The pharmacokinetics and pharmacodynamics of the MTX demonstrate immense inter-patient variability even with a similar treatment protocol [4]. The current management of MTX therapy is complicated due to its narrow therapeutic window and significant variability in pharmacokinetics. Toxicities associated with MTX can be minimized by urine alkalinization, vigorous hydration, and rescue with folinic

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acid. The concentration of MTX in plasma and other biological fluids helps to predict and prevent its toxicity. An increased risk of toxicity is associated with the concentrations of MTX $> 1 \mu\text{mol/L}$ at 48 h or $> 0.1 \mu\text{mol/L}$ at 72 h after the start of the MTX infusion [5]. MTX concentration monitoring is the gold standard for identifying MTX toxicity. However, monitoring plasma MTX concentrations is carried out in many clinical settings; there are limited reported studies from developing countries like India. Therefore the purpose of our study is to estimate the serum MTX level and correlate with MTX induced toxicity in pediatric ALL patients receiving HDMTX infusion.

Method

The study was conducted in Bharati and KEM Hospital and Research Center, Pune from a period from April 2017 to October 2018. The study was approved by the Institutional ethics committee. Prior to the study every patient's parents or guardian received extensive standardized information material about the aim and the content of the study. The informed consent was obtained from the patient's parents or guardian and assent was obtained from children above 13 years. Children of either sex below 18 years with Acute Lymphoblastic Leukaemia (ALL) at the time of enrollment who received high dose Methotrexate intravenous infusion over 24 h as a 2 g/m^2 , 3 g/m^2 and 5 g/m^2 dose was included in the study.

Treatment Protocol and Drug Administration

The selected patients were treated as per the BFM based treatment protocol that includes four courses of HDMTX. For each course, 10% of the total MTX dose (maximum to 0.5 g) was given intravenously in the first half an hour and the rest was administered evenly during the subsequent hours. Patients were hydrated for 48 h with intravenous dextrose 0.45% normal saline, 20 mmol KCl/l and 40 meq NaHCO_3/l until the urine pH was ≥ 6.5 before HDMTX infusion. Hydration was continued during and after HDMTX infusion.

Blood Sampling and Methotrexate Assay

2 mL blood samples were collected in a plain tube 48 h after the start of HDMTX infusion and allowed the blood to clot. Coagulated blood was centrifuged for 15 min at 3000 rpm and serum was isolated before analysis. Serum Methotrexate drug levels were estimated by using the Abbott ARCHITECT Methotrexate assay. The ARCHITECT Methotrexate Assay is utilized in clinical research laboratories for the quantitative determination of

methotrexate in human serum or plasma using Chemiluminescent Microparticle Immuno Assay (CMIA) technology. Measuring the range of Abbott ARCHITECT Methotrexate assay kit is from 0.040 to $1.500 \mu\text{mol/L}$. Specimens with MTX concentration prominent than $1.500 \mu\text{mol/L}$ is diluted with the Manual Dilution Procedure provided by the manufacturer. Following high dose methotrexate treatment with the folinic acid rescue, serum MTX concentrations above safety values, characterized as $> 1.0 \mu\text{mol/L}$ at 48 h after the start of HDMTX infusion demonstrated greater susceptibility to adverse reactions.

Assessment of Toxicity

The National Cancer Institute Common Terminology Criteria for Adverse Events v.5.0 (CTCAE) [6] score system was used for grading gastrointestinal, hepatobiliary, renal, blood lymphatic system, nervous system, and eye, etc. elevation of Alanine Transaminase, decreased platelet, white blood cell and neutrophil were categorized in investigations category.

Statistical Analysis

The data were analyzed by using Excel Analyze IT XP and SPSS version 18. Patients were grouped according to serum MTX level 48 h after the start of HDMTX infusion and difference in the incidence and severity of toxicity between groups was compared using Chi square test. The association between the presence of toxicity and the methotrexate level was assessed by logistic regression analysis. The P value of < 0.05 was found to be statistically significant.

Results

A total of 244 HDMTX infusions were delivered to 62 ALL patients. Out of 62 patients, the majority was boys 45 (72.6%) and aged between 1 and 6 years (48.4%). The mean age group was found to be 7.89 ± 4.64 years. In the diagnosis of ALL, Pre-B ALL was dominant in 56 cases (90.3%) of the overall cases. From the total patients, 60 patients received all four cycles of methotrexate. One patient received three cycles due to the development of anaphylactic reaction to HDMTX infusion in the fourth cycle. Another patient received only one cycle and moved to another centre. As per treatment, dosages, 2 g/m^2 was given in 40 (65.6%) patients. The characteristics of the children receiving HDMTX infusion are given in Table 1.

Out of 244 cycles, toxicities were reported in 97 cycles (39.7%). From the total of 244 cycles, serum methotrexate level in 35 cycles at 48 h after the start of HDMTX

Table 1 Characteristics of patients receiving HDMTX infusion

Characteristics	Number of patients (%) N = 62
Gender	
Boys	45 (72.6)
Girls	17 (27.4)
Age at the time of diagnosis (years)	
1–6	30 (48.4)
7–12	17 (27.4)
13–18	15 (24.2)
Mean age	7.69 years
BSA (m ²)	
Range	0.5–1.8
Median	0.76
Immophenotype	
T cell	6 (9.7)
B cell	56 (90.3)
Dose of high dose methotrexate infusion	
2 g/m ²	40 (64.5)
3 g/m ²	12 (19.3)
5 g/m ²	10 (16.1)

infusion was found to be ≥ 1.0 $\mu\text{mol/L}$ with reported toxicities among 31 cycles (88.6%). In 209 cycles MTX level was found to be less than 1.0 $\mu\text{mol/L}$ with reported toxicities in 66 cycles (31.6%) (Fig. 1). The logistic regression analysis of the level of methotrexate with the presence of toxicity between all cycles and patients was done. The presence of toxicity was significantly ($p < 0.0001$) associated with the methotrexate level. The odds ratio was calculated to be 12.72 with the confidence interval of 4.2303–38.2459, indicating the presence of toxicity in these patients has 12.72 times higher chances of increase in methotrexate level.

The highest reported toxicities were in the early cycle 1 (38.8%) which was found to be statistically significant when compared to other cycles toxicities ($p < 0.0001$). The least toxicities were observed in the latter cycle 4 (16.3%) as shown in (Fig. 2).

Fig. 1 Distribution of serum methotrexate level ($\mu\text{mol/L}$) in different cycles

The highest reported toxicities were gastrointestinal (96.9%). Among all GIT toxicity, vomiting in 48 cycles (49.5%) was the most frequent, followed by oral mucositis in 22 infusions (22.7%). Alanine aminotransferase elevation (SGPT) was observed in 07 cycles (7.2%). Acute Kidney Injury resulting from administration of HDMTX was presented in 1 (0.01%) infusions. Other toxicities associated with HDMTX were skin rashes 8 (8.2%), CNS (4.1%) and eye disorders (2.1%) respectively. Among the various toxicities associated with HDMTX, the Serum Methotrexate concentration of more than or equal to 1.0 $\mu\text{mol/L}$ was found to be statistically significant in oral mucositis ($p < 0.0001$), the elevation of liver enzymes ($p < 0.0100$), Febrile Neutropenia (< 0.0001) and dermatological toxicities ($p = 0.0118$) (Table 2).

The serum MTX levels more than or equal to 1.0 $\mu\text{mol/L}$ increased alanine aminotransferase (61.93 ± 20.29 IU/L), decreased TLC ($3278.88 \pm 381.9885/\text{Cumm}$) and Hb count (9.73 ± 0.26 g/dl) with no statistical significance. Whereas reduction in Platelet count ($183,961 \pm 19,823.55/\text{Cumm}$) was statistically significant in cycles with serum MTX ≥ 1.0 $\mu\text{mol/L}$ (Table 3).

When we compared the different dose of MTX infusion and toxicity, the highest toxicity was reported with dose of the 5 g/m² (90%). Dose reduction was done in 17.5% and 10% of patients receiving 2 g/m² and 5 g/m² MTX infusion. The highest number of mucositis (40%) was reported with 5 g/m² dose, whereas the highest number of dermatological (20%) and GIT toxicities (55%) were with 2 g/m² dose (Table 4).

From a total of 62 patients, toxicity due to HDMTX infusion, led to increasing hospitalization stay resulting in dose reduction among 11 patients (17.7%) and treatment delay in 20 patients (32.3%) respectively. Mean length of stay due to MTX toxicity was found to be 10.76 days. Ten out of eleven patients presented with serum MTX level more than 1.0 $\mu\text{mol/L}$ in the first cycle, whereas in one patient presented in the second cycle. The mean serum MTX level of these patients in the first cycle was 2.50 $\mu\text{mol/L}$, which after dose reduction was found to be 0.39 $\mu\text{mol/L}$. Another patient had normal serum MTX levels (0.11 $\mu\text{mol/L}$) however; dose reduction was done due to prolonged Neutropenia. (Table 5).

Discussion

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer; its survival rate has improved, with 5-year event-free survival (EFS) rates of 70–80% [7, 8]. ALL is more common in males at almost every age, and this fact remains unexplained [9]. In the present study, ALL was mostly diagnosed among boys 45 (72.6%) and aged

Fig. 2 Details of toxicities reported in each cycle

between 1 and 6 years (48.4%). A pediatric study conducted by Hewitt et al. [10] reported leukemia as the most common childhood cancer with the peak incidence between 2 and 6 years of age. B cell ALL is the major immunological subtype in acute lymphoblastic leukemia that represents more than 50% of the overall cases. The current study reported subtype B-cell in 90.3% and T cell in 9.7% of the overall ALL patients. This was similar to a study conducted by C.H. Pui, where 80% being originated from B-cell from the overall cases [11].

Methotrexate (MTX) is the most generally administered antimetabolite in pediatric cancers which can cause considerable improvements in the long-term survival of these patients [12, 13]. MTX toxicity is unpredictable and the mechanism of toxicity could be mainly explained by an inhibition of normal cells and tissue adjacent to the target abnormal cells [14]. In cancer chemotherapy, routine monitoring of drug concentrations has been practical only for methotrexate (MTX). An MTX level in plasma is the most important factor determining toxicity after HDMTX infusion. The frequency of HDMTX associated toxicity is variable. Out of the total four cycles, the present study demonstrated the highest toxicities in Cycle I (38.8%) and Cycle II (24.5%) respectively. The former cycles were found to be statistically significant ($p < 0.0001$) when compared to other cycles toxicities. Based on first cycles toxicity experience at most care was taken for prevention of toxicity in subsequent cycles such as dose reduction and vigorous hydration. In the current study, high-dose methotrexate was associated with toxicities in 97 cycles out of 244 (39.7%) which led to MTX dose reduction in 17.7% cases. In some childhood trials dose reductions have been recommended in addition for patients with preceding severe toxicity [15]. Treatment delay occurred in 32.26% patients and mean number of hospitalization due to toxicity was 10.76 days. As noted previously, HDMTX therapy can cause significant toxicity, which disrupts treatment protocol, resulting in dose reductions or treatment delays that can adversely affect control of the malignancy. Myelosuppression, grade 3 mucositis, and febrile Neutropenia resulted in treatment delays.

In the current study, the most commonly described gastrointestinal adverse-effects following HD-MTX was vomiting and mucositis. Vomiting occurred in maximum cycles irrespective of serum MTX level. A Chemotherapeutic agent such as MTX stimulates the release of serotonin from the enterochromaffin cells lining the gastrointestinal tract which sends impulses to the vomiting center when stimulated by an emetogenic substance [16]. Oral mucositis reported was statistically significant in cycles which serum MTX (at 48 h after start of infusion) greater than $1.0 \mu\text{mol/L}$. Pico et al. [17], reported that MTX may be secreted in the saliva, leading to increased direct mucotoxicity. This finding is consistent with those of Mahmoud et al. [18] who revealed higher frequency episodes of oral mucositis and nausea/vomiting during their study period.

The incidence of HDMTX-associated acute kidney injury (AKI) is approximately 1.8% (range 0–12%) [19]. Our study reported Acute kidney injury was found to be 0.02% (one patient) which was less as compared to other reported studies. The child received 5 g/m^2 of MTX for 24 h continuous infusion. At the 48th hour of infusion, the patient presented with severe vomiting with a rise in serum MTX level by 10.8 mmol/L . Serum MTX level of the patient at 72 h and 96 h was found to be 2.65 and $1.05 \mu\text{mol/L}$ respectively in spite of continuing vigorous hydration and leucovorin rescue. The child's parents took discharge against the doctor's medical advice. On the second day of discharge, the child was reported with manifestations of other related toxicities like mucositis, neutropenia, leucopenia, thrombocytopenia, anaemia, skin peeling, uremia, and high creatinine. Similar toxicities were reported by Kaveb et al. [20]. Serum methotrexate level at the time of readmission (the sixth day of MTX infusion) was found to be $0.95 \mu\text{mol/L}$. Prolonged elevation of systemic methotrexate concentrations can cause serious toxicity like Acute kidney injury. This child had severe vomiting at the time of HDMTX infusion. Volume depletion could be the reason for reversible AKI.

Kapoor et al. [21] detected neutropenia grade ≥ 3 ($\text{ANC} < 1 \times 10^9/\text{L}$) in 24.8% of the infusions in patients

Table 2 Correlation of Serum MTX level and reported toxicities

Reported toxicities	Serum methotrexate level at 48 h after start of HDMTX infusion (μmol/L)		Chi square distribution <i>P</i> value	Total number of patients	Total number of cycles
	More than or equal to 1.0 μmol/L	Less than or equal to 1.0 μmol/L			
	Number of cycles N (%)				
(i) Gastrointestinal disorders					
Vomiting Grade 1 and 2	09	39	< 0.0001	28	48
Nausea Grade 1 and 2	02	08	0.0323*	8	10
Abdominal pain Grade 1 and 2	01	10	0.0006*	4	11
Diarrhea Grade 1 and 3	00	02	–	2	02
Oral Mucositis (Grade 1–4)	20	02	< 0.0001*	21	22
Gastric hemorrhage	01	00	–	1	01
(ii) Immune system disorders					
Anaphylaxis	01		–	01	01
(iii) Renal and urinary disorders					
Acute kidney injury	01	00	–	01	01
(iv) Blood and lymphatic system disorders					
Febrile Neutopenaia Grade (1–4)	6	4	< 0.0001*	06	10
Anemia (Grade 2)	12	00	–	12	18
(v) Nervous system					
Seizure Grade 1	01	00		01	01
Headache	01	02 (66.67)	0.9999	03	
(vi) Dermatological					
Rash maculo-papular	07 (87.5)	01 (12.05)	0.0118*	08	08
(vii) Eye disorders					
Watering eyes (Grade 1)	00	01	–	01	01
Eye pain (Grade 1)	00	01	–	01	01
(viii) Investigations					
Alanine aminotransferase increased	06	01	0.0100*	07	07
Platelet count decreased (Grade 1–3)	08	00	–	05	08
White blood cell decreased	21	07	0.0002*	14	28
Neutrophil count decreased	21	07	0.0002*	14	28

*Statistically significant

receiving HDMTX at 5 g/m^2 . In our study, neutropenia $\geq$ grade 3 developed in 28.86% of the HD-MTX cycles. The toxicity rates in our study were similar to those reported in other studies [21, 22].

The administration of high doses of intravenous MTX permits high drug concentrations to be achieved within the central nervous system, which has been shown to result in acute, subacute, and long-term neurotoxicity including seizure which was present in one patient of the current

study. The precise mechanism of neurotoxicity is likely through disruption of CNS folate homeostasis and/or direct neuronal damage [23].

MTX-induced hepatotoxicity was expressed by elevated ALAT and was observed in 7.2% of cycles. HDMTX can cause an acute elevation in the serum transaminases from two to 20fold normal levels, even in patients who receive folinic acid rescue. If the level of alanine transferase (ALT) has not returned to less than 180 IU/L by the beginning of

Table 3 Correlation of Lab values and serum MTX level

	Serum MTX level < 1.0 µmol/L (n = 209 cycles) (Mean ± SE)	Serum MTX level ≥ 1.0 µmol/L (n = 35 cycles) (Mean ± SE)	P value
Serum methotrexate level at 48 h after start of HDMTX infusion (µmol/L)	0.36 ± 0.02	1.63 ± 0.28	< 0.001*
Alanine aminotransferase (IU/L)	41.20 ± 13.47	61.93 ± 20.29	0.491
TLC (/Cumm)	4778.07 ± 244.04	3273.8857 ± 381.9885	0.674
Platelet count (/Cumm)	359,577.32 ± 67,031.98	183,961.89 ± 19,823.55	< 0.001*
Haemoglobin (g/dL)	10.40 ± 0.15	9.73 ± 0.26	0.662

TLC total leukocyte count

*Statistically significant

Table 4 Comparison of proportions of various parameters between the groups receiving different dose of MTX infusion

Characteristics	Dose of MTX infusion Number of patients (%)	
	5 g/m ² (n = 10)	2 g/m ² (n = 40)
Toxicity developed	9 (90)	33 (82.5)
Dose reduced	1 (10)	7 (17.5)
GIT toxicities	5 (50)	22 (55)
Elevated alanine aminotransferase	3 (30)	3 (7.5)
Dermatological toxicities	0	8 (20)
Mucositis	4 (40)	15 (37.5)
Decreased platelet	3 (30)	3 (7.5)
Anaemia	6 (60)	12 (30)
Decreased TLC	5 (50)	8 (20)
Febrile neutropenia	3 (30)	3 (7.5)

GIT Gastrointestinal Tract, TLC Total leukocyte count

Table 5 Outcome of patients reported with MTX toxicity

	Number of patients (%)
Dose reduction	11 (17.74)
Percentage of dose reduction from standard dose	
10	1
20	1
25	5
35	1
50	2
75	1
Treatment delay	20 (32.26)
Number of patients required for hospitalization	20 (32.26)
Mean Length of hospital stay due to MTX toxicities	10.76 days

the next treatment cycle, the next dose should be reduced and/or delayed [1]. Holmboe et al. [24] suggested that 7-OHMTX, a main metabolite of MTX, was involved in the development of HD-MTX hepatotoxicity in patients with osteosarcoma treated with HD-MTX. Abnormal liver function observed in 7.2% of cycles were not comparable to those reported in other studies 35% [21]. These

abnormalities were transient and reversible as also observed by others and, in children, have not been reported to result in chronic liver disease. Consistent with the previous studies, [1, 18, 25] our study also reported rash maculopapular as dermatological toxicity in eight which is also associated with MTX- toxicities. Highest reported

dermatological toxicities were in cycles that serum methotrexate level is more than or equal to 1.0 $\mu\text{mol/L}$.

An analysis of the association of serum MTX levels > 1.0 mmol/L and its related toxicities such as mucositis, dermatological infection, the elevation of liver enzymes, neutrophilia and leucopenia emphasized that higher levels of MTX were responsible for the occurrence of toxicities in 31 cycles.

The toxicities such as elevated alanine transaminase, Mucositis, thrombocytopenia, leucopenia, and Febrile Neutropenia were reported more in MTX infusion with a dose of 5 g/m^2 . Toxicities such as Gastrointestinal and Dermatological more reported in 2 g/m^2 dose. As per the BFM 2002 protocol 5 g/m^2 dose is given for T cell ALL and CNS positive patients, wherein 2 g/m^2 is given for B cell ALL.

There was no therapy related to deaths during the HD MTX treatment cycles. The toxicity rates in our study were slightly lower than those reported in other studies.

Conclusion

This study concludes toxicities such as oral mucositis, febrile neutropenia, dermatological toxicities, myelosuppression, the elevation of liver enzymes; seizure and anaemia were reported in cycles in which serum methotrexate was greater than or equal to 1.0 $\mu\text{mol/L}$. Vomiting was found in almost all cycles irrespective of serum methotrexate level. Dose reduction was done due to febrile neutropenia, myelosuppression and Grade 3 mucositis. Treatment delay, dose reduction and the increasing length of hospital stay was documented in patients that serum methotrexate was greater than or equal to 1.0 $\mu\text{mol/L}$. Highest toxicity reported in methotrexate dose of 5 g/m^2 . Serum methotrexate level is a good predictor for identifying and thereby preventing toxicities due to HD-MTX infusion.

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Author Contributions All authors listed have contributed to the work and agreed to submit the manuscript for publication.

Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interests.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee.

Human and Animal Rights This study used blood sample from ALL patients. Ethical approval was obtained from institutional ethics

committee (BVDUMC/IEC/74 and KEMHRC/LFG/EC/2423) to conduct the study.

Informed Consent Informed consent was obtained from the parents/guardian of study subjects.

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