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Original Research Article

PROSPECTIVE EVALUATION OF TOXICITY OF HIGH DOSE METHOTREXATE IN ALL VS OSTEOSARCOMA IN A TERTIARY CARE HOSPITAL IN WESTERN INDIA

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Abstract

Background: Methotrexate (MTX) is one of the essential antimetabolite agents and HDMTX is used in the treatment of various childhood and adult cancers like ALL, Burkitt's lymphoma, osteosarcoma, PCNSL (Primary CNS Lymphoma), etc. leading to significant improvement in long term survival of the patients. HDMTX can be administered safely in patients with normal renal function by using alkalinization of urine, hydration, leucovorin rescue, and also by monitoring blood levels of MTX. **Materials and Methods:** This is an observational prospective study of clinical and biochemical variables with HDMTX in ALL and osteosarcoma. The study population included 41 patients with 244 infusions of HD-MTX. The study group A consisted of 31 patients who are diagnosed as acute lymphoblastic leukemia receiving BFM-90(Berlin Frankfurt-Munster) Consolidation with HD-MTX 5gm/m^2 over 24 hours continuous infusion (total 124 infusions). Group B consisted of 10 patients who are diagnosed as osteosarcoma receiving MAP (methotrexate, doxorubicin, cisplatin) protocol with HD-MTX 12gm/m^2 over 4 hours (total 120 infusions). **Result:** In present study of 41 patients with 244 infusions of HD-MTX showed that there were around 70% males and 30% females in both groups. The median age of group A was 15.65 years and group B was 17.9 years. There was significant difference in toxicities of leukocytopenia, neutropenia, transaminitis and the level of plasma MTX levels after 24 hours of completion of chemotherapy. There was no significant difference between anemia, thrombocytopenia, bilirubin levels, mucositis and serum creatinine between both groups. **Conclusion:** Adequate hydration, alkalinization and leucovorin rescue with therapeutic drug monitoring of MTX levels are essential to deliver the drug safely in all age groups without life threatening complications in the resource limited countries like India.

INTRODUCTION

The success in the treatment of acute lymphoblastic leukemia (ALL) has increased steadily since 1975. The five-year survival rate has increased over from 60% to 90% in children and from 28% to more than 75% for adolescents between 1975 and 2010. [1,2] The improved survival is due to intensive chemotherapy. Treatment of ALL consists of induction, consolidation, reinduction, maintenance, and CNS prophylaxis. High-dose methotrexate (HDMTX) forms the backbone of systemic and intracranial treatment during consolidation. [3,4] Recurrence is still the leading cause of failure in ALL. Tailoring treatment based on risk stratification is followed in BFM protocol to reduce relapses and also treatment-related mortality.

Initially, osteosarcoma was thought to be a chemo-resistant tumor until 1970 when the studies disproved it by showing sensitivity to HDMTX, doxorubicin, and cisplatin.[5] Favorable results were observed in disease free survival in patients treated with these agents in comparison to historical surgery alone (40% vs 20%).[6] In multivariate analysis, HDMTX was the

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most important factor in predicting outcome in non-metastatic osteosarcoma.[7]

Methotrexate (MTX) is one of the essential antimetabolite agents and HDMTX is used in the treatment of various childhood and adult cancers like ALL, Burkitt's lymphoma, osteosarcoma, PCNSL (Primary CNS Lymphoma), etc. leading to significant improvement in long term survival of the patients. HDMTX can be administered safely in patients with normal renal function by using alkalinization of urine, hydration, leucovorin rescue, and also by monitoring blood levels of MTX. Despite these precautionary measures, HDMTX induced acute kidney injury (AKI) is seen in 2-12% of patients.[8] Prolonged renal dysfunction leads to increased MTX levels causing myelosuppression, mucositis, hepatotoxicity, and finally leads to multiorgan failure. [9,10] Hence, prompt treatment of renal dysfunction is essential to prevent these life-threatening toxicities.

This is an observational prospective study of clinical and biochemical variables with HDMTX in ALL and osteosarcoma. There is limited data of HDMTX complications from developing countries like India, wherein besides the major risk factors, pharmacokinetic variables, socioeconomic status and also environmental factors might play a significant role.

The aim and objective is to study toxicity profile of high dose methotrexate in treatment of ALL and osteosarcoma, i.e. renal toxicity, liver toxicity, anemia, leukocytopenia, neutropenia, thrombocytopenia, mucositis and skin toxicity.

Background and Rationale

Methotrexate is an antimetabolite that is polyglutamated intracellularly and the methotrexate polyglutamates binds to dihydrofolate reductase (DHFR) with 1000 times more affinity than folate leading to competitive inhibition of dihydrofolate to tetrahydrofolate. Tetrahydrofolate is required for synthesis of purines and thymidine and its deficiency leads to decrease in DNA synthesis, repair and cellular replication. [11,12] Methotrexate is cell cycle specific and acts in the S phase of cell cycle.

Methotrexate is an antimetabolite whose dosage ranges from 12mg for intrathecal injection, 20mg/m^2 orally or 0.5-12gm/m^2 intravenously for various cancers.[10] HDMTX is defined as doses of 500mg/ m^2 or higher given as intravenous infusion over 1-36 hours for a variety of childhood and adult cancers like ALL, osteosarcoma, PCNSL, and Burkitt's lymphoma.[11,12] The rationale for using HDMTX is based on: 1) the potential to overcome the mechanisms of resistance such as impaired cellular uptake, dose dependent increased formation of MTX polyglutamate derivatives, 2) the achievement of high concentration in sanctuary sites, and 3) selective rescue of normal tissues. HDMTX treatment can cause significant toxicity leading to increased morbidity, occasional mortality and also delay treatment leading to inferior outcomes.[10] This can be prevented by rigorous standardized supportive care, that differs for various cancers and treatment protocols.[10]

The critical determinants of MTX cytotoxicity are drug concentration and also duration of exposure. High concentrations of MTX may be well tolerated for short periods of time, whereas prolonged exposure can result in life-threatening toxicity. The toxicity observed with MTX is a function of this concentration-time dependence. Exposure to high concentrations of MTX for minutes to hours may lead to acute renal, central nervous system, and liver toxicity, whereas exposure to MTX concentrations as low as 0.01 and 0.005 μM for >24 hours may result in bone marrow and gastrointestinal epithelial toxicity, respectively.[13]

Prevention and management of dose intense MTX toxicity is by hydration, urinary alkalinization, avoiding concomitant drugs that interfere with methotrexate clearance, leucovorin rescue and by monitoring plasma MTX levels.

MATERIALS AND METHODS

The present study was a prospective observational study consisting of cancer patients diagnosed with ALL and osteosarcoma, those who are treated with HD-MTX. The study sample consists of patients who are attending Cancer Research Institute, to the Department of Medical Oncology during the two-and half-year period of observation. The necessary permission and approval were taken from Institutional Review Committee to conduct the study. The study details were explained to the patients and consent for participation in the study was taken.

Inclusion criteria

1. Patients who underwent treatment with High Dose Methotrexate in various cancers like ALL & Osteosarcoma.
2. Age group:<60years
3. Patients who were willing to give the consent.

Exclusion criteria

1. Patients with preexisting renal dysfunction-Serum Creatinine value >1.4 mg/dl or Creatinine clearance less than 70 ml/minute
2. History of any neurological disorder including epilepsy
3. Patients who did not complete planned HD-MTX were excluded from the study

The study population included 41 patients (Figure 1) with 244 infusions of HD-MTX. The study group A consisted of 31 patients who are diagnosed as acute lymphoblastic leukemia receiving BFM-90 Consolidation with HD-MTX 5gm/ m^2 over 24 hours continuous infusion (total 124 infusions). Group B consisted of 10 patients who are diagnosed as osteosarcoma receiving MAP protocol with HD-MTX 12gm/m^2 over 4 hours (total 120 infusions). Leucovorin rescue was started after 18-24 hours after completion of MTX infusion at 15mg/m^2 and

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continued till total of 16 doses. The dose adjusted according to the clearance of MTX.

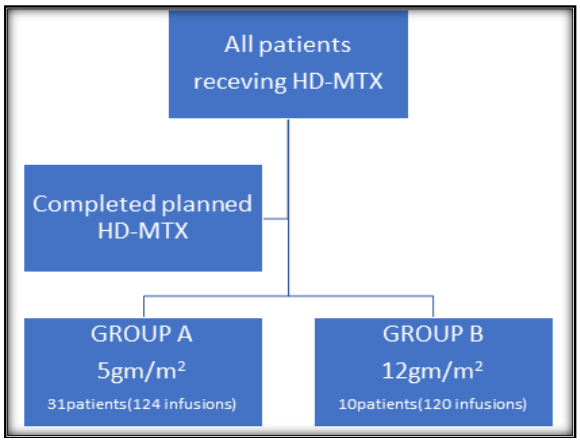

Figure 1: Total patients (ALL -Group A vs Osteosarcoma-Group B) receiving HD-MTX infusions.

Following HD-MTX infusion, toxicities like mucositis, cytopenia's, liver function tests, febrile neutropenia and days to complete treatment schedule were noted. G-CSF and blood components are given as and when required. Febrile neutropenia was treated with antibiotics and supportive care. Chemotherapy was delayed until the recovery of counts or liver function tests.

Methotrexate Assay: Quantitative analysis of methotrexate in plasma was done by EDTA sample on Roche Cobas 6000 using photometric measuring unit c-501 module and siemens Dade Methotrexate reagent. Plasma methotrexate levels are measured after 24 hours after completing MTX infusion. If the plasma MTX level was ≥0.6, then the test is repeated after 24 hours.

All statistical analysis was performed by using SPSS trail version 16 and MS-Excel 2007. Qualitative variables were expressed as frequencies and in percentages and quantitative variables were expressed as in mean and standard deviations. Chi-square test was used for examining the qualitative data. Student t test was used for means comparison. For all statistical analysis $p<0.05$ was considered as statistically significant.

RESULTS

This study consisted of two groups: Group A and Group B. Group A consisted of 31 patients (total 124 infusions) who are diagnosed as ALL and who received BFM-90 protocol with consolidation consisting of HD-MTX given at 5 gm/ m^2 as 24 hours infusion. Group B had 10 patients (total 120 infusions) who were diagnosed as non-metastatic osteosarcoma and received MAP protocol with HD-MTX given at 12gm/ m^2 over 4 hours infusion.

[Table 1] There are 87.1% patients with age ≤30 years and 12.9% with age >30 to ≤60 years in group A. group B consisted of 100% patients between 16 to 30 years.

[Table 2] Males were about 70.9% and females were about 29.1% in group A. There are 30% females and 70% males in group B.

[Table 3] On comparison of group A (124 infusions) and B (120 infusions), there was significant difference in toxicities of leukocytopenia, neutropenia, transaminitis and the level of plasma MTX levels after 24 hours of completion of chemotherapy. There was no significant difference between anemia, thrombocytopenia, bilirubin levels, mucositis and serum creatinine between both groups.

Table 1: Comparison of age distribution of patients studied.

Age group(years)	Group A(n=31)	Group B(n=10)
≤5	8(25.8%)	-
6 to 15	5(16.1%)	-
16 to 30	14(45.2%)	10(100%)
>30 to ≤60	4(12.9%)	-
Total	31	10
Mean± SD	15.65± 10.95	17.9± 2.60

Table 2: Comparison of gender distribution of patients studied.

Gender distribution	Group A	Group B
Female	9(29.1%)	3(30%)
Male	22(70.9%)	7(70%)
Total	31	10

Table 3: Comparison of various parameters between group A and B.

Parameter		GROUP A 124 Infusions	GROUP B 120 Infusions	p- value
Creatinine(>1mg/dl)		2(1.6%)	1(0.8%)	0.9772
MTX (≥0.6µmol/L)		48(38.7%)	18(15%)	0.00003*
Haemoglobin (gm/dl)	<8	1(0.8%)	3(2.5%)	0.5466
	8-10	22(17.7%)	23(19.2%)	
	>10	101(81.5%)	94(78.3%)	
TLC	Normal	72(58.1%)	88(73.3%)	0.0188*
	Grade I	19(15.3%)	20(16.7%)	
	Grade II	19(15.3%)	8(6.7%)	
	Grade III	13(10.5%)	4(3.3%)	
	Grade IV	1(0.8%)	-	

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ANC	Normal	72(58.1%)	91(75.8%)	0.0100*
	Grade I	14(11.3%)	11(9.1%)	
	Grade II	12(9.7%)	9(7.5%)	
	Grade III	23(18.5%)	7(5.8%)	
	Grade IV	3(2.4%)	2(1.7%)	
Platelets	Normal	119(96%)	111(92.5%)	0.2526
	Grade I	4(3.2%)	3(2.5%)	
	Grade II	-	3(2.5%)	
	Grade III	1(0.8%)	1(0.8%)	
	Grade IV	-	2(1.7%)	
Transaminitis	Normal	113(91.2%)	85(70.8%)	0.0005*
	Grade I	8(6.4%)	28(23.3%)	
	Grade II	1(0.8%)	5(4.1%)	
	Grade III	2(1.6%)	2(1.7%)	
	Grade IV	-	-	
Bilirubin	Normal	120(96.8%)	117(97.5%)	0.9174
	Grade I	3(2.4%)	2(1.7%)	
	Grade II	1(0.8%)	1(0.8%)	
	Grade III	-	-	
	Grade IV	-	-	
Mucositis	Grade I	89(71.8%)	76(63.3%)	0.2076
	Grade II	30(24.1%)	31(25.8%)	
	Grade III	5(4%)	11(9.1%)	

* p value <0.05 statistically significant.

DISCUSSION

HD-MTX is defined as usage of greater than 500mg/m^2. Acute toxicities of HD-MTX treatment are often unexpected and not dose dependent. In this study, we evaluated the toxicity profile of HD-MTX in various cancers.

Renal function derangements: MTX is excreted primarily by renal excretion. The majority of patients with renal dysfunction are asymptomatic and present with non-oliguric renal failure. So, MTX induced renal dysfunction leads to delay in elimination of MTX leading to various toxicities like mucositis, myelosuppression, transaminitis and finally organ failure.[14-16] Treatment should include continued monitoring of plasma methotrexate levels, administration of leucovorin and alkalinized intravenous fluids until plasma levels is less than 0.05 micromol/L.[16]. In the present study, serum creatinine was raised after HD-MTX in 1.6% and 0.8% infusions in group A and B respectively. There was no significant difference between the two groups. Warrier A. R. et al,[17] in their study showed doubling of creatinine in 0.9% of the 240 infusions. In another study by Holmboe L. et al,[18] there was grade I creatinine toxicity in 3% and grade II in 2% (out of 288 cycles).

Myelosuppression: HD-MTX is myelosuppressive and leucovorin is required to decrease the toxicity.[19] Myelotoxicity was 32.3%in group A and 20% in group B in the current study .In the study done by Ozdemir Z. C. et al,[20] there was myelotoxicity of 35.2% ,37.5% and 33.8% in the 1gm/ m^2, 2gm/ m^2 and 5gm/ m^2 respectively which are nonsignificant.

1. Haemoglobin

Among group A, there are 18.5% (23 infusions) with haemoglobin less than 10gm/dl. In group B, there are 21.7% (26 infusions) with haemoglobin less than 10gm/dl. There is no significant difference between both groups (p-value=0.5466). Ozdemir Z. C. et al,[20] in their study showed that the haemoglobin< 10gm/dl occurred in 30.8% in 1gm/ m^2 group, 30.3% in 2 gm/ m^2 and 33.8% in 5 gm/ m^2.

2. Total leukocyte count

In group A, grade I, II, III and IV leukocytopenia are seen in 15.3%, 15.3%, 10.5% and 0.8% respectively (62 infusions), whereas in group B, the grade I, II and III leukocytopenia are 16.7%,6.7% and 3.3% respectively (32infusions) with p-value of 0.0188.

3. Absolute neutrophil count

The neutropenia grade I, II, III and IV were 11.3%, 9.7%, 18.5% and 2.4% respectively in group A and 9.1%, 7.5%, 5.8% and 1.7% respectively in group B. The p-value is 0.01. Holmboe L. et al,[18] in their study showed that leukocyte nadir was 5% (grade I), 30% (grade II), 27% (grade III) and 6% (grade IV). Ozdemir Z. C. et al,[20] in their study of ALL patients showed that ANC< $1*10^9$/L was seen in 23.5%, 19.6% and 20.5% in 1gm/ m^2 group, 2 gm/ m^2 and 5 gm/ m^2 respectively. In an Indian study by Kapoor G et al,[21] neutropenia was 40.15% (grade I and II) and 2.22% (grade III and IV).

4. Platelet count

The thrombocytopenia in group A with grade I and III was 3.2% and 0.8%. In group B, grade I, II, III and IV toxicity were 2.5%, 2.5%, 0.8% and 1.7% respectively. The p- value was 0.2526. Warrier A. R. et al,[17] compared toxicity and tolerability between 3gm/ m^2 and 5gm/ m^2 HD-MTX in 60 ALL patients using BFM 86 protocol, in which thrombocytopenia grade 2 or more was 6.6%. Holmboe L. et al,[18] in their study showed that Thrombocyte nadir was 20% (grade I), 7% (grade II), 10% (grade III) and 10% (grade IV). Ozdemir Z. C. et al,[20] in their study of ALL patients showed that Platelets< $75*10^9$ /L was seen in 4.4%, 10.7% and 1.4% in 1gm/m^2 group, 2 gm/ m^2 and 5 gm/ m^2 respectively.

Liver function derangements: There was no significant difference in the bilirubin levels between

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two groups and the toxicity was grade I and II with 3.2% in group A and 2.5% in group B.

The transaminitis grade I, II and III occurred in 6.4%, 0.8% and 1.6% respectively in group A and 23.3%, 4.1% and 1.7% respectively in group B with a p-value of 0.0005. Mandal. P et al,[14] in their study showed that hepatotoxicity was 0.8% in Capizzi MTX and 1.9% in HD-MTX. A study by Ozdemir Z.C. et al,[20] showed that hepatotoxicity grade ≥3 occurred in 13.2%, 12.2% and 11.2% in the respective groups of 1gm/ m^2 group, 2 gm/ m^2 and 5 gm/ m^2. Another study by Kapoor G. et al,[21] transaminitis grade I,II was 31.78% and grade III,IV was 3.1%.

Mucositis: Mucositis is the most common complication in all protocols of HD-MTX. Grade 1 was seen in majority of the infusions (71.8% in group A and 63.3%in group B), grade II in 24.1% in group A and 25.8% in group B, whereas grade III was seen in 4% and 9.1% respectively in group A and B.In a study by Kapoor G et al,[21] there was mucositis grade I and II in 33.6% and grade III and IV in 5.37% of the infusions. Various factors for mucositis are inadequate hydration, delay in elimination of MTX, old age, and co administration of 6-MP are present.[22] Ferdousi S. et al,[23] in their study showed that oral mucositis was seen in 56.2% of patients and grade III occurred in 8.3%. Rask C. et al,[24] in their study showed mucositis in 52% of the infusions.

Other toxicities: There was febrile neutropenia in 3 infusions (2.5%) and rash in 2infusions (1.6%).In group B, febrile neutropenia was seen after 4 infusions (3.3%) and rash was seen in 3 infusions (2.4%). There was no mortality associated with toxicity due to HD-MTX in group A and B. Tiwari et al,[25] in their study showed that HD-MTX caused 5.6% neutropenia and 4.4% rash.

Analysis of MTX levels: Plasma MTX levels is most important in determining toxicity in treatment with HD-MTX. The MTX levels ≥0.6 µmol/L were seen between group A and group B and there was 38.7% in 5gm/ m^2 at 24-hour infusion, and 15% in 12 gm/ m^2 at 4-hour infusion. There was statistical significance between both groups (p- value 0.00003). In spite of significant p- value there was no mortality or treatment discontinuation due to organ dysfunctions. Rask C.et al,[24] in their single arm study of HD-MTX in ALL showed that delayed MTX elimination (p-MTX42h> 1µM or p-MTX66h>0.2 µM) was seen in 36% of the cases.

In the current study, on comparison of group A (124 infusions) and B (120 infusions), there was significant difference in toxicities of leukocytopenia, neutropenia, transaminitis and the level of plasma MTX levels after 24 hours of completion of chemotherapy. There was no significant difference between anemia, thrombocytopenia, bilirubin levels, mucositis and serum creatinine between both groups. Small study sample could be a limitation of present study. Hence study results cannot be generalized to normal population. Exclusion of patients who could not complete planned HD-MTX schedule due to COVID19 pandemic and lockdown could be a draw back. Other limitations of the present study could be compliance of patients due to long course of treatment, lack of literacy and socio-economic factors in a developing country.

Future studies in therapeutic research could focus on determining the long-term follow-up necessary to understand the long-term negative effects connected to HD-MTX and usage of genetics to predict a patient's toxicity profile.

CONCLUSION

HD-MTX is an important component of therapy for a variety of malignant conditions. The major risk with HD-MTX is MTX induced renal dysfunction due to delayed excretion of the drug leading to increased plasma levels and systemic toxicity. In our study of 41 patients with 244 infusions of HD-MTX showed that there were around 70% males and 30% females in both groups. The median age of group A was 15.65 years and group B was 17.9 years. There was significant difference in toxicities of leukocytopenia, neutropenia, transaminitis and the level of plasma MTX levels after 24 hours of completion of chemotherapy. There was no significant difference between anemia, thrombocytopenia, bilirubin levels, mucositis and serum creatinine between both groups. Adequate hydration, alkalinization and leucovorin rescue with therapeutic drug monitoring of MTX levels are essential to deliver safely in all age groups without life threatening complications in the resource limited countries like India.

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