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Original Research Article

Leukoencephalopathy Secondary to Methotrexate Therapy in Patients of Acute Lymphoblastic Leukemia and its Relationship with Serum Homocysteine, Vitamin B_{12} and Folate Levels: A Prospective Study

Akshay Lahoti[1], Prabodha K Das[2], Sonali Mohapatra[3], Suprava Naik[4], Ashutosh Panigrahi[5], Biswajit Bhuyan[6]

[1]DNB, PDCC, DM, Super Speciality Hospital, MGM Medical College, Indore
[2]Md, FRCPA, AIIMS, Bhubaneswar
[3]Md, DM, AIIMS, Bhubaneswar
[4]MD, AIIMS, Bhubaneswar
[5]Md, DM, AIIMS, Bhubaneswar
[6]Md, DM, KIMS, Bhubaneswar

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Corresponding Author: Dr. Akshay Lahoti
Conflict of interest: Nil

Abstract:

This study was done to analyze: 1) Risk of Leukoencephalopathy (LE) in Acute Lymphoblastic Leukemia (ALL) receiving Methotrexate (MTX). 2) Safety of re-administration of MTX. 3) Relationship of Homocysteine, B_{12}, Folate with LE. Thirty four pediatric ALL patients were enrolled. Chemotherapy was initiated as per Modified ALL IC BFM 2002 Protocol. All patients underwent MRI Brain and serum Homocysteine, B_{12}, Folate at four occasions. There was no difference in incidence of LE due to mode of MTX administration. There was no increase in incidence after four courses of HD-MTX. MRI at baseline was not a predictor of development of LE. Three of five LE Patients had abnormal B_{12}/Folate/Homocysteine with corresponding abnormal MRI Brain. t(1:19) and t(9:22) were associated with Leukoencephalopathy but t(12:21) was not associated with LE. Patients of MTX induced LE can be re-challenged with further course of MTX. Baseline MRI is not a predictor for development of LE.

Keywords: Homocysteine, B_{12}, Folate In MTX-LE

Introduction

Acute lymphoblastic leukemia (ALL) is characterized by excess production and accumulation of immature WBC's. ALL comprises of 15 – 35 % of all childhood cancers and is also the leading cause of death related to cancer in childhood [1,2].

Current protocols recommend methotrexate (MTX) as oral, intrathecal or high dose as key chemotherapeutic agent having efficacy against CNS and systemic disease [3]. Severe adverse effects are observed with MTX. Neurotoxicity is the most common organ toxicity observed with use of MTX [4]. Major form of neurotoxicity is leukoencephalopathy (LE) which may be symptomatic or asymptomatic [5]. MTX induced LE occurs within 2 – 14 days of intrathecal or high-dose MTX use. It presents as seizures, stroke like symptoms and aphasia. white matter hyperintensities on MRI brain are diagnostic [6].

Methotrexate inhibits formation of methyl tetrahydrofolate (MTHF). MTHF is involved in the synthesis of methionine from homocysteine using methionine synthase, which is catalyzed by vitamin B12 and zinc [7]. Thus any coexisting deficiency of folate or B12 would result in enhanced homocysteine and decreased methionine levels. Both these contribute towards neurotoxic effects of methotrexate therapy [7,8].

This study was aimed at identifying:

1) Risk, incidence, prevalence of LE in ALL receiving methotrexate therapy.

2) Whether methotrexate can be re-administered in documented leukoencephalopathy.

3) To identify whether low serum folate or b12 and high homocysteine increases risk of methotrexate induced leukoencephalopathy.

Patients And Methods

This was an open label, prospective, cohort study done in children with ALL undergoing treatment at Medical Oncology and Hematology departments, AIIMS, Bhubaneswar from June 2019 to December 2020. Prior consent was taken from IEC of AIIMS, Bhubaneswar. All the study procedures were conducted after obtaining voluntary written informed consent from legal guardians of patients.

Inclusion Criteria

- Age≤8years
- Newly diagnosed ALL

Exclusion Criteria

- History of neurological or psychiatric illness
- MRI incompatible patients.

All patients were treated as per BFM Chemotherapy protocol backbone. Patients were first stratified into 3 risk groups – standard, intermediate and high according to BFM risk stratification with COG modification (Table 1a) [9,10]. Treatment was given according to risk criteria based on protocol in Table 1b [10–12]:

Investigations

All patients underwent MRI Brain, Serum homocysteine, Vitamin B12 and Serum Folate sequentially. Complete Blood Count, Peripheral Blood smear, Flowcytometry, Cytogenetics and Molecular studies were checked at baseline. Cutoff points to define low B12, low folic acid and High homocysteine were kept at < 200 pg/ml, < 3 ng/ml and >16 umol/l respectively.

- Time point 1 – AT DIAGNOSIS.
- Time point 2 – POST CONSOLIDATION.
- Time point 3 – POST EXTRACOMPART-MENT THERAPY.
- Time point 4 – IN MAINTENANCE.

Thus analyzing neurotoxicity secondary to different modes of methotrexate therapy.

Mtx-Related Clinical Neurotoxicity was defined when neurologic symptoms occurred within 2 weeks of receiving MTX and other causes were excluded.

Mtx Induced Leukoencephalopathy was defined when diffuse or focal hyper intensity on T2WI and FLAIR sequence ± diffusion restriction in the deep white matter and/or cortex noted after methotrexate administration.

SYMPTOMATIC LE – Focal or diffuse T2WI and FLAIR hyperintensity showing diffusion restriction on DWI in the periventricular white matter (PVWM) along with white matter, changes in atypical locations [5,8,13,14].

ASYMPTOMATIC LE – Deep white matter T2/FLAIR hyperintensity without diffusion restriction on DWI [5,6,8].

Detection of Leukoencephalopathy

MRI by 1.5T or 3T MRI scanner (Siemens) using the following protocol. Axial T2WI, FLAIR, T1WI, DWI, SWI, coronal T2WI and post contrast T1WI done in each patient. Abnormal MRI changes were identified according to radiographic criteria of Common Terminology Criteria for Adverse Events (v4.0).

Statistical Analysis

Data was entered in Microsoft excel and analyzed using XLSTAT. Descriptive statistics was presented in numbers and percentages. Association between two non-parametric variables was checked using Pearson Chi-square test and spearman rho test. p-value of <0.05 was taken as statistically significant.

Results

34 children were enrolled. One patient who had baseline LE changes was excluded from analysis. Final analysis was done on 33 patients.

Among these 33, 13(39.4%) patients were <10 years and 20(60.6%) were >10 years. There were 7(21.2%) females and 26(78.8%) males with male:female ratio of 3.7:1 (Table 2).

LEUKOENCEPHALOPATHY IN MRI (Table 3; Graph 1)

Leukoencephalopathy was seen in zero patients at time point 1; 2(6.7%)patients at time point 2; 3(9.4%)patients at time point 3; and 2(6.7%)patients at time point 4.

SERUM LEVELS OF VITAMIN B12, FOLATE and HOMOCYSTEINE

Higher prevalence of low B12 was seen at Time-Point 1 in 6(18.2%) patients and at Time-Point 4 in 4(13.3%) patients. Higher prevalence of low folate was seen at Time-Point 1 in 3(9.1%) patients and at Time-Point 4 in 3(10.0%) patients. Same trend was also seen with high homocysteine, higher prevalence at Time-Point 1 in 8(24.2%) patients and at Time-Point 4 in 18(53.3%) patients. [Table 3; Graphs 2,3,4].

Association of MRI LE findings with hematological parameters were assessed using Chi-square test. We had 106 MRI scans of all participants taken at 4 different time-points along with corresponding blood parameters. There was no association of B12 with LE (p=0.214). There was significant association between folate and leukoencephalopathy (p=0.015). There was no association seen between homocysteine and leukoencephalopathy (p=0.677). [Tables 4,5,6]

WHITE BLOOD CELL (WBC) COUNT, CD20 EXPRESSION AND CHROMOSOMAL TRANSLOCATIONS

There was statistically significant association between low WBC count, CD20 expression and leukoencephalopathy (p=0.035), showing that leukoencephalopathy is dependent on WBC count and CD20 expression at baseline. [Table 7]

There was statistically significant association seen between t (1:19) and t (9:22) with leukoencephalopathy (p=0.03 & 0.0004 respectively), showing that leukoencephalopathy is dependent on t (1:19) and t (9:22) translocations. No association was found between t (12:21) and with LE. t (4:11) was not seen in any patient in our study [Table 8].

Table 1A: Risk stratification

Standard risk group:
* Leukemic cells < 1000 blasts /uL in the peripheral blood on day 8 of phase 1 part 1 * WBC < 20000 ul/L and age >1 <6 years * A complete remission on day 33 (M1-marrow) * No BCR/ABL recombination or translocation t (9;22) * No MLL/AF4 recombination or translocation t (4;11) * No T-cell ALL * All six criteria must be met **PLUS** * MRD negative (i.e., <0.01%) at both time points.
Intermediate risk group:
* Leukemic cells < 1000 blasts/uL in the peripheral blood on day 8 of phase 1 part 1 * A complete remission on day 33 (M1-marrow) * No BCR/ABL recombination or translocation t (9;22) * No MLL/AF4 recombination or translocation t (4;11) * All 4 criteria must be met as well as at least one of the following: * Leukocytes > 20000 cells/uL * Age < 1 year * Age > 6 years **PLUS** * MRD >0.01% but <1% at end of induction and low MRD <0.01% at end of consolidation.
High risk group:
* >1000 blasts/uL leukemic cells in the peripheral blood on day 8 * No complete remission on day 33(M2 or M3 marrow) * BCR/ABL recombination or translocation t (9;22) * MLL/AF4 recombination or translocation t (4;11) * Each criterion alone qualified as high risk regardless of age and WBC **PLUS** * MRD levels >1% at end of induction and/or >0.01% at end of consolidation.

Table 1b: Treatment protocol [13–15]

Induction therapy:	
Methotrexate	IT Intrathecal Day 1, 12 (18, 27 CNS Disease at diagnosis only), 33 (Dose : <1 year – 6mg, 1-2 year – 8 mg, 2-3 year – 10mg, >3 year – 12mg)
Prednisolone	60 mg/m2 orally daily days 1 to day 29
Vincristine	1.5 mg/m2 IVI Days 8, 15, 22, 29
Daunorubicin	30 mg/m2 IVI Days 8, 15, 22, 29 (SR BALL only 2 doses were given day 8, 15)
L-asparaginase	5000 units/m2 deep IM/ IVI over 60 minutes (ensure test dose has been given) given on days 12,15,18,21,24,27,30,33
	OR Peg asparginase 1000 units/m2 deep IM on days 12 and 24
Consolidation: (SR/IR ALL)	
6-Mercaptopurine	60 mg/m2 orally daily from day 1 to 28 (28 days total)
Cyclophosphamide	1000 mg/m2 by IV infusion Day 1, 29
Mesna	400 mg/m2 by IV infusion hours 0, 4 and 8 post cyclophosphamides
Cytarabine	75 mg/m2 by IV infusion daily days 3 to 6, 10 to 14, 17 to 20, 24 to 27
Methotrexate	IT Intrathecal Days 10, 24 (Dose : <1 year – 6mg, 1-2 year – 8 mg, 2-3 year – 10mg, >3 year – 12mg)
Consolidation (Augmented): (HR ALL)	

Methotrexate	IT Intrathecal Day 1, 8, 29 (Dose : <1 year – 6mg, 1-2 year – 8 mg, 2-3 year – 10mg, >3 year – 12mg)
Cyclophosphamide	1000 mg/m2 by IV infusion Day 1, 29
Mesna	400 mg/m2 by IV infusion hours 0, 4 and 8 post cyclophosphamides
Cytarabine	75 mg/m2 by IV infusion daily days 2 to 5, 9 to 12, 30 to 33, 37 to 40
L-asparaginase	10,000 units/m2 deep IM / IVI over 60 minutes (ensure test dose has been given) given on days 15,18,21,24,43,46,49,52
	OR Peg asparginase 1000 units/m2 deep IM on days 16 and 44
Vincristine	1.5 mg/m2 IVI Days 16, 23, 44, 51
6-Mercaptopurine	60 mg/m2 orally days 1 to 14 and 29 to 42
Extracompartment Therapy:	
6-Mercaptopurine	25 mg/m2 orally days 1 to 56
Methotrexate	2000 mg/m2 (SR/IR B-ALL) / 5000 mg/m2 (HR-BALL / T-ALL) by IV infusion over 24 hours days 8, 22, 36, 50
Methotrexate	IT intrathecal days 8, 22, 36, 50 (Dose : <1 year – 6mg, 1-2 year – 8 mg, 2-3 year – 10mg, >3 year – 12mg)
Leucovorin	15 mg/m2 IVI 18 hours post completion of MTX infusion, 6 doses at 6 hourly interval.
Reinduction-A:	
Dexamethasone	10 mg/m2 orally days 1 to 21
Methotrexate	IT only for patients with CNS disease at diagnosis days 1, 18
Vincristine	1.5 mg/m2 IVI Days 8, 15, 22, 29
Doxorubicin	30 mg/m2 IVI Days 8, 15, 22, 29
L-asparaginase	10000 units/m2 deep IM / IVI over 1 to 2 hours Days 8, 11, 15, 18
Reinduction-B:	
Thioguanine	60 mg/m2 orally daily from day 36 to 49 (14 days total)
Cyclophosphamide	1000 mg/m2 by IV infusion over 1 hour Days 36
Mesna	400 mg/m2 by IV infusion hours 0, 4 and 8 of cyclophosphamide infusion
Methotrexate	IT intrathecal Day 38, 45 (Dose : <1 year – 6mg, 1-2 year – 8 mg, 2-3 year – 10mg, >3 year – 12mg)
Cytarabine	75 mg/m2 by IV infusion days 38 to 41, 45 to 48 (over 30 to 60 minutes each day)
Cranial RT	
Prophylactic RT (12 GY)	HR B- ALL / T- ALL
Therapeutic RT (18 GY)	CNS 3 patients
Standard Maintenance Therapy:	
6-Mercaptopurine	50 - 90 mg/m2 orally daily
Methotrexate	20 - 30 mg/m2 orally weekly once
Methotrexate	IT intrathecal once every 3 months (Dose : <1 year – 6mg, 1-2 year – 8 mg, 2-3 year – 10mg, >3 year – 12mg)
	(Not administered in whom Cranial RT was administered)
Duration	104 weeks in total

Table 2: Baseline Demographics

	Number	Percentage
AGE		
<10 years	13	39.4
>= 10 years	20	60.6
GENDER		
Female	7	21.2
Male	26	78.8

Table 3: Investigational parameters in study subjects at 4 time points.

Abnormality	Time Point 1 (N=33)		Time Point 2 (n=30)		Time Point 3 (n=32)		Time Point 4 (n=30)	
	No.	%	No.	%	No.	%	No.	%
LEUKO ENCEPHA-LOPATHY	0	0	2	6.7	3	9.4	2	6.7
LOW SERUM B12	6	18.2	2	6.7	2	6.3	4	13.3
LOW SERUM FO-LATE	3	9.1	1	3.3	0	0	3	10.0
HIGH SERUM HO-MOCYSTEINE	8	24.2	7	23.3	6	18.8	16	53.3

Table 4: Association between vitamin B12 with Leukoencephalopathy

		LEUKOENCEPHALOPATHY		Total
		LE	No LE	
B12 level	Low	2	12	14
	Normal	5	87	92
Total		7	99	106
$\chi 2$ value = 1.543, df=1, p value = 0.214				

Table 5: Association between Folate with LE

		LEUKOENCEPHALOPATHY		Total
		LE	No LE	
Folate level	Low	2	5	7
	Normal	5	94	99
Total		7	99	106
$\chi 2$ value = 5.864, df=1, p value = ***0.015****				

Table 6: Association between homocysteine level with leukoencephalopathy

		LEUKOENCEPHALOPATHY		Total
		Absent	Present	
Homocysteine level	Abnormal	34	3	37
	Normal	63	4	67
Total		97	7	104
$\chi 2$ value = 0.174, df=1, p value = 0.677				

Table 7: Association between leukoencephalopathy and White blood cell (WBC) count at baseline

		LEUKOENCEPHALOPATHY		Total
		Absent	Present	
WBC count at baseline	<20000/UL	14	5	19
	>20000/UL	14	0	14
Total		28	5	33
$\chi 2$ value = 4.342, df=1, p value = ***0.037****				

Table 8: Association between leukoencephalopathy and CD20 expression at baseline

		LEUKOENCEPHALOPATHY		Total
		Absent	Present	
CD20 expression at baseline	Absent	8	4	12
	Present	11	0	11
Total		19	4	23
$\chi 2$ value = 4.439, df=1, p value = ***0.035****				

Table 9a: Association between leukoencephalopathy and translocation (12:21)

		Leukoencephalopathy		Total
		Absent	Present	
t(12:21)	Negative	16	4	20
	Positive	3	0	3
Total		19	4	23
Spearman's Rho: rs = -0.1777, p (2-tailed) = 0.41725.				

Table 9b: Association between leukoencephalopathy and translocation (1:19)

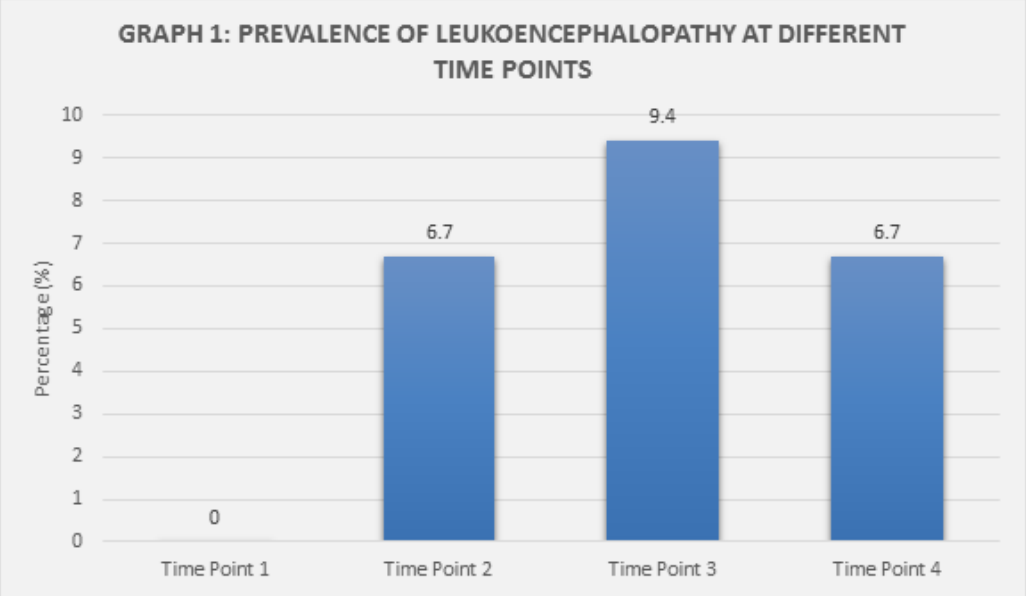
		Leukoencephalopathy		Total
		Absent	Present	
t(1:19)	Negative	19	3	22
	Positive	0	1	1
Total		19	4	23
rs = 0.46466, p (2-tailed) = ***0.02549.*** By normal standards, the association between the two variables would be considered statistically significant				

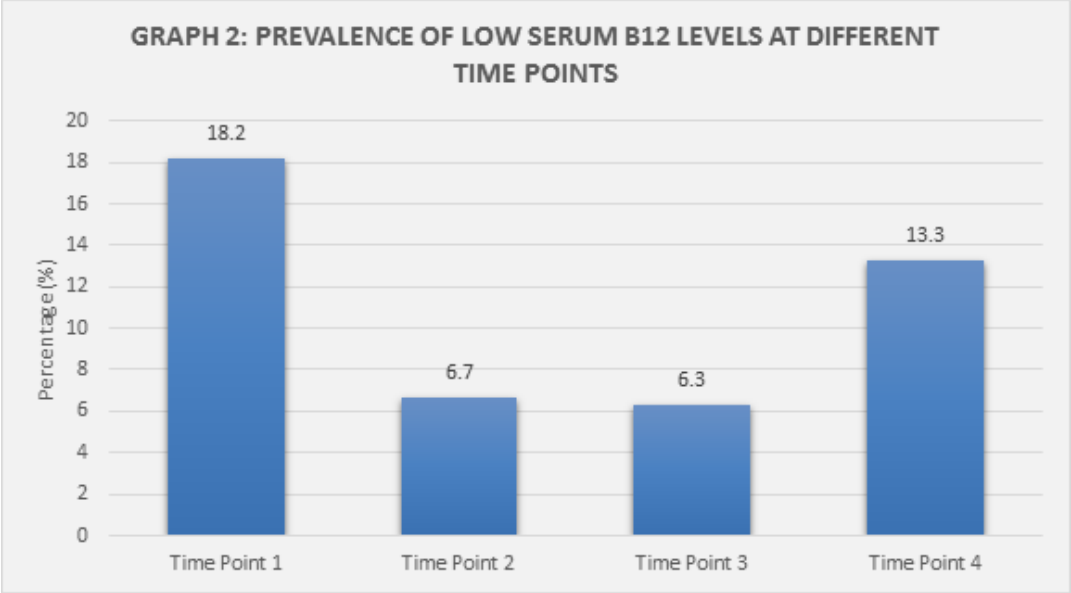
Table 9c: Association between leukoencephalopathy and translocation (4:11)

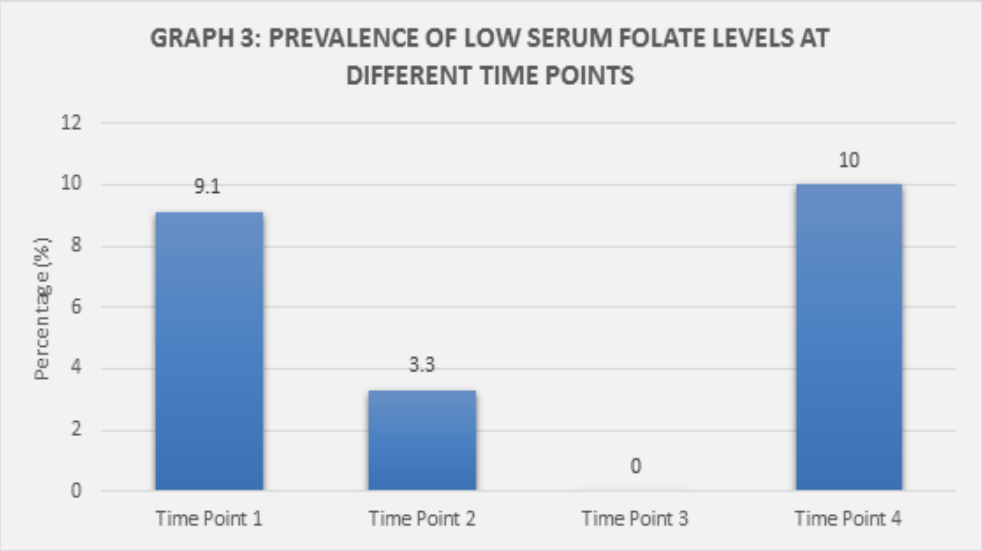
		Leukoencephalopathy		Total
		Absent	Present	
t(4:11)	Negative	19	4	23
	Positive	0	0	0
Total		19	4	23

Table 9d: Association between leukoencephalopathy and translocation (9:22)

		Leukoencephalopathy		Total
		Absent	Present	
t(9:22)	Negative	17	4	21
	Positive	2	0	2
Total		19	4	23
rs = 0.67259, p (2-tailed) = ***0.00044.*** By normal standards, the association between the two variables would be considered statistically significant.				




GRAPH 2: PREVALENCE OF LOW SERUM B12 LEVELS AT DIFFERENT TIME POINTS
Percentage (%)
20
18
16
14
12
10
8
6
4
2
0
18.2
6.7
6.3
13.3
Time Point 1
Time Point 2
Time Point 3
Time Point 4


GRAPH 3: PREVALENCE OF LOW SERUM FOLATE LEVELS AT DIFFERENT TIME POINTS
Percentage (%)
12
10
8
6
4
2
0
9.1
3.3
0
10
Time Point 1
Time Point 2
Time Point 3
Time Point 4

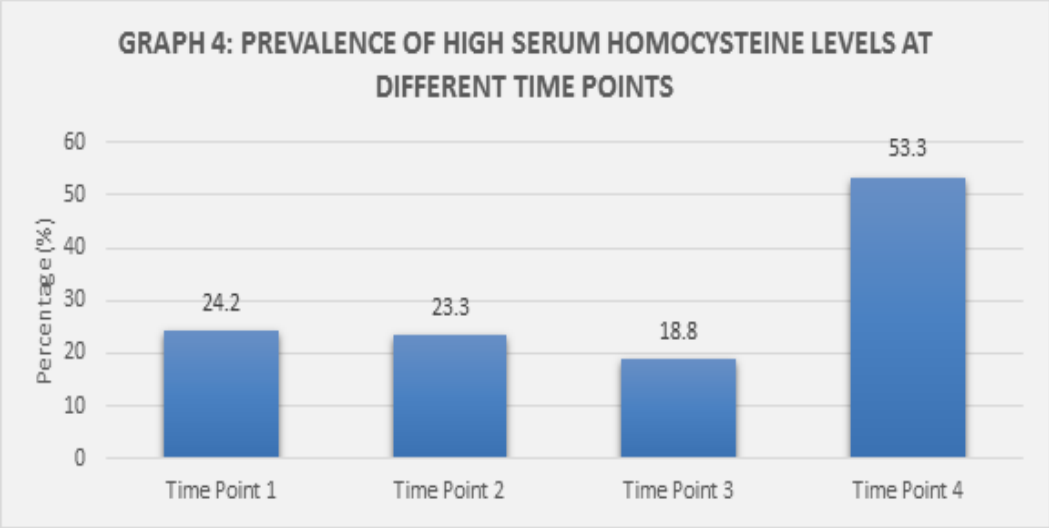

GRAPH 4: PREVALENCE OF HIGH SERUM HOMOCYSTEINE LEVELS AT DIFFERENT TIME POINTS
Percentage (%)
60
50
40
30
20
10
0
24.2
23.3
18.8
53.3
Time Point 1
Time Point 2
Time Point 3
Time Point 4

Figure set I: MRI images of asymptomatic le

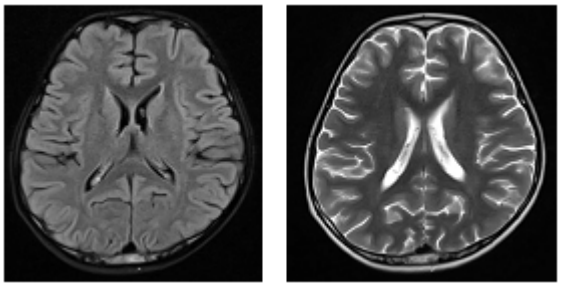


Figure 1: Baseline cranial MRI of an ALL patient. FLAIR (a) Axial T2WI (b) shows no abnormality

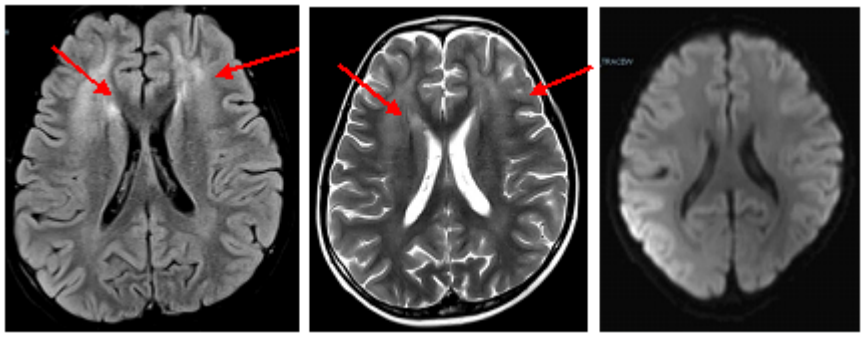


Figure 2 (Asymptomatic LE): MRI brain after administration of high dose methotrexate. FLAIR (a) and Axial T2WI (b) shows hyper intensity involving the deep white matter in bilateral frontal lobes suggesting leukoencephalopathy (c) Axial DWI no diffusion restriction noted.

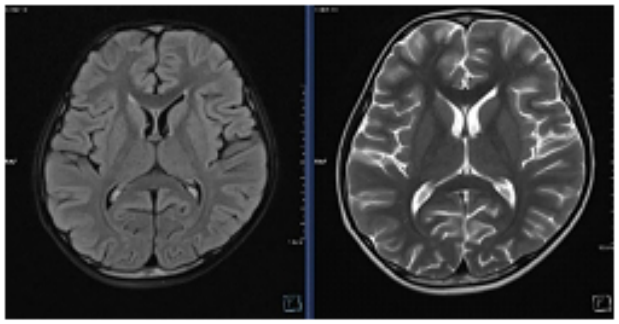


Figure 3: Cranial MRI done in maintenance of ALL patient. FLAIR (a) and Axial T2WI (b) and shows no abnormality, complete resolution of previously developed LE

<u>Figure set II: MRI images of symptomatic le:</u>

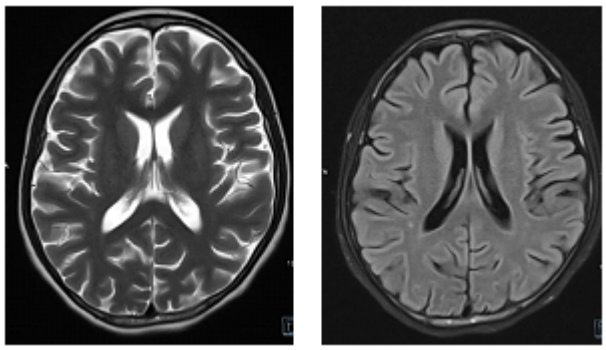


Figure 4: Baseline cranial MRI of ALL patient. Axial T2WI (a) FLAIR (b) shows no abnormality

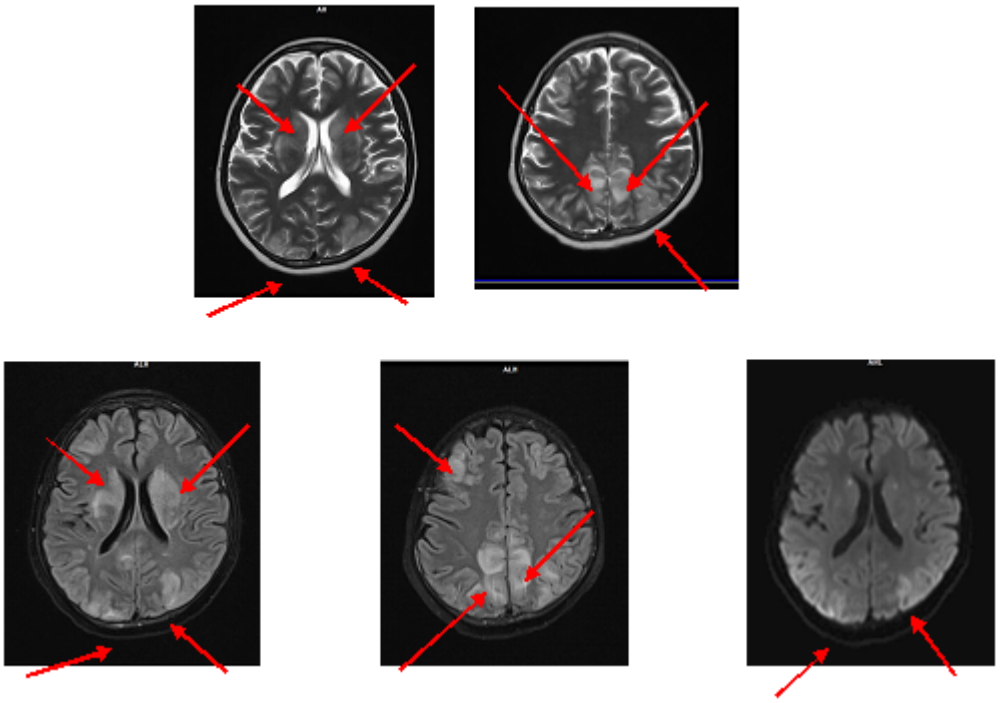


Figure 5 (Symptomatic LE): MRI brain after administration of IT methotrexate. Axial T2WI (a, b) and FLAIR (c, d) shows hyper intense lesions in bilateral basal ganglia, deep white matter, posterior frontal, parieto temporal cortex and subcortical white matter. Corresponding axial DWI (e) shows patchy areas of diffusion restriction noted within the lesions.

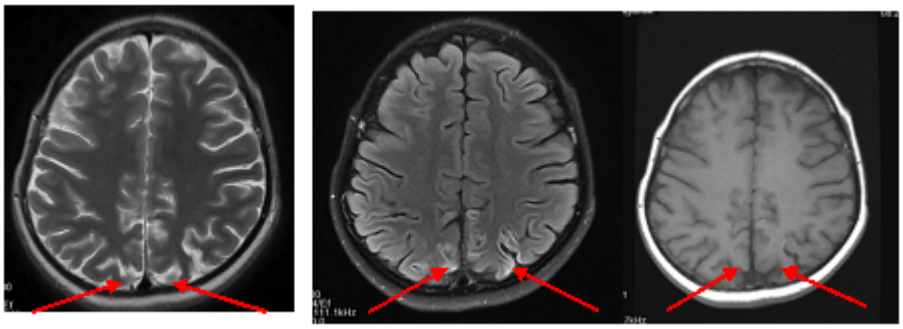


Figure 6: MRI Brain at time point 4 showing Axial T2WI (a), FLAIR (b) shows hyperintensity in high frontoparietal cortex with gyral pattern of hyperintensity in axial T1WI (c) suggestive of cortical laminar necrosis as a sequel.

Discussion

Male predominance was seen in ALL. These results are comparable to previous studies [10,15].

Incidence of MTX induced LE:

We identified Leukoencephalopathy secondary to methotrexate in 7of116 (6.03%)cases and on MRI brain in 5of33(15.15%)patients. Of these, 1(3.03%) had symptomatic LE, which is consistent with previous studies which had reported 0.8to3.8% of symptomatic LE (16–18). 4(12.12%) were asymptomatic, this is similar to other studies having reported as 11to68% of asymptomatic LE (5,19). All LE patients were in age group more ≥10 years, which corroborates with previous report (18). Age association might be due to lower MTX clearance in adolescents [17].

Description of MTX induced LE in 5 patients as follows:

1 patient with symptomatic LE was a 14-year-old female B-ALL IR with t [1;19], received 4 drug induction, baseline MRI was normal, baseline b12 was normal, folate was low and homocysteine was high. She received 2 uneventful intrathecal methotrexate. 7 days after receiving 3rd IT MTX she presented with status epilepticus, MRI at this time showed hyperintense lesions in bilateral basal ganglia, deep white matter, parietotemporal cortex

and subcortical white matter with patchy areas of diffusion restriction within the lesions. After stabilization, chemotherapy was resumed, she could tolerate further doses of IT and HD MTX with no recurrence of LE. Prospective MRI showed resolution of lesions and 4th MRI revealed T1 hyperintensity in bilateral parieto-tempero-occipital lobes suggestive of cortical laminar necrosis. Patient had early systemic relapse during maintenance and succumbed to disease.

This case is similar to earlier reported cases of atypical presentation of LE [5,8,17]. Important hallmark was involvement of cortex and subcortical white matter, few have suggested it to be an overlap between PRES and LE [20].

Of the 4 other patients, 1st patient was a 14-year male with B-ALL IR, normal baseline MRI, normal b12, folate, homocysteine levels. He received 4 drug induction A with 5 doses of IT-MTX, 2nd MRI was done before 1st HD-MTX which showed LE changes. At that time, folate was low, with normal b12 and homocysteine. Subsequently he received 4 doses of HD-MTX. 3rd MRI after last dose of HD-MTX revealed decrease in intensity of LE and it disappeared by 4th MRI.

2nd patient was a 10-year-old male B-ALL HR (poor prednisolone response), received 4 drug induction f/b augmented consolidation f/b 4 cycle of HD-MTX. His baseline and 2nd MRI along with b12, folate, homocysteine at both points were normal. 3rd MRI was done after 4 doses of HD-MTX revealed LE changes. B12, folate and homocysteine at this time-point were normal. LE changes disappeared in the 4th MRI.

3rd patient was a 12-year-old male patient of B-ALL HR (MRD2=0.02%), received 4 drug induction A, induction B, 4 doses of HD-MTX, re-induction A and B f/b maintenance with oral low-dose MTX and 6MP. His 1st, 2nd, 3rd MRI were normal, had low b12 and high homocysteine at 1st time-point and isolated high homocysteine at 3rd time-point. His 4th MRI done after 2months of oral low-dose MTX revealed LE, corresponding b12, folate and homocysteine were normal.

4th patient was 13-year-old male T-LBL IR, received 4 drug induction A, induction B, 4 doses of HD-MTX, re-induction A and B f/b maintenance with oral low dose MTX and 6 MP. His 1st, 2nd, 3rd MRI Brain were normal, had low b12, folate, high homocysteine at 1st and 4th time-points, corresponding MRI at 4th time-point showed LE changes.

All the patients with asymptomatic LE diagnosed on MRI revealed hyperintensity involving the deep white matter without diffusion restriction. All 5 patients with different disease characteristics developed LE at different time during therapy, thus we could not predict in whom LE will develop and after which dose and mode of methotrexate. Baseline MRI was not predictive of development of LE. 3of5 patients with LE had abnormal b12/folate/homocysteine with corresponding abnormal MRI. We found significant association of LE with low folate in accordance with previous reports [21,22]. 2 patients who had LE in 4th MRI, it might be the delayed MTX toxicity and needs to be followed up longer to look for any cognitive disturbances or risk of relapse.

We gave 4 courses of IV HD-Mtx to SR, IR B-ALL, HR B-ALL, T-ALL. MTX levels were not available. Therefore HD-MTX administration was followed by leucovorin rescue @15 mg/m2 IVI 18 hours post completion of MTX infusion, 6 doses at 6 hourly interval [23]. We found no increase in incidence of leukoencephalopathy secondary to methotrexate : leucovorin ratio as reported by previous studies [6,16]. Also there was no difference in incidence with respect to mode of administration of Mtx, even there was no increased incidence after 4 courses of HD-MTX contrary to previous reports [24,25].

Re-challenge with Methotrexate in LE:

All patients with leukoencephalopathy were exposed to all doses of IT, HD and Oral Methotrexate and was not associated with progression or recurrence of LE. Thus, from our findings, we recommend not to withhold methotrexate, as supported in previous studies [16,18,26].

Relation of folate, b12, homocysteine levels with MTX-LE

Severe folate deficiency can cause pancytopenia and megaloblastic anemia [27]. We found abnormal b12, folate and homocysteine levels in considerable patients. There was prevalence of low b12 in 10of33(30.3%), low folate in 6of33(18.18%), high homocysteine in 21of33(63.63%) cases which was less compared to previous studies probably because of lower cut-offs [28,29].

Higher prevalence of low B12, low folate was found at baseline and during oral methotrexate therapy. High homocysteine was found during oral MTX therapy. Significant patients had high homocysteine at 4th time-point. i.e. during oral methotrexate administration compared to other time points because assays were done after gap 14 days of last dose of MTX. MTX levels and its effect on homocysteine might have normalized till then [30,31].

B12 and folate deficiencies negatively influence the outcome and occurrence of complications during induction chemotherapy in children with ALL. Homocysteine in children with ALL is elevated

prior to therapy, probably because of occasional folate deficiency and increased burden of proliferating cells [32]. Low folate aggravates MTX effect secondary to homocysteine accumulation and is also responsible for hematological toxicity [28,33,34]. There are very few articles on association of MTX induced neurotoxicity with low folate [21,35] or low b12 levels [7].

Homocysteine increased following methotrexate, and is associated with methotrexate induced neurotoxicity [22,30–32]. Homocysteine causes neurotoxicity by direct vascular endothelial damage [8,17]. Hyperhomocysteinemia is precipitated by presence of low folate or MTHFR mutations which may be biomarkers of methotrexate induced neurotoxicity [30,33]. Thus, methotrexate therapy, decreased MTHFR enzyme activity, low b12 or low folate, all result in increased homocysteine and decreased methionine levels which are responsible for neurotoxic effects.

We found significant association of low folate with methotrexate induced leukoencephalopathy($p<0.05$). There is suggestion that serum folate should be measured and deficiency should be corrected prior to methotrexate therapy to reduce toxicity [28]. We also saw increased systemic toxicity in few patients post methotrexate therapy having low folate or b12. Initially b12 and folate supplements were not given even in patients with very low levels with a fear that it might aggravate leukemia, as reported by previous author [34]. But looking at the severity of cytopenias and their prolonged duration causing chemotherapy interruptions especially after HD-MTX, b12 and folate supplementation was initiated and this resulted in improvement in blood counts. Leukoencephalopathy was seen in patients with low WBC count at diagnosis, absence of CD20 expression and with positive t [1:19] and t [9:22].

Therefore, it may be worth doing serum folate, b12, and homocysteine in ALL patients at diagnosis and consider these to correct in those with deficiency so as to limit systemic and neurotoxicity secondary to chemotherapy. However multi-institutional prospective studies with large number of patients would be able to answer the question conclusively.

Conclusion

MTX-induced leukoencephalopathy is transient, and most patients can be rechallanged with subsequent MTX without recurrence. MRI at baseline is not a predictor for LE. Low folate is associated with increased risk of methotrexate induced leukoencephalopathy.

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