Original Article

Monitoring of Blood Methotrexate Level after High Dose of Methotrexate in Childhood Hematological Malignancy

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Abstract

Background: In childhood cancer chemotherapy, regular monitoring of drug concentration has been practical only for methotrexate. The primary settings for pharmacokinetics monitoring of MTX is its use in high dose for adjuvant therapy of Non-Hodgkin lymphoma and acute lymphoblastic leukemia. Monitoring of blood level of MTX after high dose is not only aimed to monitoring effectiveness but also safety aspects of the administration high dose because the use of high doseMTX is one of the problems associated with severe toxicity in various organs.

Methods: A prospective observational study was conducted in the Department of Pharmacology, BSMMU in collaboration with the Departments of Pediatric Hematology & Oncology of and DMCH from March 2017 to July 2018. 18 patients through purposive sampling suffering from hematological malignancy (ALL and NHL) were enrolled. All of them received 3-gram MTX over 3 hours with leucovorin (LV) rescue. Plasma MTX levels were measured at 0, 24, 48 and 96 hours and serum creatinine concentrations were measured at 0, 24 and 48 hours. Correlation between plasma MTX concentrations with serum creatinine concentrations at 0, 24 and 48 hours were determined.

Result: The levels of methotrexate in plasma before high dose were 53.84 ± 72.98 pmol/L (mean ± SD). After 24 hours of high dose this was 718.41 ± 1756.23pmol/L (mean ± SD). The change was not statistically significant (p=0.308). At 48 and 96 hours after high dose the same parameters were 117.33 ± 225.72 pmol/L (p=0.470) and 64.88± 139.24 pmol/L (p=0.424) respectively. The mean± SD of serum creatinine concentrations before HDMTX was 39.15 ± 13.72 µmol/L. At 24 and 48 hours the same were 38.70 ± 21.63 µmol/L (p=0.962) and 39.88 ± 23.07 µmol/L (p=0.653) respectively which were not statistically significant.

Conclusion: At the dose (3 gram/m^2) of methotrexate used in the present study, the plasma methotrexate levels were not elevated to toxic concentrations. MTX associated toxicity could be prevented. Evaluation of renal functions is an important tool for monitoring of high dose MTX.

Key words: hematological malignancy, methotrexate

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Introduction

ALL is the most common malignancy in children and accounts for one-fourth of all childhood cancers and 72% of all cases of childhood leukemia [1]

Overall cure rates for pediatric ALL have improved from virtually zero in the 1950s to the current Event Free Survival (EFS) rates of 75% to 85% for this disease. The advances in this regard been due to the development of active chemotherapeutic agents, to improvement in our understanding of how to dose and combine these agents more effectively and to significant advances in supportive care.[1]

While Non-Hodgkin lymphoma result from malignant proliferation of cells of lymphocytic lineage and represent 8-10% of all malignancies in children between

5-19 years of age. It accounts for approximately 60% of all lymphoma in children and adolescents.[2]

In Bangladesh, cancer in children is emerging as a significant threat to life while at the same times morbidity and mortality from infections and malnutrition have begun to decrease due to concerted maternal and child health care and initiatives taken by physicians and this represents a blessing of advancement in our scientific knowledge. There is no national population based cancer registry but using worldwide incidence rates an estimated rate of 6000-9000 new cases per year in Bangladesh are being assumed.[3] The relative incidence of malignancies seen at BSMMU in 2012 was acute lymphoblastic leukemia (ALL) 58%, non-Hodgkin lymphoma (NHL) 11% of the total cancer patients admitted in that year.

Methotrexate (amethopterin) is an antifolate agent used in the treatment of autoimmune diseases and various types of cancers. Methotrexate first demonstrated efficacy as a chemotherapeutic agent in acute lymphoblastic leukemia (ALL) in 1947. [4]. Since that time, it became one of the most widely studied anticancer agents. In many such deadly instances methotrexate has been the drug of choice because in addition to its efficacy, it can be administered safely in a multitude of dosing strategies, its toxicity can be antagonized by administering leucovorin (LV) and alkalization of urine can ameliorate its toxic actions to a considerable extent.

Diversified uses of MTX is worth mentioning because this drug is indicated for use in pediatric neoplastic disorders such as ALL, meningeal leukemia, osteosarcoma, some brain tumors and non-Hodgkin lymphoma[5,6]. These neoplastic indications are well established and researched almost always required combination therapy. [7]

Prior to routine monitoring of plasma methotrexate concentrations and pharmacokinetically guided adjustment of folinic acid rescue were advocated, mortality associated with HDMTX recorded as 4.6% - 6.0%. [8,9]. Since then serious cases of the disease for which MTX was indicated, the HDMTX regime has been a protocol to be followed in selected well-guided management of severely affected patients.

The HDMTX usually delivered over 4 to 36 hours infusion and require 2-3 days period where leucovorin (LV) is being administered to terminate the toxic effects of MTX (the leucovorin rescue). Successful rescue by leucovorin depends on rapid elimination of MTX by kidneys which requires aggressive pretreatment as well as post treatment hydration and urinary alkalization[6]

Methotrexate is eliminated primarily by renal excretion undergoing glomerular filtration and renal tubular reabsorption and secretion. Approximately 70% to 90% of a dose is excreted unchanged in the urine, most within the first 6 hours.[1]In patients with significant renal dysfunction MTX clearance is delayed resulting in prolonged drug exposure and a greater risk of severe toxicities especially to the kidneys. Any patient who is suspected of having renal dysfunction and who receives MTX should have the plasma MTX concentrations closely monitored and must receive LV if clearance of MTX is delayed.[10,11]

Successful and quality management of HDMTX demands that each course of HDMTX should be closely monitored by following renal function and plasma MTX concentrations to determine the dose and duration of LV rescue.[1,12] However, in the absence of plasma MTX level monitoring, many centers carry on HDMTX programs by monitoring of renal and hepatic functions and GIT toxicities e.g. mucositis and LV doses are corrected accordingly. It may be worth mentioning at this instance that CNS toxicities of MTX and HDMTX appear later and can be well prevented by successful LV rescue therapy.

The primary toxic effects of MTX following IV therapy are myelo-suppression and gastrointestinal mucositis which usually occur 5 to 14 days after administration of the drug. The development of toxic reactions is related to the concentration of drug and the duration of exposure. [13] In patients receiving a 6-hour infusion of methotrexate, a 48-hour methotrexate concentration above 1μM was observed to be associated with the development of significant toxicity[13]. These toxicities can be prevented by administration of LV. With the use of therapeutic drug monitoring and continuation of leucovorin rescue until plasma methotrexate concentration has fallen below 0.05 to 0.1μM, the toxicity of HDMTX can be avoided in most patients. LV dosing should be started after 1-2 days of HDMTX therapy as otherwise the efficacy of the drug may be impaired. Despite these precautionary measures, nephrotoxicity still may occur in almost 2% of patients receiving HDMTX infusions. The development of renal

dysfunction during HDMTX is a medical emergency. Therefore, such patients must be closely monitored and the dose of LV increased in proportion to the plasma methotrexate concentrations.[12]

In situations where there are well established conditions for plasma MTX measurements that are in a rescue rich setting, measurement of a minimum of three MTX levels are recommended. These measurements of MTX are performed at 24, 42 and 48 hours from the start of MTX infusion. [14,15] Based on the plasma MTX levels, the volume and duration of hydration, as well as the dose and frequency of leucovorin are titrated. Along with plasma MTX levels, serum creatinine, urine output and urine p^H should to be estimated.

However, there is limited discussion in the literature on the administration of HDMTX without monitoring TX levels. [12,16,17] The reported alternative measures include monitoring renal function (e.g. by creatinine clearance, serum creatinine or change in creatinine from baseline to assess MTX or HDMTX administration as possible indirect indicator of MTX level or restricting the dose of MTX.[12]

Methods:

A prospective observational study was conducted in the Department of Pharmacology, BSMMU in collaboration with the Departments of Pediatric Hematology & Oncology of BSMMU and DMCH from March 2017 to July 2018. Clearance from institutional review board of BSMMU was taken earlier. Children aged between 5 to17 years diagnosed cases of ALL and NHL parents or guardians permitted for administration of protocol-based chemotherapy. Children with impaired renal function, having any serious systemic infection and malignancies other than ALL and NHL had been excluded from the study. Demographic data (age, sex) were collected and documented. All patients received standard routine medical care throughout the study. With all aseptic precaution 5 ml blood was collected from patient before giving chemotherapy by venipuncture from the antecubital vein and kept it in 1 X 5 ml EDTA (anticoagulant) containing test tube. EDTA tube was centrifuged at 3500 rpm for 10 minutes. Plasma was collected in labeled eppendorf by micropipette and stored at -20^0 C in refrigerator at the department until analysis. Estimation of plasma methotrexate (Modification of Moghbel.)

In this study, HPLC methodology was used for detection of methotrexate in plasma. Estimation of serum creatinine concentration by automated Analyzer (Architect Plus ci4100) in the department of Biochemistry, BSMMU.

Data were processed and analyzed using computer software SPSS (Statistical Package for Social Science) version 22. Wilcoxon Signed Rank test was excellent alternative to paired t- test in case of non-parametric data and when data shows asymmetric distribution. Wilcoxon Signed Ranked test was used to compare the continuous data within groups. Correlation was done using Pearson correlation statistics to observe relationship between parameters. $p< 0.05$ was considered statistically significant.

Results:

18 children with hematological malignancy were enrolled for the present study following inclusion and exclusion criteria. The mean age of the male patients were 7.08 ± 2.06 years and female patients were 10.50 ± 4.13 years. The mean height of male patients were 116.5 ± 17.0 cm and female were 122.0 ± 23.1 cm. The mean weight of male and female were 23.6 ± 13.3 kg and 28.4 ± 14.3 kg respectively. The mean BSA of male and female were 0.88 ± 0.32 m^2 and 1.12 ± 0.32 m^2 respectively.

Demographic profile of the patients (n=18)

	n	Age (years)	Height (cm)	Weight (kg)	BSA (m^2)
Male	12	7.08 ± 2.06	116.5±17.0	23.6 ±13.3	0.88 ± 0.32
Female	6	10.50 ± 4.13	122.0±23.1	28.4 ± 14.3	1.12 ± 0.32
Total	18	8.22 ± 3.25	118.3 ± 18.7	25.2 ± 13.4	0.97 ± 0.33

Plasma methotrexate levels before and after HDMTX administration

The levels of methotrexate in plasma before high dose were 53.84 ± 72.98pmol/L (mean ± SD). After 24 hours of high dose (3 g/m^2) this was 718.41 ± 1756.23pmol/L (mean ± SD) . The change was not statistically significant (p =0.308). At 48 and 96 hours after high dose the same parameterswere 117.33 ± 225.72pmol/L (p = 0.470) and 64.88 ± 139.24pmol/L (p =0.424) respectively.

Plasma methotrexate concentrations before and after administration of HDMTX (n=18)

Plasma methotrexate concentrations (μmol/L)	mean ±SD	p-value
Before HDMTX administration (a)	53.84±72.98	
After 24 hours of HDMTX administration(b)	718.41±1756.23	0.308 (a vs. b)
After 48 hours of HDMTX administration(c)	117.33± 225.72	0.470 (a vs. c)
96 hours after HDMTX administration (d)	64.88±139.24	0.424 (a vs. d)

Wilcoxon Signed Ranks test was done to measure the level of significance

Serum creatinine concentrations before and after administration of HDMTX

The mean ± SD of serum creatinine concentrations before HDMTX was 39.15 ± 13.72 μmol/L. At 24 and 48 hours the 0same were 38.70 ± 21.63 μmol/L (p = 0.962) and 39.88 ± 23.07 μmol/L (p = 0.653) respectively.

Serum creatinine concentrations before and after administration of HDMTX (n=18)

Serum creatinine concentrations (μmol/L)	mean ±SD	p-value
Before HDMTX administration (a)	39.15 ± 13.72	
After 24 hours of HDMTX administration (b)	38.70 ± 21.63	0.962 (a vs. b)
After 48 hours of HDMTX administration (c)	39.88 ± 23.07	0.653 (a vs. c)

Wilcoxon Signed Ranks test was done to measure the level of significance

Discussion:

Methotrexate (MTX) is one of the folate antagonists used in different childhood malignancies like ALL, NHL, in some other malignant conditions as well as in some autoimmune diseases and has been a mainstay of treatment ever since. The drug (commonly known as antimetabolite anticancer drug) exerts its cytotoxic effects by competitively inhibiting dihydrofolate reductase (DHFR), the enzyme responsible for converting dihydrofolates into tetrahydrofolate (the reduced folate carriers which function in the transfer of carbon units). The primary setting for pharmacokinetic monitoring of MTX done only if uses in high doses as adjuvant therapy for osteosarcoma, for single agent treatment of intracranial lymphomas and in combination therapy of childhood leukemia as well as adult and pediatric non-Hodgkin lymphomas.[18,19] Although plasma MTX concentrations are monitored in most large medical centers in developed countries, empirical administration of HDMTX is mostly lacking in most of the developing countries, including Bangladesh. Serum concentrations and pharmacokinetics parameters MTX are not related with outcome of the diseases. Prognoses based on single drug pharmacokinetic estimates within a complex multiple agent protocol appeared to be unreliable [20]However therapeutic drug monitoring of HDMTX remains a useful tool for early detection of impaired or delayed elimination of MTX. [20] and avoiding systemic toxicities. High levels of MTX persisting after administration might lead to systemic toxicities including nephrotoxicity, hepatotoxicity, neurotoxicity and mucosities.[21] Moreover, in some situations such as pre-existing impaired renal functions, elimination of MTX may be prolonged (i.e. delayed elimination of MTX) and enhancing nephrotoxicity. Observations from the present study state that creatinine clearance rates at 24 hours was positively correlated with plasma methotrexate concentrations and at 48 hours it was also positively correlated with plasma MTX concentrations. The p values were (p=0.486) and (p=0.243) respectively which were not statistically significant. Correlation of plasma methotrexate concentrations with serum creatinine concentrations at 24 hours was negative and at 48 hours it was also negative. P values were 0.827 and 0.444 respectively at those time which were not statistically significant. Assessment of renal function may be useful means of monitoring plasma MTX concentrations during HDMTX of ALL and NHL[12] The present study had assessed serum creatinine as a method of renal function. No significant deterioration of

renal functions were observed in both ALL and NHL patients (Table 3.3, 3.4 and Fig. 3.2, 3.3) and the observation bears resembled to those observed. [22] Majority of MTX is cleared by the kidneys (more than 90%) using hyper hydration of fluids to induce high urinary flow rates to protect the kidney from injury during treatment with HDMTX.

Proper monitoring and supportive care along with tailoring treatment is vital in improving cure rates and minimizes toxicities in childhood ALL. In our study, plasma MTX monitoring, serum creatinine monitoring, creatinine monitoring along with clinical monitoring, hydration, urine alkalization and leucovorin rescue remain essential to HDMTX administration. To achieve optimal efficacy and low toxicity in the clinical treatment of ALL and NHL, the plasma MTX concentration should be sufficiently high after 24 hours and relatively low, indicating timely excretion after 48 hours. Therefore, we examined factors associated with plasma MTX concentrations before and at 24, 48 and 96 hours after the start of HDMTX. Nephrotoxicity is one of the most frequently reported side effects of HDMTX. [22] There are however only few previous reports about MTX induced renal adverse effects in pediatric populations. In the largest reported survey by the relationship between MTX elimination time and renal functions was studied retrospectively in 264 consecutive children with ALL.[23] Serum creatinine was found to increase after 0.02% (28/1, 164) of the MTX courses, which is in accordance with the present results.[24]

A study is whether serial monitoring creatinine can predict HDMTX related toxicities. The evidence is variable.[23]conducted a study in 1164 HDMTX courses in 264 Swedish children with ALL and concluded that elevation of serum creatinine by more than 50% was a better predictor of delayed elimination than the level of serum MTX at the end of MTX infusion[23]. From China conducted a study in 105 children with ALL/NHL to assess the correlation of markers of renal function with plasma MTX level[12] They found serum creatinine and creatinine clearance rate to correlate with plasma MTX concentrations after HDMTX. The author suggested that it may be possible to indirectly monitor plasma MTX concentrations by assessing renal function. Such indirect monitoring would be of utilities in centers where monitoring of plasma MTX is not available. [12]

Conclusion:

At the dose (3 gram/m^2) of methotrexate used in the present study, the plasma methotrexate levels were not elevated to toxic concentrations. Serum creatinine concentrations did not increase significantly which suggest that the filtration rate of the glomeruli of the kidneys had maintained. Coincidently, the MTX levels at 48 and 96 hours were lowered down and this would suggest that the HDMTX administered to the pediatric ALL and NHL patients of the present study were not toxic. This supports that the supportive measures (hydration, alkalization of urine, LV rescue) had been maintained properly. Ideally plasma methotrexate levels should measure after administration of HDMTX. Thus, MTX associated toxicity could be prevented. Evaluation of renal functions is an important tool for monitoring of high dose MTX.

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