Original Article

Infections during high dose methotrexate therapy in childhood acute lymphoblastic leukemia (ALL)

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Abstract

High-dose methotrexate (HDMTX) therapy is an effective therapy for childhood ALL, which is frequently associated with side effects like infection in our centre. It prolongs hospital stay, delays chemotherapy and causes more economic burden on patients. This study was done to identify the incidence, risk factors and severity of infection among children with ALL during HDMTX therapy. This prospective observational study was conducted among 50 patients suffering from acute lymphoblastic leukemiascheduled to receive HDMTX at the Department of Pediatric Hemato-oncology of BSMMU. It was carried out from January 2012 to June 2012. The end result showed, 19 (38%) patients suffered from infection; among them microbiologically documented infections found in 9 occasions, where gram negative bacilli - E.coli 4(44.4%) & Pseudomonas 3(33.33%) were predominant organisms. Gastrointestinal tract (GIT) was the most common site (8,42.11%). The rate of infection is significantly higher in children <5 yrs(78.94%). Mortality rate was 6%. As the infection and case fatality rate is quite high with HDMTX therapy, we recommend to use this drug with caution.

Key wards: Childhood ALL, high-dose methotrexate therapy, infection

Introduction:

Childhood leukemias are the most common malignant neoplasm, accounting for about 41% of all malignancies.[1,2] The incidence of leukemia in children up to the age of 15 years is approximately 1.5 in 1,00,000 in western countries.

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Acute lymphoblastic leukemia (ALL) accounts for about 77%, acute myelogenous leukemia (AML) 11% and chronic mixed leukemia 3-5% of all cases of childhood leukemia.[1] ALL has a peak incidence between 2-6 years age and occurs more frequently in boys than girls at all age.

Only 20 years ago, this disease was fatal within 6 months of diagnosis in more than 80% of cases and within 2 years almost all patients died. With the modern multimodal therapy including improved supportive care 70%-80% patients have attained remission lasting more than five years and many of them are cured.[3] Survival in acute lymphoblastic leukemia (ALL) has improved in recent decades due to recognition of the biologic heterogeneity of ALL, utilization of risk-adapted therapy and development of protocols that include optimized chemotherapy combinations, effective central nervous system (CNS) prophylaxis, post-induction intensification of therapy and a prolonged maintenance phase of treatment.[4] High-dose methotrexate (HDMTX) therapy with long-term intrathecal injection has been generally accepted as an effective regimen for the prevention of central nervous system involvement in children with acute lymphoblastic leukemia (ALL) because similar results can be obtained in preventing central nervous system leukemia as radiotherapy with relatively lower adverse effects.[5,6,7]

The anti folates (e.g. Methotrexate) produce the first striking remission in leukemia and produce fast cure of a solid tumor choriocarcinoma.[8] Higher systemic doses have contributed to improved control of testicular and medullary disease.[9] For cancer, methotrexate allosterically inhibits dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolate synthesis[10] thereby, inhibits DNA, RNA, and protein synthesis. It has a greater toxic effect on rapidly dividing cells (gastrointestinal or oral mucosa malignant and myeloid cells) which replicate their DNA more frequently and thus inhibits the growth and proliferation of these noncancerous cells.[11] It can be taken orally or administered by injection (intramuscular, intravenous, subcutaneous, or intrathecal).

Myelosupression is the most common side effect due to cytotoxic chemotherapy including MTX therapy in children with ALL.[1,12,13] Fever in acute leukemia is generally due to

infections. Patients with neutropenia secondary to chemotherapy induced marrow failure are at great risk of overwhelming bacterial infection especially Gram positive skin flora including Staphylococcus Albus, Staphylococcus Aureus and staphylococcus Epidermidis & Gram negative bowel organisms including E coli, Protease, klebseilla and Pseudomonas.[3] Empirical antibiotic therapy should be administrated promptly to all neutropenic patients at the onset of fever.[14] Infection generally occur when absolute neutrophil count (ANC) is <500/cmm and those with ANC <100/mm3 are at greater risk. Prolong and severe neutropenia increases risk of fungal infections.[15,16]

Bacterial and fungal infections are major causes of morbidity & mortality in children during chemotherapy for ALL. Infections increase the risk of death and may also increase the risk of relapse due to interruption of treatment and need for a decrease in the dose of chemotherapeutic agent.[17] Nosocomial infections are important clinical complication as it can cause considerable morbidity and mortality; thus increase hospital stay and healthcare cost. Monitoring nosocomial infection rate is of great importance to establish preventing measures.[18]

Commonly used regimens for childhood ALL in our centre include high-dose methotrexate, where, the problem of HDMTX associated infection is frequently faced. It prolongs the hospital stay, delays the chemotherapy and causes more economic burden on patients. In our limited resource and limited hospital seats and other facilities, it imparts a negative impact on treatment of other children as well. Surprisingly, published data about the incidence and characteristics of infectious complications in patients undergoing HDMTX therapy for ALL are limited. The results of this prospective observational study will help to improve supportive care strategies in patients undergoing therapy for ALL to decrease morbidity and mortality and thus to improve overall survival.

Methods

This was a prospective observational study performed in the inpatient ward of the department of Pediatrics Hemato-oncology, BSMMU to identify the incidence, common site, course, severity and main pathogens of infection during HDMTX therapy from January 2012 to June 2012 over a period of six month. Fifty children with a age range of 1-15 yrs who were diagnosed as ALL and scheduled to receive HDMTX with Leucoverin rescue at the Department of Pediatric Hematology and Oncology, BSMMU during the study period were enrolled in this study prospectively. Patients with ALL under treatment who already had fever or other signs of infections were excluded. Baseline investigations were done prior to administration of MTX therapy. Prehydration and posthydration was done with 3 Lit/m^2 i/v saline over 24 hours before and after giving MTX. Total dose of MTX was 2.5gm/m^2 in B cell leukemia and 5gm/m^2 in T cell leukemia; given over a period of 24 hours. 10% of total MTX was given in 1st hour and rest was given over 23 hours. Leucoverine rescue therapy was started after 42 hours of starting MTX at the dose of 15 mg/m^2/dose every 6 hourly total 6 doses. After enrolment, detailed history and physical examination was recorded in a pre-designed questionnaire. Complete blood count with differential count was done in the department of Pediatric Hemato-oncology. Blood culture and sensitivity and were done in the department of Microbiology of the same hospital. Blood culture was done by conventional method with 3 ml of venous blood drawn with strict asepsis in a sterile culture bottle. Follow up was done in daily basis starting from the day of infusion up to recovery or discharge from the hospital. Reports of all the relevant investigations were recorded in the questionnaire. Empirical antibiotic was started if infection was suspected (mostly 4th generation cephalosporin in addition to aminoglycosides) & changed subsequently according to culture sensitivity. Each infectious episode was correlated to the degree of neutropenia at its onset. The patients were classified as having very severe, moderate or no neutropenia when ANC was less than 100/mm^3(very severe), 100-500/mm^3(severe), 500-1000/mm^3(moderate), >1000/mm^3(no neutropenia).

If patient showed partial improvement or failure, modification of treatment was done by adding ceftipime and vancomycin in majority of episodes. Carbapenem with aminoglycoside were also given in few episodes. Amphotericin B was also given to few patients on clinical suspicion. After collection data was statistically analysed by using SPSS-16.

HDMTX was defined, in this study, when the dose of MTX was 2.5gm/m^2 body surface area in B cell leukemia and 5gm/m^2 in Tcell leukemia. Any patient having fever requiring antibiotic therapy; and/or any patient having clinical signs and symptoms of infection associated with isolation of a pathogen, or any patient having an identifiable site of infection by physical examination or imaging study was considered as having infection.

Results

A total of 50 patients of 1 year to 15 years of either sex were studied during the period. Among them maximum number (31, 62%) were found in the age group 1-5 years, 11(22%) belonged to 6-10 years and 8(16%) belonged to 11-15 years. Mean weight of patient was 19.09±8.69kg with a

range from 9-45kg. Regarding sex distribution, 32 (64%) were male and 18 (36%) were female. Male female ratio was 1.77:1. (Table-I)

Table-I: Demographic distribution of patients (n=50)

Factors	Infection		Mean±SD
Age	Number	Percentage	
1-5 years	31	62	5.78±3.66
6-10 years	11	22	
1-15 years	8	16	
Sex			
Male	32	64	
Female	18	36	
Weight			
<10kg	2	4	19.29±8.38
10-20kg	37	74	Range-
21-30kg	6	12	(9-45kg)
31-40kg	2	4	
41-50kg	3	6	

Infection occurred in 19 (38%) patient among total 50 patients. Twelve episodes (63.1%) of those had a particular foci, in 7 episodes (36.9%) fever occurred due to unknown origin.

Total 19 patients (38%) developed infection during HDMTX therapy. In maximum cases (63%) there was an obvious focus of infection. GIT was the most common site (8,42.11%). Other primary sites were without focus 7 (36.84%), respiratory system 6(31.58%), thrombophlebitis 2(10.53%), genito-urinary system 2(10.53%) and soft tissue infection 2(10.53%). (Table-II)

Table-II: Distribution of primary site of infections (n=19)

Primary site of infection	No of patients	Percentage
GIT	8	42.11
Respiratory system	6	31.58
No focus	7	36.84
Genitourinary System	2	10.53
Soft tissue infection	2	10.53
Thrombophlebitis	2	10.53

Culture report showed microbiologically documented infections in 9 occasions. Gram negative bacilli as E. coli 4(44.4%) and Pseudomonas 3(33.33%) were the predominant organisms here. Gram positive organism was also found. Staphylococcus aureus contributed in 2 (22.22%). (Table-III)

Table-III: Distribution of isolated bacteria according to the site of culture (n=9)

Organism	Blood culture	Swab from the skin lesion	Urine culture	Stool culture
E.coli	1	0	1	2
Pseudomonas	3	0	0	0
Staphylococcus aureus	0	2	0	0

Association among various factors related to infection showed that rate of infection is significantly higher (p value =0.01) in children of <5 year of age (78.94%). Male child (15, 78.94%) were more prone to infection than female child (4, 21.05%). (p=0.01). Among total 19 patient who suffered infections, 13(68.42%) had B-cell leukemia and rest 6(31.58%) had T-cell leukemia. Thirteen (68.42%) out of 19 patients got HDMTX at the dose of 2.5gm/m^2 and 6(31.58%) got at the dose of 5gm/m^2. (Table-IV)

Table-IV: Association of various factors with infection (n=19)

Factors	Infection		P value
Age	Number	Percentage	
< 5 years	15	78.94	Z = 2.226
> 5 years	4	21.05	P = 0.01
Sex			
Male	15	78.94	Z = 2.226
Female	4	21.05	P = 0.01
Type of leukemia			
B-cell	13	68.42	Z = 1.488
T-cell	6	31.58	P = 0.06
Dose of MTX			
2.5gm/m^2	13	68.42	Z = 1.488
5gm/m^2	6	31.58	P = 0.06

Data were analyzed by using Z-test

The analysis of the treatment outcome showed that in 6 patients (31.58%) infections resolved with the first line antibiotic combination (4th generation of Cephalosporin with or without aminoglycoside e.g. Ceftipime, Amikacin) while 10 (52.63%) required modification of treatment. Usually modification of therapy was done by adding ceftipime and vancomycin in majority of episodes. Carbapenem with or without aminoglycoside were also given in few episodes. Few episodes were treated with amoxicillin-clavulanic acid or ciprofloxacin with or without carbapenem. All these modifications were done according to culture & sensitivity results or on clinical ground. Amphotericin B was given in 4 episodes. Three (15.79%) patients out of 19 infected patients died in this study. Overall death rate was 6%. (Figure-1).

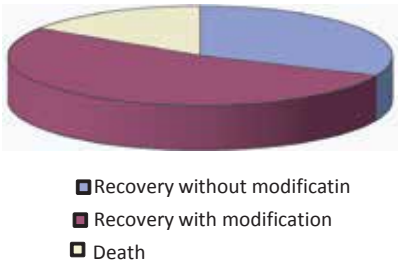

Figure- 1: *Outcome of the infected patients (n=19)*

About half 10(52.6%) of the infected children received treatment for 7 to 10 days, 4(21.1%) received 8-14 days, 2(10.5%) received 15-21 days and 3 (15.8%) of the infected children received treatment for >21 days. The mean (±SD) duration of treatment received was 12.05±7.18 days with a range from 7-28 days.(Figure-2).

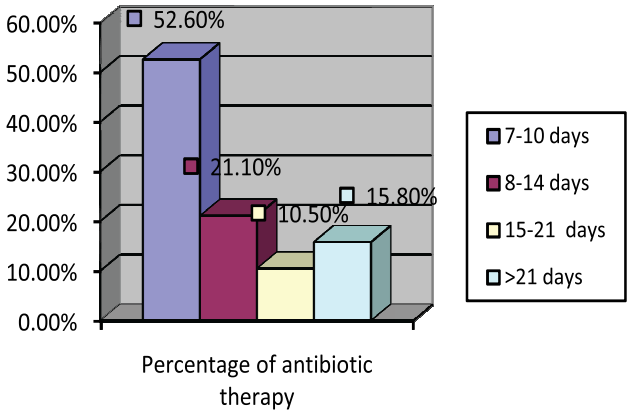

Figure-2: *Duration of antibiotic received during infectious episodes(n=19)*

All 19(100%) patients developed vomiting after receiving HDMTX. Among other features of infection, 8(42.10%) patients presented with oral mucositis, 7(36.84%) patients with abdominal pain, 6(31.58%) with diarrhea and 5(26.31%) patients with cough. Other features included hematemesis-melena in 4(21.05%), swollen cannula site in 3 (15.78%), respiratory distress in 3(15.78%), skin lesion in 3 (15.78%), convulsion in 2 (10.52%) and headache in 1(5.26%) patient. (Table-V)

Table-V: Clinical presentation of infected patients (n=19)

Clinical presentation	No of patients	Percentage
Cough	5	26.31
Vomiting	19	100.0
Abdominal pain	7	36.84
Diarrhoea	6	31.58
Mucositis	8	42.10
Skin lesion	3	15.78
Headache	1	5.26
Convulsion	2	10.52
Respiratory distress	3	15.78
Swollen cannula site	3	15.78
Haematemesismalena	4	21.05

This study showed very severe thrombocytopenia (<5000/mm^3) in 4 episodes (21.1%), severe thrombocytopenia (5000-10000/mm^3) in 4 (21.1%), moderate thrombocytopenia (10000-150000/ mm^3) in 1 (5.3%) and no thrombocytopenia (>150000/mm^3) in 10(52.6%) episodes. Twenty nine (58%) patients had total leukocyte count 4000-11000, 16 (32%) patients had total leukocyte count <4000 and rest 5 (10%) patients had total leukocyte count >11000/mm^3. (Table -VI).

Table-VI: Absolute neutrophil count (ANC) of the infectious episodes (n=19)

ANC/mm^3	No of infectious episodes	Percentage
0-100	4	21.1
100-500	2	10.5
500-1000	1	5.3
> 1000	12	63.2
Total	19	100.0
Mean±SD	2752.26±2542.72	
Range	(20-7690)/mm^3	

Discussion

Leukemia is the most common form of childhood malignancy having a high mortality rate due to disease process as well as due to chemotherapy induced complication. A retrospective study was done by Joseph VS, Elizabeth H and Warren J[19] to find out the cause of death among the patients who died during course of intensive combination chemotherapy including MTX. They found the proximate cause of death was infection (24 of 26 children in complete remission) and Pneumocystis carinii pneumonia was the primary cause of death. Where as bacterial infection was the primary cause of death during marrow remission reported by Nies et el[20], Jacques N and Judith MC[21] had done another retrospective study showing similar result. Children younger than 5 years had a higher risk of infection in this study. Here death due to infection occurred in 8% patients. Respiratory tract infections, both lower and upper, were the most common. Septicaemia was mostly due to gram negative agents. In 8.5% patients there was no obvious clinical cause of infection and microbiological investigations were negative.

Xu Weiqun et al[22] carried out a retrospective study in order to observe the morbidity of elimination delay in Chinese children with acute lymphoblastic leukemia during high-dose methotrexate (HDMTX) therapy and the toxicities in Children's hospital of Zhejiang University School of Medicine of Chaina between January 2000 and January 2003. The overall morbidity was 12.1% among a total of 497 infusions. Patients with elimination delay had lower platelet count (P<0.01) and greater cumulative CF rescuing intensity (P<0.001).

Another study done by R Jagarlamudi et al published on 2002 included 240 infective episodes in ALL patients showed that clinically documented infections were found in 52% cases with 48% isolated febrile episodes without focus. They found respiratory system as the most common site (35.7%) of infection.[23] A prospective study done on 1980 by Chessell MJ and Leiper AD which included both ALL and AML patient during their induction therapy showed that the incidence of infection was 40%; only 1% patient died. The organism responsible for most infections were Pseudomonas aeroginosa and Staphylococcus aureus. E.coli was also responsible for infection in their study.[24] Cwiekliska M et al also found 40%-50% incidence of infection in their study.[25] In another previous study done by Katsimpardi K et al[26] showed that Pseudomonas aeroginosa, Klebsilla pneumonia and E coli were the organisms most commonly isolated. Meir et al[27] found 37% microbiologically documented infection where gram positive cocci followed by gram negative bacilli were isolated in most of the cases.

In UK, a study conducted by Hutchison J H et al[28] to analyze treatment in childhood leukemia with methotrexate included 78 patients receiving CNS prophylaxis and 90 patients not receiving CNS prophylaxis. Study showed that proportion of patients with neutrophil counts below $1x10^9$/l were more in the prophylaxis group and emphasized the correlation between neutropenia and serious pyogenic infection in acute leukemia patient.

In an attempt to find out the incidence of infection in pediatric Acute Lymphoblastic Leukemia during High dose Methotrexate, this prospective observational study was done in 50 patients. Nineteen (38%) of them developed infection during HDMTX therapy which is comparable to the estimation of 40% reported by Chessells and Alison DL[24]during remission induction period of ALL patient and 40%-50% reported by Cwiekliska M et al.[25] The mean age of study population was 5.78±3.66 years. Study done by Santolaya M et al showed the mean age was 7±4 years.[29] Among the study group 64% cases were male; indicating male preponderance and among them 31[62%] were younger than 5 year; similar to the study done by Jacques N and Judith MC.

In a previous study done by Jagarlamudi R et al showed that clinically documented infections were 52% and 48% was isolated febrile episodes without focus.[23] Our study was different from most of the studies where respiratory system was the most common site of infection, whereas in our study, it is the second most common site.[23,30]

Microbiologically documented infections found in 9 occasions with predominance of gram negative bacilli such as E coli (4,44.4%) & Pseudomonas (3, 33.33%). Gram positive organism Staphylococcus aureus also contributed in 2 (22.22%) occasions in this study. This is comparable to Katerina Katsimpardiet al[26] which also showed predominance of gram negative organism (Pseudomonas , K pneumonia and E coli). Chessell MJ and Leiper AD showed that the organism responsible for most infections were Pseudomonas aeroginosa followed by Staphylococcus aureus and E coli[24], whereas Meir et al[27] found gram positive cocci followed by gram negative bacilli in most of the cases.

In our study we found that 31.58% episodes of infections resolved with the first line antibiotic combination (ceftipime, amikacin) and 52.63% episodes required modification of treatment.S.Mahmood et al[35] reported that,

72% episode resolved with first line antibiotics and 24% need modification. The main cause of high rate of modification of treatment in our centre may be due to rapid growth of bacterial resistance as well as due to co infection with other pathogen. The mean (±SD) duration of treatment received was 12.05±7 days in our study. More than half of the patient received antibiotics for 7-10 days. Average length of antibiotic therapy was 8.4 days in a study by Mahmood S et al.[31] Rodriguez V et al[32] suggests in their study that it is important in patients apparently responding to antibiotics to continue treatment for a minimum of 7-10 days even if cultures prove sterile; too early cessation of treatment may cause relapse of infection and death.

Here 3 patients died due to infectious complication during study period. Death due to infection occurred in 6% of patients among study population. Jacques N and Judith M C [21] found infection caused the death in 8% of patient which was almost similar to our result. Simone et al[33] in 1972 reported 16% fatality and Craft et el[34] in 1977 found 18% death due to infection. But in the study of Judith M C[21] and Leiper AD[24] only 1 patient (0.5%) died due to infection in 1980. Although mortality rate was decreased than previous years; still infection related death was unacceptably high.

Children younger than 5 years and male patients had a significantly higher rate of infection in our study. Jacques N and Judith M C[21] also found higher rate of infection in child below 5 years of age, although they did not found any significant difference of incidence of infection among male and female sex. As immune system is not well developed in this group of patient and upper respiratory tract infections are more severe in younger children[21], this may be the probable cause of high rate of infections in this age group.

In this study vomiting was the presenting feature in almost all the patient 19(100%), eight (42.10%) patients presented with oral mucositis, abdominal pain in 7(36.84%) patient, diarrhea in 6(31.58%), cough in 5(26.31%) patients. Other features include hematemesis-melena 4(21.05%), swollen cannula site 3(15.78%), respiratory distress 3(15.78%), skin lesion in 3(15.78%), convulsion in 2(10.52%) and 1(5.26%) patient presented with headache. These clinical features are almost similar to the study done by Charlotte R et al.[35] Mucositis was the most common GIT side effect according to them. Cheng KK[36] also found oral mucositis as a major toxicity associated with HD-MTX.

In our study we found only 16 out of 50 patient had WBC count below 4000/mm^3. Charlotte R et al[35] also did not found any clinical or pharmacokinetic correlation with drop of WBC count.

Near about half of the patients with infection showed mild to severe thrombocytopenia. The study also showed very severe neutropenia (0-100 mm^3/) in 21.1%, severe neutropenia (100-500/mm^3) in 10.5%, moderate neutropenia (500-1000/mm^3) in 5.3% and no neutropenia (>1000/mm^3) in 63.2% infectious episodes. Meir HM et al[27] found 31%, 44% and 25% episodes were associated with ANC <100/mm^3, ANC <500/mm^3, ANC >500/mm^3 respectively.

Comparison to our results with those published by other centers in the world has shown many similar and few different findings. From the current study we should stress the importance of frequency, severity and outcome of infections in such critically ill patients over the years in order to detect changing epidemiologic patterns.

Incidence of infections during HDMTX therapy is high. Mortality rate was also unacceptably high as per this study. Children younger than 5 years and male child are more prone to infection. Gram negative organism (E coli, Pseudomonus) were the main pathogen and GIT was the commonest site of infection according to this study.

This was a single centre study and there were some other limitations as well. So in future, study with large sample size and long study period should be done and the supportive care management of ALL during HDMTX therapy should be improved.

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