EFFECT OF HIGH DOSE METHOTREXATE AND DELAYED ELIMINATION ON MYELOTOXICITY PROGRESSION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT

Background: Methotrexate (MTX) as an antineoplastic agent inhibits dihydrofolate reductase. The frequency of high dose methotrexate (HDMTX) associated toxicity is variable. In this study we investigate the frequency of myelosuppression following 5 and 9 days of HDMTX infusion and MTX delayed elimination in subsequent MTX cycles in children with Acute lymphoblastic Leukemia (ALL).

Methods: This study included 28 children diagnosed with ALLduring the period between May2014to April 2016. Complete blood counts were measured before and after 5 and 9 days of HDMTX infusion and MTX levels at 42hour in 28 children with ALL. The HD-MTX dose is 5 gm/m^2 during 102 infusion of HD MTX at consolidation phase of ALL therapy. The MTX levels at 42 h in patients with and without toxicity were compared to evaluate the correlation between MTX levels and myelotoxicity.

Results: MTX infusion induced significant reduction in levels of TLC, ANC, RBCs, Hb and significant elevation of PLT count after 5 days of MTX administration. Additionally, after 9 days of MTX infusion, there is significant decrease inTLC, ANC, and RBCs levels. However, no significant difference in the PLT count and Hb level occurred. There is gradual decrease in myelotoxicity after 5 days and increase after 9 days of MTX administration with regard to MTX cycles. There is no statistical difference in MTX level at 42 h between patients with and without myelotoxicity after 5 and 9 days of MTX infusion. MTX delayed elimination observed in MTX cycles 1, 2, 3 and 4 was 42.8% (n=12), 42.8% (n=12), 57.1% (n=16) and 72% (n=13) respectively.

Conclusion: Myelotoxicity was decreased after 5 days of MTX administration and increased after 9 days with regard to MTX cycles. There is no correlation between MTX plasma concentration after 42 h and hematologic toxicity. Therefore, we cannot depend on MTX levels at 42 h to anticipate and predict hematologic toxicity.

Keywords: HDMTX, ALL, myelosupression, blood count

INTRODUCTION

Methotrexate (MTX) is an analogue of aminopetrinand the most widely antifolate used in the treatment of certain neoplastic disease, sever psoriasis and adult rheumatoid arthritis[1]. Methotrexate (MTX) was firstapplied as a treatment for malignant diseases in oncology in 1948[2].It is an important component of the consolidation and maintenance therapy of childhood ALL[2, 3].It inhibits dihydrofolate reductase and was initially developed as an antineoplastic agent[4].

High-dose methotrexate (HDMTX) chemotherapy withleucovorin (LV) rescue is administered to prevent extramedullary infiltration and it is very important ALL therapy[5]. Unfortunately, MTX therapy may lead to myelosuppression, acute liver toxicity, nephrotoxicity, mucositis, and neurotoxicity[4,

[6-11]. MTX toxicity is associated with several factors including dose, the duration of administration, patient risk factors, and genetic factors[12, 13]. There are no sufficient data involving the use of HDMTX pharmacokinetic and toxicity information to anticipate hematologic toxicity in children with ALL.

Delayed MTX elimination was defined by either MTX concentration >1µmol/L at 48 h or > 0.1µmol/L at 72 h[14,15]. Kidney and/or liver dysfunction, bone marrow suppression, oral mucosallesions, secondary infection, and delays in the following course of chemotherapy may be a consequence of MTX delayed elimination[6,16]. Therefore, adjustments of MTX and leucovorin dose, hydration and alkalization were made to minimize the risk of elimination delay/MTX toxicity[16]. Previous reports referred to the effect of some drugs as proton-pump inhibitors, non-steroidal anti-inflammatory drugs, trimethoprim, sulfamethoxazole, penicillins , ciprofloxacin, anticonvulsants such as phenobarbital on delaying MTX elimination[17-19].

In this study, we proposed to determine first: the hematologic toxicity and MTX delayed elimination frequency and second:evaluate the relationship between hematologic toxicity and MTX level at 42 hour in children taking 5 gm/m^2 MTX infusion during the consolidation phase of ALL therapy.

METHODS

Patient Selection

This study was approved by the committee of Medical Ethics of Zagazig university (**IRB number: 2184**).The patients were recruited from Pediatric Hematology and Oncology unit Zagazig University during the period between May 2014 and April 2016. ALL subjects include 28 patients, 16 female (57.1%) and 12 male (42.9%) where their ages ranged between 2-18

Protocol of Study

According to TOTAL XV protocol, all patients in this study received four HD-MTX doses (5 g/m^2) at 2-week intervals on days 1, 15, 29 and 43 of consolidation therapy and 6-mercaptopurine (50 mg/m^2/day) on days 1 to 56 of consolidation therapy. These chemotherapeutics were administered when ANC is ≥ 300/µL, and platelet count is ≥ 50x10^9/L. HDMTX will be held if total bilirubin > 2mg/dl and direct bilirubin > 1.4 mg/dl. However, 6- mercaptopurine may be held in the presence of ANC < 300/ µL, platelet count < 50000/ µL or grade 3 or 4 mucositis. Dosage of 6-mercaptopurinesubsequent courses may be reduced to 25 mg/m^2/day in patients who have prolonged neutropenia after HDMTX and 6-mercaptopurinetreatment.At least two hours before HDMTX, prehydration IV fluid (D_5W+ 40 mEq $NaHco_3$ /L) will be administered at the rate of 200 ml/m^2/hr provided that urinary pH is ≥ 6.5. Leucovorin [15mg/m^2 IV or PO for standard/high-risk] will be started at 42 h after the start of MTX and repeat every 6 h. The dosage of leucovorin will be increased in patients with high plasma MTX concentrations (> 1.0 µM at 42 hrs) and continued until the MTX concentration is less than 0.10 µM.

Complete Blood Counts and MTX Level Assessments

In the course of 102 infusions of HD-MTX, the MTX plasma level was measured at 42 h after HD MTX infusion. Hemoglobin (Hb), absolute neutrophil count (ANC), platelet (PLT) count, red blood count (RBCs) and TLC (total leukocytes count) were determined before MTX administration and on the 5^{th} and 9^{th}day followingMTX infusionusing Automated Hematology Analyzer. MTX concentration at 42 h was measured by high performance liquid chromatography (HPLC) assay.

Evaluation of Myelotoxicity

Hematological toxicity or myelotoxicity signs were determined by absolute neutrophil count and hemoglobin according to *Common Terminology* Criteria for Adverse Events (CTCAE)[20](table 1).

STATISTICAL ANALYSIS

Statistical analyses of data were done by Prism 6, Graph pad, CA, USA. Results were expressed as mean ± standard deviation. Statistical differences were sought using Student's t-test or one way analysis of

variance (ANOVA) followed by Fisher's least significant difference (LSD) and or post hoc test (if more than two sets of data were being compared). Differences were considered significant at a P<0.05.

RESULTS

In this study, there are 102 infusions of HD-MTX delivered to twenty eight newly diagnosed acute lymphoblastic leukemia (ALL) patients aged 2-18 years. There is a statistically significant reduction in TLC (23%, *P*<0.0001), ANC (22.8%, *P*<0.0001), RBCs level (8.3%, *P*<0.0001), Hblevel (5.01%, *P*<0.05) and elevation in PLT count (19.8%, *P*<0.01) after 5 days of MTX infusion compared to before administration data. Additionally, there is a statistically significant reduction in TLC (18.6%, *P*<0.0001), ANC (20.3%, *P*<0.0001), RBCs level (5.5%, *P*<0.05) after 9 days of MTX infusion. Whereas, there is no statistically significant difference in the PLT count and Hb level after 9 days of MTX infusion (table 2).

Gradual decrease in myelotoxicity after 5 days of MTX administration was shown. However, there is gradual increase in myelotoxicity after 9 days of MTX administration with regard to MTX cycles (tables 3-4, figure1).

In addition, Table 5 and 6show that there is no statistical difference in MTX level at 42 h between patients with or without myelotoxicity after 5 and / or 9 days of MTX infusion. Figure 2 also illustrates that there is a gradual increase in% MTX delayed elimination with regard to MTX cycles.

DISCUSSION

Consolidationphase is considered avaluable and important step in the treatment of children with ALL. The use of high dose methotrexate, sometimes in combination with 6-mercaptopurine (6-MP) has significantly contributed to cure children (70-80%). In this study administration of MTX infusion for 5 days induced significant reduction in TLC, ANC, RBCs, Hblevels and PLT count after 5 days of MTX infusion. Similar data was observed during 9 days administration except the effect on PLT count and Hblevel. These results are in agreement with previous studies [21].This effect onblood counts especially RBCs and TLC is attributed to higher concentration of MTXPG in patients whose blast count decreased within 24 h . Blast cells are the origin of platelets, red blood cells, neutrophils and other types of white blood cells in the myeloid cell line [22]. Therefore, decrease in blast concentration of MTXPGLC (MTX polyglutamate long chain) may be strongly related to the rate of inhibition of de novo purine synthesis in ALL blasts [23].

The significant reduction of Hb after 5 days and the non statistical significant difference ofHb after 9 days may be justified by the results of **de Rotteet al.**[24].Methotrexate can affect HbA1c where, methotrexate use and higher concentrations of erythrocyte MTXGlu (erythrocyte methotrexate polyglutamate) are associated with decreased levels of HbA1c. Therefore, concentrations of erythrocyte MTXGlu may be still high after 5 days of MTX infusion. However, after 9 days, concentrations of erythrocyte MTXGlu may be decreased.

Lexicomp[25]has been reported that not all patients should suffer low blood counts in RBCs, WBCs and platelets but this low blood counts is only common in more than 30% of patients supporting the present finding wherethere is no statistically significant difference in the PLT count and Hb level after 9 days of MTX infusion.

The current studyillustrated gradual decrease in hematologic toxicity and myelotoxicity after 5 days of MTX infusion with repeated MTX administrations. These results are consistent with others[26].Attenuation of hematologic toxicity and myelotoxicity with repeated MTX administrations may be related to HDMTX may be held if total bilirubin > 2mg/dl and direct bilirubin > 1.4 mg/dl[27] so the myelotoxicity will be decreased. Additionally, dosage of 6- mercaptopurine may be reduced to 25 mg/m^2/day in patients who have prolonged neutropenia after HDMTX and 6-mercaptopurine treatment [27]and this may be a good reason for the gradual decrease of myelotoxicity[21], especially while the degree of myelosuppression and duration of

treatment interruptions following HD-MTX is related to the dose of concurrently administered oral 6MP [21, 28-30]and can be avoided by reductions of the dose of 6MP in the weeks before and after HD-MTX[31].

On the other hand, there is gradual increase in hematologic toxicity and myelotoxicity after 9 days of MTX infusion with repeated MTX administrations. These results were compatible with other previous reports[6, 32].**Rask, C., et al**[6]have been demonstrated that increase in myelosuppression in subsequent cycles of MTX may be related to the accumulation of cytotoxic metabolites of MTX and 6MP. In addition, other studies [21, 29]referred to using high dose MTX with increased doses of 6MP may also increase hematologic toxicity.

Interestingly, this study revealed that there is no correlation between MTX plasma concentration after 42 hour and hematologic toxicity.These results were supported by **Özdemir et al.**[32]. Theydocumented that the MTX levels at 42 h in patients with myelotoxicity were not different from patients without toxicity. Additionally, **Csordas et al.**[33]did not find any correlation between myelotoxicity and the levels of serum MTX. However, other studies reported that there is a relationship between elevated serum MTX levels and hematologic toxicity[6].

This study explore that there is a gradual increase in MTX delayed elimination with regard to MTX cycles. This may be consistent with the results of **Bauters et al.**[34]. They reported that high MTX levels (72 h) were frequently observed upon intake of cola beverages. Higher MTX levels were more common after intake of cola during the first and/or second day after the start of HD-MTX infusion. **Santucciet al.**[35]explained thatCola beverages have a low pH due to their phosphoric acid content and that may explain its effect on MTX elimination.

CONCLUSION

There is gradual decrease in myelotoxicity after 5 days of MTX administration with regard to MTX cycles. However, there is gradual increase in myelotoxicity after 9 days of MTX administration with regard to MTX cycles. There is no correlation between MTX plasma concentration after 42 h and hematologic toxicity. Therefore, we cannot depend on MTX levels at 42 h to anticipate and predict hematologic toxicity. Moreover, there is a gradual increase in MTX delayed elimination with regard to MTX cycles.

Table1: Toxicity criteria according to the Common Terminology Criteria for Adverse Events 2010 guideline

	Grade 1	Grade 2	Grade 3	Grade 4
Hb (g/L)	LLN-10	8-10	<8	Life threateninganemia
ANC(x10^9//L)	LLN-1.5	1.5-1	1-0.5	<0.5

Hb: Hemoglobin, ANC: Absolute neutrophil count and LLN: lower limit of normal.

Table 2: TLC, ANC, RBCs, Hb, and Platelets concentrations before and following 5 and 9 days of administration of MTX in ALL patients. All results were expressed as mean ± SD.

	Before MTXInfusion	5 days	9 days
TLCx10^3/µL	3.6 ± 1.07	2.76 ±1.03*	2.93 ±1.13*
ANC/µL	1835 ±709.9	1416 ±538.9*	1462±578*
RBCs x10^6/µL	3.6 ±0.5	3.3 ±0.46*	3.4±0.53 [a]
Hb(g/dL)	10.56 ±1.36	10.03 ± 1.422[a]	10.37 ±1.28
PLTx10^3/µL	291 ±111.9	348.7 ±138.2[b]	303.5 ±120.5

TLC, Total leukocyte count, ANC, Absolute neutrophil count, RBCs, Red blood count, Hb,Hemoglobin.

Significantly different from before MTX administration at *p< 0.0001, [a]p<0.05and [b]p<0.01

Table 3: Toxicityfrequencies after 5 days of MTX administration with regard to MTX cycles in ALL patients.

	1st MTX (n=28)	2nd MTX (n=28)	3rd MTX (n=28)	4th MTX (n=18)
ANC<1 x10^9/L	2(7.1%)	4(14.3%)	2(7.1%)	1(5.5%)
Hb< 10 g/L & ANC<1 x 10^9/L	9(32.1%)	7(25%)	3(10.7%)	2(11.1%)
Myelotoxicity%	39.2%	39.3%	17.8%	16.6%

Hb, Hemoglobin and ANC, Absolute neutrophil count.

Table 4: Toxicity frequencies after 9 days of MTX administration with regard to MTX cycles in ALL patients.

	1st MTX (n=28)	2nd MTX (n=28)	3rd MTX (n=28)	4th MTX (n=18)
ANC<1 x109/L	1(3.6%)	4(14.3%)	7(25%)	3(16.7%)
Hb< 10 g/L & ANC<1 x 109/L	7(25%)	7(25%)	3(10.7%)	3(16.7%)
Myelotoxicity%	28.6%	39.3%	35.7%	33.4%

Hb, Hemoglobin and ANC, Absolute neutrophil count.

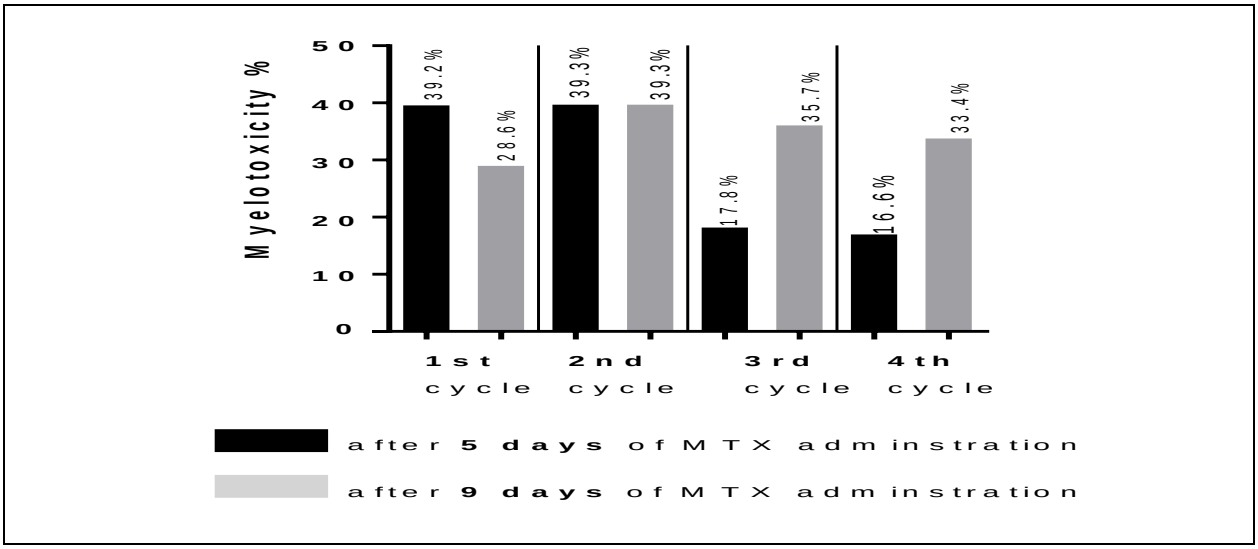


Figure1: Myelotoxicity percent in different high dose MTX cycles after 5 and 9days of MTX administration in ALL patients

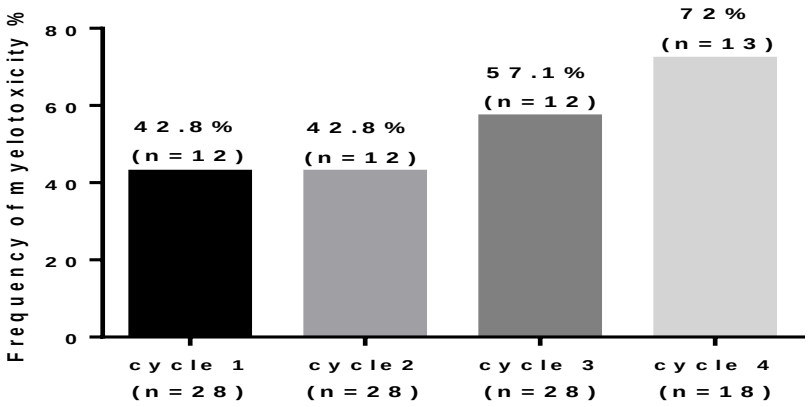
Table 5: Correlation of MTX levels at 42 hour in patients with and without hematologic toxicity after 5 days of MTX infusion and distribution with reference to the cycles. All values were expressed as mean ± SD.

	Patient with Myelotoxicity	Patient without Myelotoxicity	*P* value
Cycle 1(n=28)	1.01 ± 0.37**(n=11)**	0.96 ± 0.37**(n=17)**	>0.05
Cycle 2(n=28)	0.99 ± 0.35**(n=11)**	0.82 ±0.32**(n=17)**	>0.05
Cycle 3(n=28)	0.88 ± 0.27**(n=5)**	0.93 ±0.34**(n=23)**	>0.05
Cycle 4(n=18)	0.96 ± 0.33**(n=3)**	1.04 ±0.29**(n=15)**	>0.05

Table 6: Correlation of MTX levels at 42 hour in patients with and without hematologic toxicity after 9 days of MTX infusion and distribution with reference to the cycles. All values were expressed as mean ± SD.

	Patient with Myelotoxicity	Patient without Myelotoxicity	*P* value
Cycle 1(n=28)	0.94 ±0.40**(n=8)**	0.96 ±0.38**(n=18)**	>0.05
Cycle 2(n=28)	1.05 ±0.36**(n=11)**	0.79 ±0.29**(n=17)**	>0.05
Cycle 3(n=28)	1.00 ± 0.32**(n=10)**	0.88 ±0.33**(n=18)**	>0.05
Cycle 4(n=18)	0.85 ±0.28**(n=6)**	1.11 ±0.25**(n=12)**	>0.05

Figure (2): Percentage of delayed MTX elimination with regard to MTX cycles in ALL patients



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