HIGH DOSE METHOTREXATE IN TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA: TOXICITY PROFILE AND COMPARISON OF TOLERABILITY BETWEEN TWO DOSAGE SCHEDULES

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CERTIFICATE

This is to certify that this dissertation on "**High Dose Methotrexate in treatment of Acute Lymphoblastic Leukemia:Toxicity Profile and Comparison of tolerability between two dosage schedules**" is a bonafide work done by **Dr. Arun R.Warrier,** in the Department of Medical Oncology, College of Oncological Sciences,Cancer Institute(WIA), Chennai, under my supervision and guidance, to my satisfaction

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CONTENTS

INTRODUCTION

The rate of success in the treatment of acute lymphoblastic leukemia (ALL) has increased steadily since the 1960s. The five-year event-free survival rate is nearly 80 percent for children with ALL and approximately 40 percent for adults.(1,2) Up to 80% of pediatric patients with acute lymphoblastic leukemia (ALL) can be cured if intensive therapy is applied. Severe side effects may be encountered in all patients of which, however, only the minority is life-threatening. The leading cause of failure in childhood ALL is still recurrence of disease.Deciding about treatment protocol after risk stratification as followed by BFM group is aimed at reducing relapses as well as treatment morbidity. One of the important components is the introduction of High dose Methotrexate in the consolidation phase. This combined with cranial radiotherapy in High risk group is postulated to prevent CNS relapses. (5,12).

Methotrexate (MTX) is one of the most widely used anti-cancer agents, and administration of high-dose methotrexate (HDMTX) followed by leucovorin (LV) rescue is an important component in the treatment of a variety of childhood and adult cancers. HDMTX can be safely

administered to patients with normal renal function by the use of alkalinization, hydration, and pharmacokinetically guided LV rescue. (15).Despite these measures, HDMTX-induced renal dysfunction continues to occur in approximately 1.8% of patients with osteosarcoma treated on clinical trials. Prompt recognition and treatment of MTX-induced renal dysfunction are essential to prevent potentially life-threatening MTX-associated toxicities, especially myelosuppression, mucositis, and dermatitis.(26,27).

Since the initiation of monitoring of MTX plasma concentrations and appropriate alterations in leucovorin dosage and hydration, the frequency of serious toxicity has been reduced and toxic deaths following HD-MTX have been virtually eliminated. This is an observational nonrandomized study of clinical and biochemical variables to identify the risk factors associated with high-dose MTX therapy.

There are limited data of complications resulting from treatment of ALL incorporating high dose Methotrexate from developing countries like India, wherein besides the major risk factors, pharmacokinetic variables, socioeconomic factors ,and other environmental factors also might play an important role.

AIMS

- To study the toxicity profile of high dose methotrexate in treatment of Acute Lymphoblastic Leukemia
- To compare between tolerability of 3gm/m^2 and 5 gm/ m^2 of high dose methotrexate

REVIEW OF LITERATURE

Background and Rationale

Acute Lymphoblastic leukemia has a cure rate nearing 80% in the developed world. Patients are broadly classified and treated as standard risk or high risk ased on age and white blood cell count (WBC).(1,2) Despite almost constant progress in the treatment of this disease, there are major concerns about the predictive values of current prognostic factors and the universal intensification of therapy.(3,4) .

Prognostic factors in ALL

Risk factor	Favorable	Unfavorable
Age	≥1 and ≤9 years	<1 or >9 years
Gender	Female	Male
WBC count at diagnosis	<50,000/mm^3	≥50,000/mm^3
DNA index	>1.16	≤1.16
Chromosome number per leukemic cell	>50	<45, especially 24-28
Induction response to prednisone on day 8	No peripheral blasts	Peripheral blasts
CNS status	CNS 1	CNS 2 or 3
Cytogenetics	Trisomies 4 and 10	t(4;11), t(9;22)
Molecular genetics	*TEL-AML1*	*MLL* gene rearrangements
Immunophenotype	Precursor B	T cell, mature B cell

Strategies responsible for the improvement in results include:

1) Prolongation of treatment with two or more sequential combinations of multiagent chemotherapy,
2) CNS prophylaxis
3) Stratification of patients according to prognostic factors that predict risk of relapse,
4) Risk-based intensification of therapy,
5) Large, controlled and usually randomized clinical trials.

Risk Directed Therapy in ALL

A breakthrough in the treatment of children with acute lymphoblastic leukemia (ALL) came with the introduction of treatments that could penetrate the CNS. Trials in the late 1960s and early 1970s established that children who received effective CNS-directed therapy had substantially superior event-free survival (EFS) and overall survival. (4,5)The treatments used were initially craniospinal irradiation and then cranial irradiation, usually at a dose of 24 Gy, with short-term intrathecal therapy. However, with long-term follow-up of large numbers of

children, it became apparent that there were late adverse effects, including growth and endocrine problems,(6,7) an increased risk of developing secondary tumors,(8,9) and possible neuropsychological sequelae.(10,11) With the development of alternative CNS-directed strategies, including variations in the radiotherapy dose and combinations of intrathecal treatment and high-dose intravenous methotrexate, the question is now whether alternatives expected to have fewer such side effects might be as effective for disease control.

BFM 86 PROTOCOL

The trial ALL-BFM 86 is the sixth multicenter trial in childhood acute lymphoblastic leukemia ,conducted by the Berlin-Frankfurt-Munster (BFM) group. 928 evaluable patients were enrolled in study ALL-BFM 86. The estimated 6-year EFS was 72% for the whole study population including all subsets of childhood ALL. Patients with T-cell ALL had a favorable EFS with this intensive treatment regimen The contribution of HD-MTX to the overall treatment results of the trial is difficult to judge. A major contribution of HD-MTX as a 24-hour infusion may be

protection of the CNS because cytotoxic steady-state concentrations are achieved in the cerebrospinal and are boosted by an intrathecal MTX application. Only 1.8% of the patients suffered from isolated CNS relapses.

METHOTREXATE

Methotrexate (MTX), a classical antifolate, is one of the most widely used and studied anticancer agents. Unlike other anticancer agents, MTX can be safely administered over a wide dose range, ranging from 20 mg/ m^2 per week in maintenance chemotherapy for acute lymphoblastic leukemia and when combined with leucovorin (LV) rescue, to doses of 1,000–33,000 mg/m^2 (13). The latter, termed high-dose methotrexate (HDMTX) is usually administered as a prolonged i.v. infusion and is an important component in the treatment regimens for a variety of cancers, including acute lymphoblastic leukemia, lymphoma, osteosarcoma, breast cancer, and head and neck cancer (14,15). HDMTX can be safely administered to patients with normal renal function by vigorously

hydrating and alkalinizing the patient to enhance the solubility of MTX in urine and through the use of pharmacokinetically guided LV rescue to prevent potentially lethal MTX toxicity.

MTX PHARMACOLOGY

Knowledge of MTX's mechanism of action and metabolism are important for understanding MTX-associated toxicities and treatment. MTX enters the cell via the reduced folate carrier and undergoes polyglutamation catalyzed by folyl-polyglutamate synthetase. Once polyglutamated, MTX is retained in cells for prolonged periods of time. Methotrexate and its polyglutamates block de novo nucleotide synthesis primarily by depleting cells of reduced tetrahydrofolate cofactors through inhibition of dihydrofolate reductase (DHFR) (Fig. 1) (17.) MTX polyglutamates and dihydrofolates that accumulate as a result of DHFR inhibition also inhibit thymidylate synthase and other enzymes involved in the purine biosynthetic pathway.

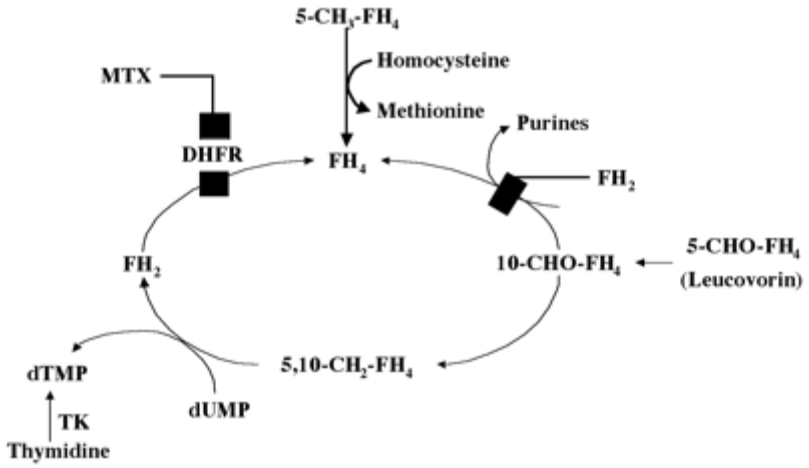


Figure 1. Folate pathway. Sites of action of methotrexate (MTX) and of the rescue agents leucovorin and thymidine. MTX primarily inhibits dihydrofolate reductase (DHFR). This results in the depletion of reduced folates (FH4), which are required for deoxythymidine monophosphate (dTMP) synthesis from deoxyuridine monophosphate (dUMP), and in accumulation of dihydrofolates (FH_2), which inhibit purine synthesis. The MTX rescue agent leucovorin restores the reduced folate pool after conversion to its active metabolite 5-methyltetrahydrofolate (5-CH_3-FH4). Thymidine is directly converted to thymidine monophosphate by the enzyme thymidine kinase (TK).

Similar to other antimetabolites, critical determinants of MTX cytotoxicity are not only drug concentration but also duration of exposure. High concentrations of MTX may be well tolerated for brief periods of time, whereas prolonged exposure to low concentrations can result in life-threatening toxicity. The type of toxicity observed with MTX is also a function of this concentration–time dependence. Exposure to millimolar concentrations of MTX for minutes to hours may lead to

acute renal, central nervous system, and liver toxicity; 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(18).

Following administration of HDMTX, two metabolites, 7-hydroxy-methotrexate (7-OH-MTX) and 2,4-diamino-N^{10}-methylpteroic acid (DAMPA), are observed in plasma. Within 12–24 hours of the start of a HDMTX infusion, the plasma concentration of 7-OH-MTX, formed by the action of the enzyme aldehyde oxidase, exceeds the concentration of MTX.Intracellular polyglutamation of 7-OH-MTX results in prolonged retention and enhanced cytotoxicity. DAMPA, a minor, inactive(19,20) metabolite of MTX, accounting for <5% of the total dose of drug that is excreted in urine, is presumably formed from MTX that is excreted into the intestinal tract, hydrolyzed by bacterial carboxypeptidases, and then reabsorbed.

Methotrexate Induced Renal Dysfunction

As MTX is primarily cleared by renal excretion, MTX-induced renal dysfunction leads to delayed elimination of MTX, and the resulting sustained, elevated plasma MTX concentration may lead to ineffective rescue by LV and a marked enhancement of MTX's other toxicities.Since the introduction of HDMTX with LV rescue more than 25 years ago by Djerassi et al.(16), the ability to safely administer this regimen to patients has improved, and there have been a number of advances in the treatment of HDMTX-induced renal dysfunction over the past 20 years. The etiology of MTX-induced renal dysfunction is believed to be mediated by the precipitation of MTX and its metabolites in the renal tubules or via a direct toxic effect of MTX on the renal tubules. More than 90% of MTX is cleared by the kidneys. MTX is poorly soluble at acidic pH, and its metabolites, 7-OH-MTX and DAMPA, are six- to tenfold less soluble than MTX, respectively. An increase in the urine pH from 6.0 to 7.0 results in a five- to eightfold greater solubility of MTX and its metabolites(22) ·Several drugs have

been associated with increased toxicity when coadministered with MTX. The most significant interactions involve agents that interfere with MTX excretion, primarily by competing for renal tubular secretion, such as probenecid, salicylates, penicillins, and nonsteroidal anti-inflammatory agents.MTX-induced renal dysfunction results in sustained, elevated plasma MTX concentrations, which in turn may lead to ineffective rescue by LV and a marked enhancement of MTX's other toxicities, especially myelosuppression, mucositis, hepatitis, and dermatitis.

Vomiting and diarrhea during or shortly after the administration of MTX have been observed in patients who developed MTX toxicity, but the majority of patients with renal dysfunction are initially asymptomatic, and most present with nonoliguric renal dysfunction . Although the risk for MTX toxicity is dependent upon the dose and schedule of administration, plasma MTX concentrations should be 1.0 µM at 42 hours after the start of the HDMTX infusion, and plasma MTX concentrations 10 µM at this time point are associated with a high risk

for the development of toxicities (24,25).

Prevention and management of HDMTX toxicity

The guiding principles for prevention of HDMTX toxicity, namely maintaining urine output, urinary alkalinization, monitoring serum creatinine, electrolytes, and plasma MTX concentrations, and pharmacokinetically -guided leucovorin rescue, are also the cornerstones of management for patients who develop early signs of renal dysfunction and delayed MTX elimination.

1) Hydration and urinary alkalinization

Maintaining adequate hydration and urine output are essential for rapid clearance of MTX. Most protocols recommend at least 2.5 to 3.5 liters/m2 of IV fluid hydration per day, starting four to 12 hours prior to the initiation of the MTX infusion.

The pH of the urine should be measured at baseline. MTX precipitates in acid urine; maintaining the urine pH 7.0 or higher increases MTX solubility, prevents drug precipitation in renal tubules, and drastically decreases the chance of renal damage. In clinical practice, it is customary to begin the MTX infusion only after the urine pH is ≥7.0, and to maintain it in this range until plasma MTX levels have declined to less

than 0.1 microM.

Urinary alkalinization is most easily accomplished by adding ampules (amps) of sodium bicarbonate to each liter of IV fluid hydration. This accomplishes both fluid hydration and urinary alkalinization.

A typical choice is IV D5W with 2 to 3 amps (100 to 150 meq) of sodium bicarbonate per liter, administered by continuous infusion at 125 to 150 mL/hour. A cation concentration of 80.5 meq/L is roughly equivalent to one-half normal saline. The amount of bicarbonate in each liter and the IV fluid composition can then be modified according to the urine pH, and serum sodium. If only one amp of bicarbonate is added to each liter of IV fluid, physicians should be aware that the resultant fluid will be hypotonic if D5W is used

2) Leucovorin administration

Leucovorin rescue should be started within 24 to 36 hours of the start of the MTX infusion. A variety of dosing schedules have been published, but most administer 10 mg/m2 IV or 15 mg/m2 orally every six hours until plasma MTX levels are less than 0.05 to 0.1 micromol/L. The size and number of leucovorin doses do not appear to be critical in patients who have normal MTX clearance(27). Even doses of 10 to 15 mg/m2 are

often in excess of those required to achieve rescue in such patients. In contrast, higher concentrations of leucovorin are needed if rapid elimination of MTX is compromised by renal insufficiency.

Early studies conducted in the 1970s revealed that the following drug levels after MTX infusion indicated a high risk for bone marrow and gastrointestinal mucosal toxicity (28,29)

Levels above 5 to 10 microM at 24 hours

Levels above 0.9 to 1 microM at 48 hours

Levels above 0.1 microM at 72 hours

Leucovorin in patients with delayed elimination and prolonged elevated plasma MTX levels is as follows(Uptodate)

Plasma methotrexate (MTX) level	**Leucovorin dose and/or schedule adjustment**
24 hours	
>1-5 micromolar	25-50 mg/m^2 every 6 hours IV
≥10 micromolar	50-100 mg/m^2 q 6 hours IV
48 hours	
≥1 micromolar	50-100 mg/m^2 q 6 hours IV
≥10 micromolar	100-200 mg/m^2 q 3-4 hours IV•
72 hours	
0.1-0.9 micromolar	50-100 mg/m^2 every 6 hours IV
≥1 micromolar	50-100 mg/m^2 q 3-4 hours IV

Alternative rescue techniques have been utilized in an attempt to enhance MTX clearance and/or minimize severe systemic toxicity.

These include extracorporeal removal of MTX by means of peritoneal dialysis, hemodialysis, hemoperfusion, and/or charcoal hemofiltration administration of leucovorin in conjunction with thymidine, and administration of the metabolizing enzyme carboxypeptidase G2(30,31)

Oral Mucositis

Oral mucositis is a common problem after high-dose methotrexate (HD-MTX) treatment. Seveal studies have been carried out to identify factors associated with the development of mucositis in children with ALL who had no delayed elimination of methotrexate (MTX)). Pharmacokinetic studies of MTX and the metabolite 7-hydroxymethotrexate (7-OHMTX) in plasma and saliva is an indicator of risk for mucositis.(26). Grade of mucositis was found to correlate significantly with low systemic clearance of MTX during the infusion and a low p-7-OHMTX/p-MTX ratio at 66 hours after the start of infusion. The MTX concentration in saliva didnot show any correlation with the development of mucositis. The present conventional criteria for high-risk MTX concentrations might need to be reevaluated because a high percentage of patients still

suffer from oral toxicity despite "normal" elimination. A reduced ratio between the simultaneous concentrations of 7-OHMTX and MTX in plasma may be a possible mechanism of this unpredictable oral toxicity.

Hepatotoxicity

MTX has the potential for hepatotoxicity at all doses. The association between MTX and hepatic dysfunction has been studied most extensively in patients receiving chronic oral low-dose MTX. Hepatotoxicity can be manifested as a mild transaminitis, but patients are at risk for fibrosis and cirrhosis when the total dose exceeds 1.5 to 2 grams.

HDMTX can cause an acute elevation in the serum transaminases from two to twenty-fold normal levels, even in patients who receive leucovorin rescue. Acute transaminitis occurs in as many as 60 to 80 percent of patients, and typically resolves spontaneously within one to two weeks. If the level of alanine transferase (ALT) has not returned to less than 180 IU/L by the beginning of the next treatment cycle, the next dose should be reduced and/or delayed.

Rarely, HDMTX causes a temporary elevation in serum bilirubin, which

usually normalizes within a few days. Subsequent cycles do not require dose reduction unless the peak serum bilirubin level exceeds 3 mg/dl (32).Among patients receiving IV MTX for treatment of cancer, hepatic fibrosis (with a subsequent risk for hepatocellular cancer) is reported only rarely. All cases have been in children receiving MTX for acute lymphoblastic leukemia.

FEBRILE NEUTROPENIA

Neutropenia. When the neutrophil count decreases to <1000 cells/mm^3, increased susceptibility to infection can be expected, with the frequency and severity inversely proportional to the neutrophil count. Patients with neutrophil counts of <500 cells/mm^3 are at considerably greater risk for infection than are those with counts of <1000 cells/mm^3, and patients with counts of ≤100 cells/mm^3 are at greater risk than are those with counts of <500 cells/mm^3.

The criteria for defining a infection related fever in the presence of grade IV neutropenia(lancet) (absolute neutrophil count < 0.5 x 10^9 / L) are

1. Oral temperature of > 38˚C on at least one occasion, associated with a clinical focus of infection.

2. Oral temperature >38˚C but < 39˚C sustained upto 12 hrs in the absence of other non-infectious pyrogenic processes and without a clinical focus of infection
3. Oral temperature >39˚C on at least one occasion with chills but in the absence of other non-infectious pyrogenic processes

In addition to the number of circulating neutrophils, the duration of neutropenia is an important determinant of infection. A low nadir in the neutrophil count and protracted neutropenia (i.e., neutrophil count of <500 cells/mm^3 for 10 days) are major risk factors for impending infection (33).

Recommendations for evaluation.

Initial evaluation should consist of a thorough physical examination; a complete blood cell count; measurement of serum levels of creatinine, urea nitrogen, and transaminases; and culture of blood samples (obtained from a peripheral vein and/or a catheter). A chest radiograph is indicated for patients with respiratory signs or symptoms or if outpatient management is planned.

Specimens should be obtained immediately for culture for bacteria and fungi. If a central venous access device is in place, some authorities,

including the new "IDSA Guidelines for the Management of Intravascular Catheter–Related Infections"(34), recommend that ⩾1 set of blood samples be obtained for culture from the device lumen(s) as well as from a peripheral vein. Other investigators believe that culture only of a blood sample obtained from a central venous catheter is adequate [idsa). Quantitative blood cultures, although not necessarily recommended routinely for all patients, may be helpful for those suspected of having a catheter-related infection, for whom specimens obtained from a central venous catheter and a peripheral vein should be compared

TREATMENT

The initial regimen chosen should be a broad spectrum covering majority of the Gram negative and Gram positive organism either in the form of monotherapy of polymicrobial therapy. This initial regimen should be continued until 5 days unless the clinical condition deteriorates substantially. The observations of Elting and colleagues, that the median time to clinical response of febrile neutropenia due to bacterial infections was 5-7 days after introduction of antibiotics, must always be kept in mind.(35)

The second important point is the duration of therapy; most patients with febrile neutropenia do not have documented infections, so decision on the duration of treatment cannot be made on the basis of sterilization of cultures or presence of specific organisms

Pizzo and colleagues suggested that antibacterial therapy should be continued until neutropenia resolves, though there is lack of strong evidence that shorter duration would lower the risks of development of fungal infections, antibiotic resistance, and drug-related toxic effects. (36)

Selection of antibiotics was based on the hospital Antibiotic Policy

Table 1:

1st line :	Cefaperazone/sulbactam+ Amikacin
2nd line :	Piperacillin+ Tazobactam + Amikacin
3rd line :	Meropenam/Imipenem
Gram+ive:	Vancomycin
Antifungal:	Amphoterecin B

USE OF COLONY-STIMULATING FACTORS IN TREATMENT

Hematopoietic growth factors have been studied as adjunctive therapy to antimicrobial therapy for febrile neutropenic patients in several randomized, controlled trials (37,38). These studies show that G-CSF or granulocyte-macrophage colony-stimulating factor used as part of the

treatment of febrile neutropenic patients can consistently shorten the duration of neutropenia, but these agents have not consistently and significantly reduced other measures of febrile morbidity, including duration of fever, use of anti-infectives, or costs of management of the febrile neutropenic episode. No study has demonstrated a decrease in infection-related mortality rates. The 2001 update of the American Society of Clinical Oncology guidelines recommends against the routine use of hematopoietic growth factor in uncomplicated cases of fever and neutropenia.

Dose intensity of 6 Mercaptopurine

Since their introduction to leukemia treatment in the 1950s, the thiopurines mercaptopurine and thioguanine have played an essential role in treatment protocols for ALL. TPMT is a cytosolic enzyme ubiquitously expressed in the human body and catalyzes the S-methylation of thiopurines. The TPMT locus is subject to genetic polymorphism. To date 20 variant alleles (TPMT*2-*18) have been identified, which With regard to treatment outcome in childhood ALL, Lennard and colleagues(50) described in 1990 a higher relapse rate in children with lower thioguanine nucleotide concentrations measured in

erythrocytes and suggested a substantial role for genetically determined TPMT activity in the predisposition to the cytotoxic effects of mercaptopurine and, consequently, ALL outcome. Their hypothesis is supported by the work of Relling and colleagues(51) who demonstrated in a study of 182 children with ALL that mercaptopurine dose intensity was the strongest predictor of outcome are associated with decreased activity compared with the TPMT*1 wild-type allele., The association of TPMT genotype with minimal residual disease levels before and after application of a 4-week cycle of mercaptopurine during induction consolidation treatment in BFM protocols thus having an impact on outcome has also been reported(52).

Neurotoxicity following HDMTX

The incidence of various neurological events following lntrathecal or intravenous high dose Methotrexate is estimated around 4-15%

(1).Three distinct clinical patterns of neurotoxicity have been observed (a) an acute toxicity, occurring within hours of IT chemotherapy, probably resulting from chemical arachnoiditis; (b) sub acute toxicity occurring within days or weeks, and characterized by symptoms of seizures, transient paresis, or cerebellar abnormalities; and (c) a delayed

leukoencephalopathy form that is more commonly observed when IT MTX is used with cranial irradiation (53).

The mechanism of methotrexate induced neurotoxicity is poorly understood. Both high-dose intravenous MTX and intrathecal MTX are proposed to have association with demyelination, white matter necrosis, loss of oligodendroglia, axonal swelling, microcystic encephalomalacia, and atrophy relatively selective for the deep cerebral white matter (54). An altered myelin metabolism disturbance induced by Mtx (55) or elevated levels of homocysteine and its excitatory amino acid neurotransmitter metabolites (homocysteic acid and cysteine sulfinic acid) may mediate, in part, methotrexate-associated neurotoxicity (56). The absence of significant imaging abnormalities has raised the possibility of cyotoxic edema.

SUBJECTS AND METHODS

Inclusion criteria

1. Patients undergoing BFM 86 protocol treatment at Cancer Institute,Chennai
2. Age group:3- 25 years
3. Patients who have attained complete remission after induction chemotherapy

Exclusion criteria

1) Pre existing renal dysfunction-Serum Creatinine value >1.4 mg/dl OR Creatinne clearance less than 70 ml/minute
2) History of any neurological disorder including epilepsy
3) CNS involvement at presentation as defined by the ROME criteria

The study population included 60 patients who were analysed in two groups , Group A (30 patients who received 3gm/m^2 of high dose methotrexate) and Group B (30 patients who were treated with 5 gm/ m^2 of high dose methotrexate).

BFM 86 protocol

Consolidation treatment is with High dose Methotrexate,4 doses given on days 8,22,36 and 50. 6MercaptoPurine(6 MP) is started at 25 mg/m^2 on day 1 and continued till day 56.4 doses of intrathecal methotrexate are also given along with HDMTX. HDMTX was given as a 24 hour infusion with 3 litres /m^2 /day of intravenous hydration and alkalanisation. HDMTX was given only if urinary pH was determined as alkaline prior to infusion. Creatinine clearance was checked prior to 1 st dose of HDMTX .Urea and creatinine were monitored daily. Serum Methotrexate levels were done at 48 and 72 hours after starting of infusion. Leucovorin rescue was started at 48 hours at 15 mg/m^2 iv q 6 th hourly and continued for a total of 16 doses ,unless there was any renal toxicity necessitating dose adjustment .

Following HDMTX infusion, toxicities like mucositis, myelosuppression, Liver Function Test abnormalities were noted ,graded and managed appropriately .GCSF and blood component support given as required. Febrile episodes were treated as per protocol.6 MP was continued unless there was Grade 3/4 myelosuppression, febrile neutropenia or transaminitis.

Methotrexate Assay

Emit Methotrexate assay, which is a homogenous enzyme immunoassay intended for use in the quantitative analysis of methotrexate in human serum or plasma was used. With this assay, samples containing 0.3-2600 micromol/L can be analysed. Primary protocol can analyse up to 2 micromol/L methotrexate and those more than that can be brought within range of standard curve by serial dilution.Each assay requires 50 microlitre of serum or plasma. Whole blood cannot be used. Acceptable anticoagulants are heparin, EDTA and oxalate. Serum or plasma is to be kept at 2-8degree centigrade and protected from sunlight. Leucovorin or Trimethoprim do not interfere with this assay at maximum pharmacological or physiological concentrations.(Source-Product insert of Dade Behring Syva Emit Methotrexate Assay)

MTX excretion	24 hours	48 hours	72 hours
Normal	10 μ mol/L	1 μ mol/L	< 0.2 μ mol/L
Delayed late			> 0.2 μ mol/L
Delayed early	50 μ mol/L	5 μ mol/L	

Leucovorin dose

Normal 15 mg/m^2 q 6H for 16 doses

Delayed late 15 mg/m^2 q 6H till < 0.05 μ mol/L

Delayed early 150 mg/m^2 q 3H till < 1 μ mol/L

15 mg/m^2 q 3H till < 0.05 μ mol/L

Statistical Methods: Descriptive statistical analysis has been carried out

in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. ,Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis) and Student t test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale with in each group. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two groups(39,40,41)

1. Chi-Square Test

   $\chi^2 = \frac{\sum(Oi - Ei)^2}{Ei}$, Where Oi is Observed frequency and Ei is Expected frequency

2. Fisher Exact Test 2x2.Fisher Exact Test statistic=

$$\sum p = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!} \frac{1}{\sum a!b!c!d!}$$

3. Student t test (Two tailed, independent) $t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{s^2(1/n1 + 1/n2)}}$

   Where $s^2 = \frac{(n1-1)\sum_{i=1}^{n1}(x1 - \bar{x}1)^2 + (n2-1)\sum_{i-1}^{n2}(x2 - \bar{x}2)^2}{n1 + n2 - 2}$

4. Student t-test for paired comparisons

Statistical software: The Statistical software namely SPSS 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 were used for the analysis of the data

RESULTS

Table 1: Comparison of age distribution of patients studied

Age in years	*Group A*		*Group B*		*Total*	
	No	*%*	*No*	*%*	*No*	*%*
Up to 5	12	40.0	10	33.3	22	36.7
6-10	6	20.0	7	23.3	13	21.7
11-15	9	30.0	8	26.7	17	28.3
16-20	3	10.0	5	16.7	8	13.3
Total	30	100.0	30	100.0	60	100.0
Mean ± SD	10.57±7.21		10.73±7.39		10.65±7.24	

Samples are age matched with P=0.730

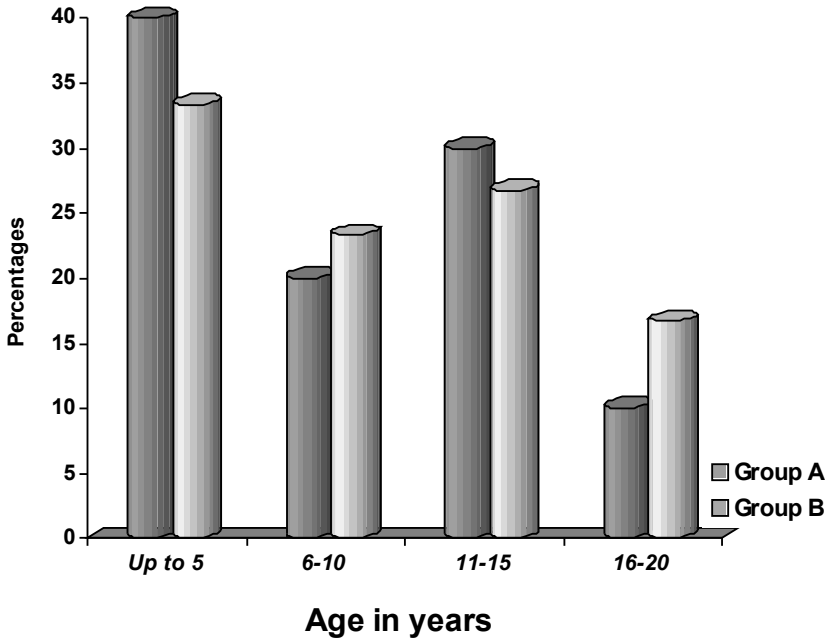


Table 2: Comparison of gender distribution of patients studied

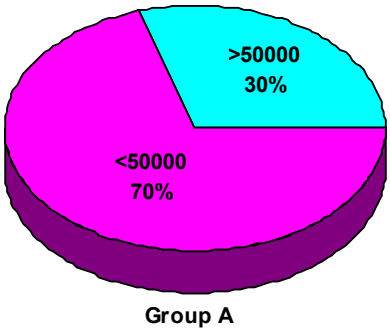
Gender	*Group A*		*Group B*		*Total*	
	No	*%*	*No*	*%*	*No*	*%*

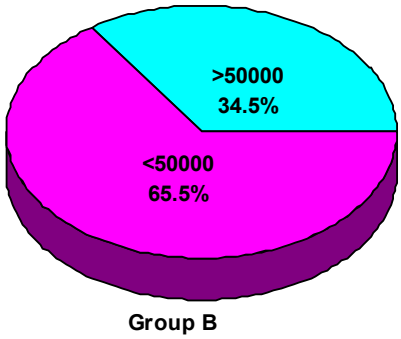
Male	20	66.7	15	50.0	35	58.3
Female	10	33.3	15	50.0	25	41.7
Total	30	100.0	30	100.0	60	100.0

Samples are gender matched with P=0.190

Table 3: Comparison of Total count of patients studied

Total count	***Group A***		***Group B***		***Total***	
	No	***%***	***No***	***%***	***No***	***%***
<50000	20	70	19	65.5	38	67.9
>50000	10	30	11	34.5	18	32.1
Total	30	100.0	30	100.0	56	100.0





Group A **Group B**

Table 4: Comparison of average number of days to complete the protocol (C – Consolidation)

Phases	***Group A***	***Group B***	***P value***
C1	17.83±5.29	16.40±3.98	0.241
C2	18.17±5.36	17.93±4.11	0.851
C3	17.27±3.94	18.17±5.03	0.449
C4	14.43±1.72	16.37±4.34	0.027*

Total	74.03±12.29	73.97±11.54	0.983

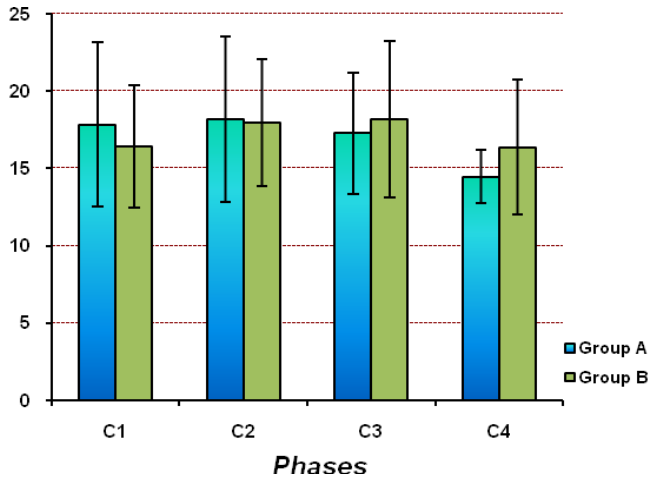


Table 5: Comparison of mucositis

Mucositis	***Group A (n=30)***	***Group B (n=30)***	***P value***
Grade I	26 (86.7%)	23(76.7%)	0.317
Grade II	3(10.0%)	5(16.7%)	0.448
Grade III	1(3.3%)	2(6.7%)	0.554

Table 6: Comparison of Rise in Creatinine by more than 50.0% at 48 hours

↑ Creatinine >50.0%	***Group A (n=120)***	***Group B (n=120)***	***P value***

C1	1	2	
C2	1	1	
C3	0	1	
C4	1	2	
Number of episodes	3 (2.5%)	6(5%)	0.499

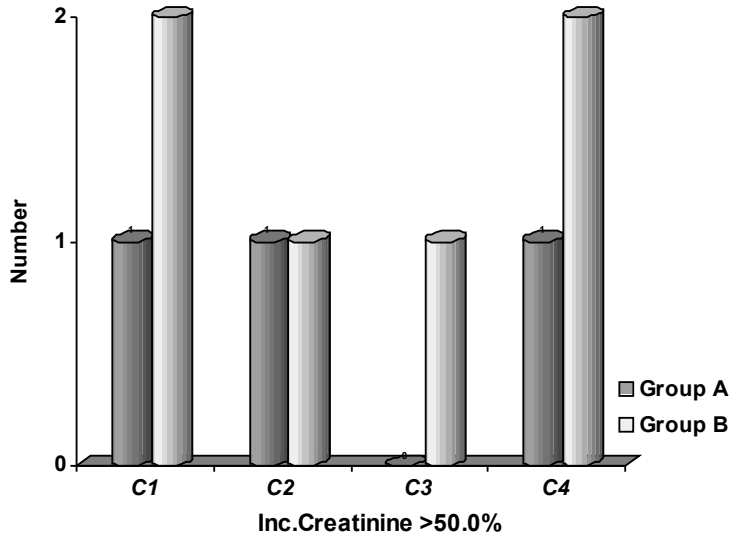


Table 7: Comparison of levels of serum Creatinine (mg/dl)

Phases	***Levels of s. Creatinine***	***Group A (n=30)***	***Group B (n=30)***	***P value***
C1	0 hour	0.59±0.12	0.60±0.09	0.555
	48 hour	0.64±0.22	0.61±0.09	0.502
	P value	0.265	0.745	-
C2	0 hour	0.59±0.09	0.59±0.11	1.000
	48 hour	0.62±0.12	0.58±0.09	0.185
	P value	0.211	0.620	-

C3	0 hour	0.60±0.12	0.59±0.12	0.835
	48 hour	0.59±0.09	0.77±0.99	0.327
	P value	0.655	0.356	-
C4	0 hour	0.60±0.11	0.60±0.13	1.000
	48 hour	0.59±0.11	0.63±0.15	0.246
	P value	0.536	0.310	-

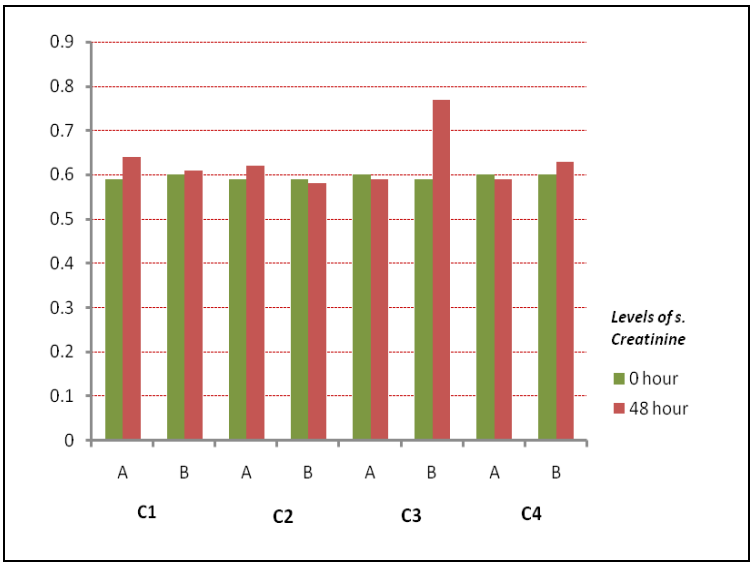


Table 8: Comparison of MTX >0.30 at 48 hours between two groups

MTX >0.30	***Group A (n=30)***	***Group B (n=30)***	***P value***
C1	2(6.7%)	0	0.492
C2	0	1(3.3%)	1.000
C3	0	2(6.7%)	0.492
C4	0	2(6.7%)	0.492
Number of episodes	2(1.7%)	5(4.2%)	0.446

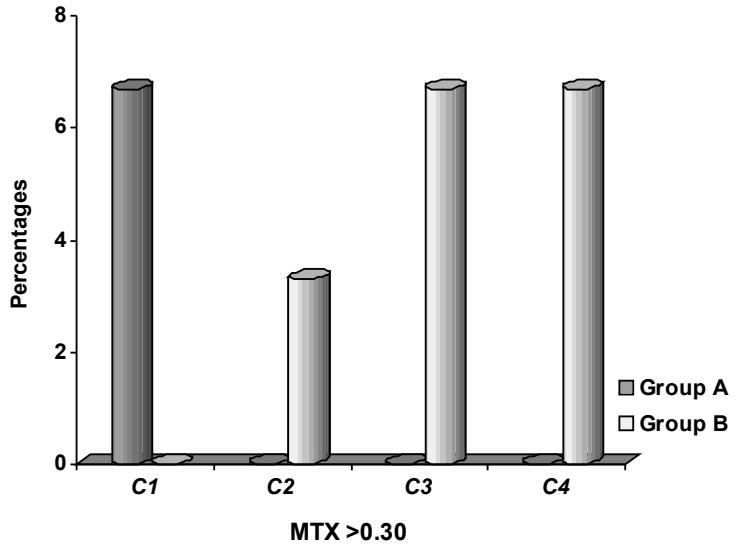


Table 9: Comparison of LFT Abnormalities

Any bilirubin>1.4mg/dl, SGOT/SGPT>2 times upper limit of normal,i.e>80 u/l taken as LFT derangement

LFT high	***Group A (n=30)***	***Group B (n=30)***	***P value***
C1	5 (16.7%)	8(26.7%)	0.347
C2	5(16.7%)	5(16.7%)	1.000
C3	4(13.3%)	1(3.3%)	0.353
C4	3(10.0%)	3(10.0%)	1.000

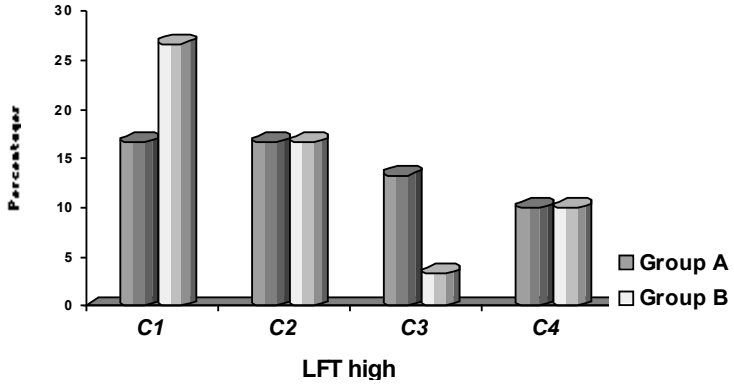


Table 9 a .Transaminitis post HDMTX

Transaminitis Grade	3 gm/m2	5 gm/m2
Grade 1	9	7
Grade 2	6	6
Grade 3	2	3
Grade 4	0	1

Table 10: Comparison of Total WBC Count (TC)

Phases	***Levels of TC***	***Group A (n=30)***	***Group B (n=30)***	***P value***
C1	501-1000	-	-	χ^2=0.800; P=0.371
	1001-1500	5 (16.7%)	6(20.0%)	
	1501-2000	1(3.3%)	3(10.0%)	
	>2000	24(80.0%)	21(70.0%)	
C2	501-1000	1(3.3%)	2(6.7%)	χ^2=1.926; P=0.165
	1001-1500	5(16.7%)	6(20.0%)	
	1501-2000	1(3.3%)	3(10.0%)	
	>2000	23(76.7%)	18(60.0%)	
C3	501-1000	1(3.3%)	2(6.7%)	χ^2=0.082; P=1.000
	1001-1500	6(20.0%)	5(16.7%)	
	1501-2000	1(3.3%)	2(6.7%)	
	>2000	22(73.3%)	21(70.0%)	

C4	501-1000	2(6.7%)	2(6.7%)	χ^2=2.700; P=0.100
	1001-1500	3(10.0%)	8(26.7%)	
	1501-2000	2(6.7%)	3(10.0%)	
	>2000	23(76.7%)	17(56.7%)	

Table 11: Comparison of days taken for TC to recover

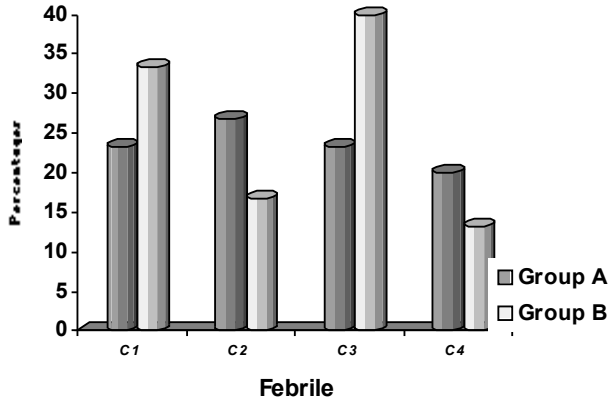
Phases	***Levels of REC***	***Group A (n=30)***	***Group B (n=30)***	***P value***
C1	Before D14	26(86.7%)	26(86.7%)	1.000
	D15-D18	2(6.7%)	2(6.7%)	1.000
	D19-D22	1(3.3%)	1(3.3%)	1.000
	D23-D26	0	0	-
	D27-D30	1(3.3%)	1(3.3%)	1.000
C2	Before D14	23(76.7%)	21(70.0%)	0.559
	D15-D18	3(10.0%)	6(20.0%)	0.472
	D19-D22	3(10.0%)	2(6.7%)	1.000
	D23-D26	1(3.3%)	1(3.3%	1.000
	D27-D30	0	0	-
C3	Before D14	23(76.7%)	25(83.3%)	0.384
	D15-D18	5(16.7%)	0	0.050*
	D19-D22	2(6.7%)	4(13.3%)	0.671
	D23-D26	0	0	-
	D27-D30	0	1(3.3%)	1.000
C4	Before D14	28(93.3%)	24(80.0%)	0.254
	D15-D18	0	2(6.7%)	0.492
	D19-D22	2(6.7%)	0	0.492
	D23-D26	0	3(10.0%)	0.237
	D27-D30	0	1(3.3%)	1.000

Table 12 Comparison of Thrombocytopenia

Thrombocytopenia	*Group A (n=30)*	*Group B (n=30)*
Normal	19 (63.3%)	20(66.7%)
Grade 1	3(10.0%)	2(6.7%)
Grade 2	4(13.3%)	5(16.7%)
Grade 3	3(10.0%)	2(6.7%)
Grade 4	1(3.3%)	1(3.3%)

Table 13: Comparison of Febrile neutropenia

Febrile neutropenia	*Group A (n=30)*	*Group B (n=30)*	*P value*
C1	7(23.3%)	10(33.3%)	0.390
C2	8(26.7%)	5(16.7%)	0.347
C3	7(23.3%)	12(40.0%)	0.165
C4	6(20.0%)	4(13.3%)	0.488
Number of episodes	28(23.3%)	31(25.8%)	0.653



Febrile neutropenia

Table 14: Comparison of Use of antibiotics

Use of antibiotics	***Group A (n=30)***	***Group B (n=30)***	***P value***
C1	8 (26.7%)	8(26.7%)	1.000
C2	8(26.7%)	5(16.7%)	0.347
C3	7(23.3%)	14(46.7%)	0.058+
C4	6(20.0%)	4(13.3%)	0.488
Number of episodes	29(24.2%)	31(25.8%)	0.682

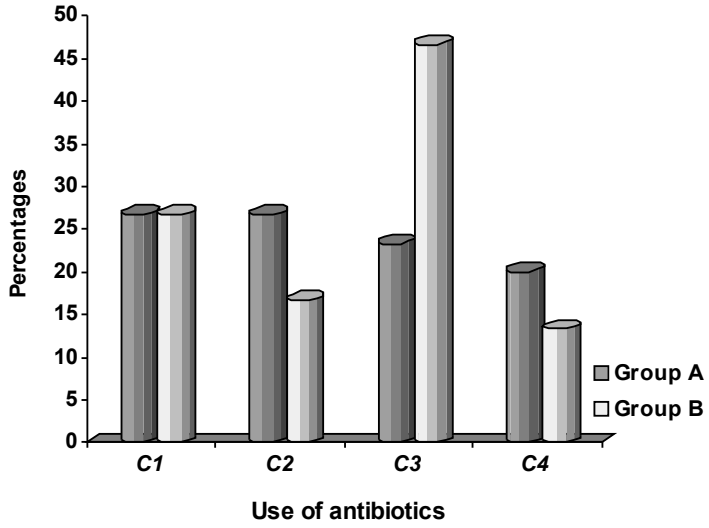


Table 15: Comparison of number of days 6MP was given

No of days of 6MP	*Group A (n=30)*	*Group B (n=30)*
<20	3 (10.0%)	0
21-30	4(13.3%)	4(13.3%)
31-40	7(23.3%)	8(26.7%)
41-50	6(20.0%)	10(33.3%)
51-60	10(33.3%)	8(26.7%)
Mean ± SD	41.20±13.39	42.83±10.53

6MP administered in number of days is statistically similar between two groups with P=0.602

Table 16.Central Nervous System Toxicity

AGE	SEX	HDMTX	ITMTX	DEFICIT	Recovery	CSF	MRI	RFT	S.MTX
4	F	D 11	D 12	Hemiparesis	1 day	N	N	N	0.15
8	F	D 9	D 9	Hemiparesis	1 day	N	N	N	0.2
13	M	D 10	D 10	Hemiparesis	2 days	N	N	N	0.18
14	F	D 12	D 12	Hemiparesis	3 days	N	N	N	0.22

Table 17 Toxicities associated with HDMTX(including both groups)

Toxicity	**Number (out of 240)**	**Percentage**
Doubling of Creatinine	2	.9%

50 % rise in Creatinine at 48 hours post HDMTX	9	3.75%
Mucositis Grade 2 or more	11	4.5%
Transaminitis Grade 2 or more	18	7.5%
Neutropenia Grade 2 or more	70	29%
Febrile Neutropenia	59	24.5%
Thrombocytopenia Grade 2 or more	16	6.6%
Mean Number of days delayed	5.6	N.A.

Base Line Characteristics

The study population was matched for age (Table 1). Approximately 60% % of the study population was less than 10 years of age. There was slight male preponderance in the study population(Table 2). But no stsistically significant difference was present between the two groups with respect to gender. Although both Total WBC count and age are considered together for risk stratification, TC was separately considered

for matching and both there was no difference between two groups (Table 3).

Delay in completion of protocol was noted in both the groups. But the mean value of days taken was 74 days for both the groups (Table 4). There was no significant change between the various consolidation cycles. Grade 1 mucositis was noted in majority of the patients (Table 5). Number of patients with either Grade 2 or 3 mucositis was more in 5 gm/m2 group.

Renal Function Test Abnormality

Any increase in Serum Creatinine values post HDMTX may point to nephrotoxicity. Increase of Creatinine by 50 % from the baseline value was compared.(Table 6).Group administered 5 gm/m2 had double the number of such cases (6 out of 240 infusions) when compared to 3 gm/m2 group. No significant change was noticed between creatinine levels at baseline and at 48 hours between the two groups. (Table 7). This remained the same throughout the continuation of protocol from Consolidation cycle 1 through 4. Serum Methotrexate levels at 48 hours >0.3 micromol/L was noted in 5 patients administered 5 gm/m2 HDMTX, (Table 8) which was more than 2 similar episodes noted in the

other group. But when taken as a side effect of 120 infusions in each, there was no statistical significance.

Liver Function Test abnormalities

If the total study population is taken,17 patients each had some LFT abnormalities in either group. (Table 9 & 9a)Further analysed as number of patients having Hyperilirubinemia, and transaminitis .Isolated hyperbilirubinemia (Bilirubin > 1.4mg/dl) was seen in 4 patients in 3 gm/m^2 group and 6 patients in 5 gm /m2 group. Maximum level was 8 mg/dl%

Myelosuppression

Neutropenia

Was categorized into 4 grades.There was no case with TC les than 500. ANC was calculated ,but as ANC was not available for all cases, in order to make the comparison uniform , total WBC count was taken for analysis (Table 10). In those with Grade 3 or 4 neutropenia , no significant difference was noticed among Group A or B. Only less than 5 % of the infusions was complicated by TC < 500.Growth factors were

used in 4 cases in 5 gm/m2 group.

Recovery of counts

80-85% of the patients had recovery of counts with in 14 days of HDMTX. More than 4 days delay before the recovery of TC was seen in 10 out of 120 infusions in 3 gm/m ^{2} and in 15 out of 120 infusions in 5 gm/m^2(Table 11). Although the absolute numbers are low, this observation was statistically significant.

Thrombocytopenia

21 episodes of thrombocytopenia of any grade was noticed in the study population. There was no significant difference between both the groups (10 in 3 gm/m2 and 11 in 5 gm/m2 (Table 12).2 patients in 5 gm/m2 group had bleeding manifestations.7 platelet transfusions were given for Grade 3 & 4 thrombocytopenia.

Febrile neutropenia

Febrile neutropenia was noted in 23.3 % of 3 gm/m2 group and 25.8 % of 5 gm/m2 group, which was not statistically significant (Table 13). .Documented infection was seen in 24% of febrile neutropenic episodes..

Among the documented infections Gram-negative organisms were isolated 42.8 %. Organisms were mostly Escherechia coli, Kleibseilla, Pseudomonas in a few Acinetobacter. Gram-positive infections were reported in 32.3%. Organisms isolated were mostly Staph.aureus, streptococci, coagulase negative staphylococcus. Antifungals were started presumptively for 3 patients. Antibiotics were used in 25% of infusions in both groups according to the hospital policy(Table 14). 5 days of antibiotics was sufficient for 80% of the cases.

Dose intensity of 6 MercaptoPurine

6 MP could be administered as per protocol in only 30 % of the patients .The study population received a mean of 42 days of 6 MP(Table 15). There was no difference between 3 and 5 gm/m2 HDMTX

CNS Toxicity

4 out of 120 5 gm/m2 HDMTX infusions were characterized by transient hemiparesis. All the patients had normal CSF studies upfront(Table 16). They had received IT MTX along with intravenous high dose MTX (HDMTX) 5 gm/m2 before the event. All the patients developed the event between Day 9 to D12 of HD MTX. The neurological event recovered within 24 hours in all of them . CSF study was normal in all

patients I/T MTX had been given for all.

Tolerability of HDMTX

Significant toxicities which were observed in the study population including both the groups is given in Table 17.Renal dysfunction characterized by a rise in serum Creatinine was seen in only 4.65%.Although mild mucositis was noted in majority, only 11 patients had grade 2 or above mucositis.Liver function tests were deranged post HDMTX in 7.5 % .Neutropenia was observed in 29% of population . 25% of the infusions were complicated by fever also. Despite these factors,the median delay before starting the next HDMTX was only 5.6 days for both the groups combined together.

DISCUSSION

In analyzing outcome for acute lymphoblastic leukemia, risk categorization according to age,total count,immunophenotyping and sex are some of the factors influencing outcome (2). Male sex is one of the poor prognostic factors in treatment of leukemia (3). When the toxicity profile of an agent is studied ,it is essential that they are matched for the above factors . In the study,patients below age of 10 years was similar in both groups.

Dose intensity

Maintaining dose intensity is an important factor in deciding outcome in acute lymphoblastic leukemia. This was particularly important in this study as the tolerability of the study population was uncertain. As per the BFM 86 protocol, each High dose Methotrexate is to be given once in 14 days.(12) Both the groups took 74 days to complete the consolidation phase. There was a mean delay of 5.6 days for the total study population.In BFM 86 pilot study,the duration of consolidation phase is 60-64 days.So the reason for the delays like mucositis,myelosuppression, transaminitis etc were analysed.

Mucositis

Mucositis is a major complication in all protocols which utilize HDMTX. (25)Various reasons like inadequate hydration, elimination delay of Methotrexate(42),co administration of 6 MP are some of the aggravating factors. Grade 1 mucositis seen in the majority (81%) was a cause of concern, but did not lead to much treatment delays.

Renal Function Derangements

As MTX is primarily cleared by renal excretion, MTX-induced renal dysfunction leads to delayed elimination of MTX, and the resulting sustained, elevated plasma MTX concentration may lead to ineffective rescue by LV and a marked enhancement of MTX's other toxicities like mucositis,myelosuppression and transaminitis(23,27). Creatinine clearance was calculated only at the beginning of consolidation. A lack of correlation between Creatinine clearance and methotrexate toxicity has previously been reported (29).

The majority of patients with renal dysfunction are initially asymptomatic, and most present with nonoliguric renal dysfunction(25). So an abrupt rise in serum creatinine during or shortly after MTX infusion indicates the development of renal dysfunction and can result in

significantly elevated plasma MTX concentrations Variations in serum Creatinine was further studied in both the groups, by taking the absolute values in each consolidation cycle .Elimination delay in Methotrexate has been described as one of the factors influencing methotrexate toxicity. (42).The intent was to analyse whether there was any progressive decline in the levels with repeated infusions. Both the groups had a very close median baseline values of 0.59/0.60.There was no marked change in the values with the continuation of regimen, thus ascertaining the tolerability of HDMTX. There was a small (<0.1) change in Creatinine after 48 hours in most of the cycles, which is of no significance. Only in 3 rd consolidation in group B was a trend towards significance seen, the baseline creatinine value being 0.59 and 48th hour Creatinine being 0.77. There is paucity of literature aboot worsening of Renal functions with successive HDMTX infusions.

Analysis of Methotrexate levels

Serum MTX levels is the most important determining toxicity after HDMTX infusion (43). The values have to be done at 24,48 and 72 hours. Only 2 patients had values more than 1 mic mol/L at 48 hours. In order to correct for any laboratory variation in Mtx assay, a lower threshold of 0.3 mic mo/L at 48 hours was chosen for comparison

between the two groups. Only 2 patients(1.7 % of all infusions)in group A and 5 patients(4.2 % of all infusions) in Group B had MTX > 0.3 mic mol/L at 48 hours. No progressive derangement was noted from C1-C4.The difference between 2 groups was also not statistically significant.

Liver function tests abnormalities

Post HDMTX, alterations in LFT line hyper bilirubinemia 2 transaminitis can occur (32). Elimination delay of MTX can result in aggravation of LFT abnormalities (42). Any bilirubin > 3 mg/dl necessitates a reduction in dose of MTX. MTX, 6MP and LV rescue can result in transaminitis. However, the alterations seen in the study population was not significant, but resulted in protocol delay. No episodes of encephalopathy were noted. Although supportive care helped in resolution of transaminitis, dose intensity was compromised as both 6 MP had to be pended and there was a delay before initiation of the next cycle.

Myelosuppression

Neutropenia

HDMTX is myeloablative and leucovorin rescue is mandatory (45). The dose of leucovorin is of importance, as studies have shown that increase

in LV dose can increase the incidence of relapse (57). As noted in previous studies (42), the incidence of TC < 2000 in the population with normal Methotrexate elimination as about 60-70%.Relling et all (24) has reported increased MTX area under the concentration time curve, low urine pH,emesis, intrathecal therapy and low Methotrexate clearance as various factors associated with increased with increased methotrexate toxicity like myelosuppresion and mucositis (24).

Recovery of counts

Any delay in the recovery of counts will compromise dose intensity as well as make the patient susceptible to febrile neutropenia(33). 80% of the patients had recovery of counts with in 14 days of HDMTX. Elimination delay of Methotrexate increases myelotoxicity. As the number of patients with increased Methotrexate levels was per se very low ,analysis for myelotoxicity in this subgroup could not be done. The percentage of patients having delay in resuming subsequent chemotherapy was similar to other studies(24).

Thrombocytopenia

Correlation between thrombocytopenia and serum methotrexate levels has been reported (47).Bleeding manifestations after HDMTX therapy is

seldom reported. Delayed methotrexate elimination has been found to be one of the risk factors for thrombocytopenia (42) .

Febrile neutropenia

The pattern of infections associated with ALL therapy in developing countries are different from that of Western literature. There is still predominance of gram negative infection unlike in the developed where there is a shift to gram posititve infection.(49). The gram negative organisms included K.pneumoniae, E Coli Ps.aeroginosa and Acinetobacter.Infections complications during intensive phases of treatment of acute lymphoblastic leukemia has previously been reported (48). 25% of HDMTX infusions were complicated by febrile neutropenia.This is comparable with the Institute data of febrile neutropenia in various protocols,i.e,MCP 841 and INCTR-02.

Dose intensity of 6 Mercaptopurine

Due to various genetic polymorphisms, there is marked variation in mercaptopurine metabolism. This has been found to affect minimal residual disease and outcome in ALL (50,51). Although molecular studies were not done, dose intensity of 6 MP was analysed.75 % dose intensity could be maintained, and there was no significant difference

between the two groups.Mucositis, transaminitis and neutropenia were the main reasons for pending 6 MP.

Central Nervous System Toxicity

Subacute toxicities like seizures, cerebellar dysfunction and hemiparesis have been described previously (53). Transient hemiparesis was an unusual toxicity described in the study population.All the events were seen in 5 gm/m2 group.All of them were exposed to multiple doses of IT MTX prior to the event. There was definite sex predilection for this event with females being more affected. Interestingly the toxicity is seen around Day 9 to day 12. Despite the event HDMTX and IT MTX was continued in all these patients but no recurrences were noted. Imaging studies were conducted in all patients within 24 hours of the onset of the event and all had normal standard MR imaging study of the brain.

Prognostic Factors Predicting Toxicity

The plasma concentration of MTX after high-dose therapy was closely related to the renal excretion(14) the exposure to nephrotoxic agents, the age of the patients, the dosage of hydration, and the intensity of urine alkalization.(15) Rask et al reported that the oral mucositis and the elevated liver enzymes were related to the slow clearance or high area

under curve(58). Other reports concerning raised hematologic side effects due to delay in methotrexate elimination are also documented in the literature (59). In the original BFM 86 protocol study (12), no difference in toxicity was noted among the patients given either 5 gm/m^2 or 8gm/m^2 HDMTX. The same rationale holds good for the identical LV rescue given after variable doses of HDMTX. After matching for confounding factors, data was analysed for any variable predicting HDMTX toxicity. As the number of infusions complicated by significant toxicity was per se small in the study population, no significant variable could be determined.

CONCLUSIONS

- Adequate hydration, alkalanisation and Leucovorin rescue with close monitoring of Serum Creatinine as well as Methotrexate levels are essential to prevent life threatening toxicities.
- High dose Methotrexate (HDMTX) could be safely administered across all age groups in the study population.
- Myelosuppression and febrile neutropenia resulted in treatment delays.
- Cumulative toxicity of HDMTX with repeated infusions was not noted.
- There was no significant difference in tolerability between 3 gm/ m^2 and 5 gm/m^2 of HDMTX.

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