EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
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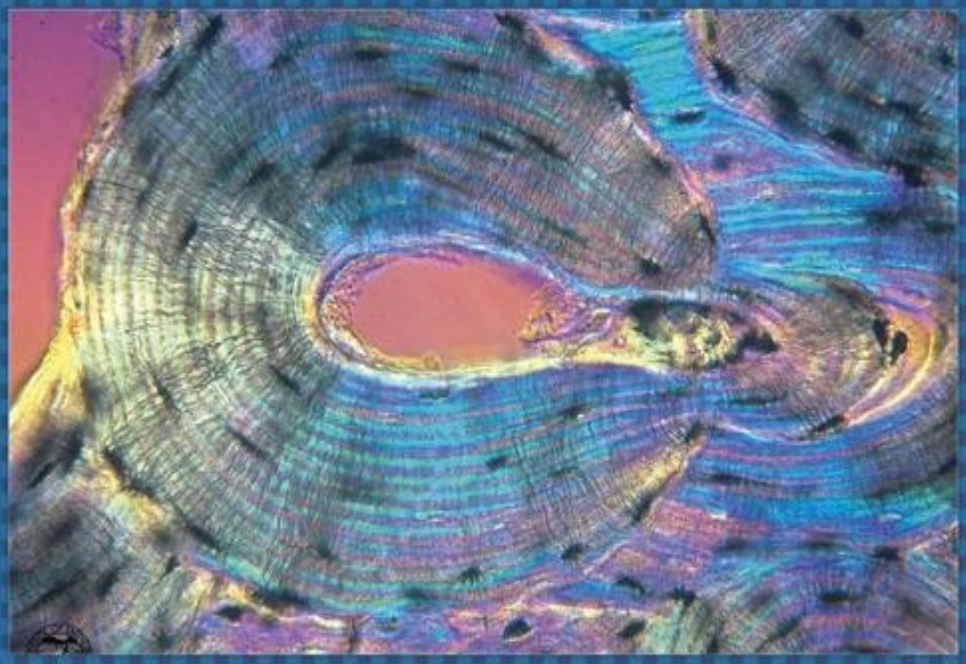
HISTOLOGY & HISTOCHEMISTRY



ISSN
2090-0775

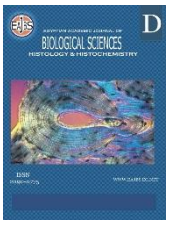
WWW.EAJBS.EG.NET

Vol. 17 No. 1 (2025)

Citation: Egypt. *Acad. J. Biolog. Sci. (D-Histology and histochemistry) Vol.16(2) pp1-9(2025)*
DOI: 10.21608/EAJBSD.2025.403509

Egypt. Acad. J. Biolog. Sci., 17(1):1-9 (2025)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg



Central Nervous System Relapse in Acute Lymphoblastic Leukemia: Incidence and Prognostic Factors

Sunitha Shankaralingappa[1] and Arundhathi Shankaralingappa*[2]
[1]M. S. Ramaiah memorial hospital,Bengaluru.
[2]All India Institute of Medical Sciences, Mangalagiri,Andhra Pradesh.
*** E-mail** : arundhathi19@yahoo.co.in

ARTICLE INFO
Article History
Received:6/7/2024
Accepted:7/1/2025
Available:11/1/2025

***Keywords*:**
Acute lymphoblastic leukaemia, Central nervous system relapse, hyperleucocytosis, bone marrow, isolated CNS relapse.

ABSTRACT

Background: The recognition of central nervous system (CNS) involvement at the time of diagnosis in acute lymphoblastic leukemia (ALL) is crucial for determining appropriate treatment strategies and reducing the risk of CNS relapse. While CNS involvement at diagnosis is relatively rare, occurring in less than 5% of children and 10% of adults with ALL, it remains an important consideration due to its potential impact on prognosis.**Materials and methods:**In the retrospective analysis of 102 patients with acute lymphoblastic leukemia (ALL) and CNS relapse, the study aimed to compare clinical, hematological, phenotypical, and molecular features at the time of diagnosis and response to treatment between patients with isolated CNS relapse (Group 1) and those with combined CNS relapse with concurrent bone marrow relapse (Group 2). Additionally, the study aimed to identify risk factors associated with relapse in these patients. **Results:** Among 720 patients with acute lymphoblastic leukemia (ALL), 102 (14.17%) experienced CNS relapse. Median blast percentage in peripheral blood was 55%. Hyperleucocytosis seen in 26.47% patients and peripheral smear blasts >50% in 50.98%. Immunophenotyping revealed that 13.73% of cases were T-cell ALL, while 83.33% were B-cell ALL. Philadelphia chromosome identified in 15.69% cases. Among patients with CNS relapse, 33.33% experienced isolated CNS relapse, while 66.67% had concurrent bone marrow relapse. Patients in Group 2 were more likely to present with hyperleukocytosis and peripheral smear blasts >50% compared to those in Group 1. **Conclusion:** In our study we found hyperleucocytosis and TLC at time of relapse were significantly associated with CNS relapse

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is one of the most common childhood malignancies, comprising about 25% of all malignancies in children and about 75% of childhood leukemias (Sung SH *et.al.,* 2014). Central nervous system (CNS) involvement at the time of diagnosis is relatively rare, occurring in less than 5% of children and 10% of adults with ALL. However, when CNS involvement does occur, it significantly impacts relapse-free survival and overall survival rates (Davis *et.al.*, 2014). In the early 1970s, before the widespread use of CNS prophylaxis, the incidence of CNS leukemia was remarkably high, ranging from 80% to 85% (Evans AE et.al., 1970). With the advent of CNS prophylaxis, the CNS relapse has reduced to 5-10% and is still a major clinical concern (Pui *et.al.,* 2000).

In the recent past, researches and clinical trials have improved the response rates in patients with ALL. Better understanding of pathogenesis of disease, implementation of risk-based induction and maintenance treatment strategies with improved supportive care have improved the overall survival of patients with ALL (Del Principe *et.al.*, 2014).

CNS relapse can be isolated, after bone marrow (BM) recurrence or with simultaneous CNS and BM recurrences (Surapaneni *et.al.,* 2002). There are many factors which have been responsible for CNS relapses in ALL according to literature search. These factors include age, gender, total leucocyte count at presentation, immunophenotype and genotype of leukemic cells and rapidity of response to early therapy (Pieters *et.al.,* 2010). This study was conducted to analyse the risk factors responsible for CNS relapse in ALL.

MATERIALS AND METHODS

Ours is a retrospective hospital-based study done over a period of two years. Out of 720 cases of ALL, patients with CNS relapse were enrolled in the study.

Inclusion criteria: Patients with CNS relapse and diagnosed in our hospital.

Exclusion criteria:

1. Patients without CNS relapse or with only medullary relapse.
2. Initial diagnosis not made in our hospital.
3. Patients lost to follow up.

Patients were allotted into two groups, group:

1-Isolated CNS relapse and group.

2- Combined relapse (CNS with concurrent medullary relapse).

The demographic details and clinical informations were obtained from patients' hospital records and information system. The clinical, hematological and molecular details were computed, analysed and compared between the two groups. The categorical variables were expressed in percentages. The results were expressed as median and Inter Quartile Ranges (IQR). The risk factors in both the groups were compared using Mann-Whitney U test as the data was non-normally distributed. p-value <0.05 was considered as statistically significant. Analysis of the data was done by SPSS version 20.0.

1-Diagnosis and Definition:

Bone marrow examination was performed on all patients at day 33following induction – phase I therapy to assess marrow remission. Complete remission is defined as bone marrow blasts <5%. Bone marrow relapse is defined as blast percentage >25%. Cerebrospinal fluid (CSF) examination was performed in all patients irrespective of presence or absence of CNS symptoms on 8^{th} day of treatment. CSF was considered as involved if the leukaemic cells were identified in cytospin preparation and this was considered as initial CNS involvement.The positive cytology patients were followed with repeat CSF examination till the CSF became negative for leukaemic cells.

CSF examination was performed on patients who presented with headache, diplopia, vomiting, altered sensorium or cranial nerve defects during course of the disease. Immunophenotyping was performed wherever possible. Isolated CNS relapse is defined as $\geq$5 cells/mm^3 with leukemic blasts in CSF without major blood contamination ($\leq$ 20 RBCs/ $mm^{3)}$ or clinical signs of CNS disease or leukemic mass found on cranial imaging studies with bone marrow blasts <5%, no blasts in peripheral blood or no leukemic infiltration elsewhere.

Total leucocyte count (TLC) and peripheral smear blast % at the time of diagnosis was collected in all patients. Hyperleucocytosis was defined as TLC $>50x10^9$/L. Immunophenotypic details of all the patients were computed. The cytogenetic profile was analysed by conventional cytogenetics and FISH. Adverse cytogenetic abnormality was defined as t (9,22) and complex karyotype. TLC at the time of relapse

was analysed for leucocytosis (>$10x10^9$/L) and leucopenia (<$4x10^9$/L).

The relapse free time, TLC at the time of diagnosis, PS blast %, TLC at relapse, organomegaly, lymphadenopathy, prescence of mediastinal mass, immunophenotype, cytogenetic abnormality, presence of relapses at multiple sites and the response to treatment were analysed and compared between the two groups to study the risk factor for CNS relapse.

2-Treatment Protocol for ALL Patients:

Berlin-Frankfurt- Munster- 90 Regimen Pre-induction: Prednisolone (orally), 60 mg/m^2/day, day 1-7

Induction Phase-I: Prednisolone (orally), 60mg/m^2/day, day 8-28; Vincristine (IV), 1.4mg/m^2 and Daunorubicin (IV), 30mg/m^2, day 8,15,22,29; L-Asparaginase (IM), 10,000 Units/m^2, day 12,15,18,21,24,27,30,33; Intrathecal methotrexate, 12mg on day 8, 15, 29.

Induction Phase-II: Cyclophosphamide (IV), 1gm/m^2, day 36, 64; Cytarabine (IV), 75mg/m^2, day 38-41, 45-48, 52-55, 59-62; 6-MP (orally), 60mg/m^2/day, day 36-64; Intrathecal methotrexate, 12mg, day 38,52.

Consolidation: 6-MP (orally), 25mg/m^2/day, day 1-7 of consolidation; Methotrexate (24 hr infusion), 5gm/m^2, day 8,22,36,50; Intrathecal methotrexate, 12mg, day 8,22,36,50.

Re-induction (delayed intensification): Dexamethasone (orally), 10mg/m^2/day, day 1-21 of Re-induction; Vincristine (IV), 1.4mg/m^2, day 8,15,22,29; Doxorubicin (IV), 30/mg/m^2, day 8,15,22,29; L-Asparaginase (IM), 10,000 units/m^2, day 8,11,15,18; Cyclophosphamide (IV), 1gm/m^2, day 36; Cytarabine (IV), 75mg/m^2, day 38-41, 45-48; Intrathecal methotrexate, 12mg, day 38, 45.

Risk stratification was done using the parameters- leukaemic cell mass (BFM-RF)*, prednisolone response, immunophenotype, mediastinal mass, CNS disease and Ph chromosome.Standard risk group is defined as <1000/µl blasts in peripheral blood on day-8 (Prednisolone good response-PPR), BFM-RF <0.8, no CNS disease, not T-ALL, no mediastinal mass, Ph-negative.Moderate risk group is defined as <1000/µl blasts in peripheral blood on day-8 (Prednisolone poor response-PPR), BFM-RF >0.8 & CNS disease or T-ALL or mediastinal mass.High risk group is defined as >1000/µl blasts in peripheral blood on day-8 (Prednisolone poor response-PPR), >5% blasts in bone marrow on day-33 and presence of Ph-chromosome.

*Leukaemic cell mass (BFM-Risk factor) is calculated as 0.2 x log (blast count in peripheral blood+1) + 0.06 x liver size in centimeters below the costal margin + 0.04 x spleen size in centimeters below the costal margin.

Treatment of CNS Involvement:

Prophylactic cranial radiation-12Gy is given in high- risk group.

Therapeutic cranial radiation- <1year-cranial radiation is not given; 1-2years-18 Gy; >2years-24 Gy.IT Methotrexate, IT Cytarabine and IT Hydrocortisone is given twice/week. This is continued till 3 consecutive CSF examinations are negative for leukaemic cells. CSF examination is done twice/ week.Treatment of testicular relapse: 2400 Gy local radiation and chemotherapy.Treatment of BMR: Cyclophosphamide, Vincristine and Prednisolone for 4-6 weeks. BM examination is done after 4 weeks to assess for marrow remission.

Primary Outcome of Study: To determine the risk factors associated with CNS relapse in ALL

Statistical Analysis: A descriptive study was carried out for all the variables included in the study. Data was entered in Microsoft Excel master sheet and analyzed using Statistical Package for Social Sciences (SPSS) version 20 software. Chi-square test was used to assess the association between these parameters. A value of $P < 0.05$ was taken as significant. analysed separately and p <0.05 was considered significant.

RESULTS

Among 720 patients with ALL, 102 (14.17%) presented with CNS relapse. Among those with CNS relapse, 72 (70.59%) were males and 30 (29.42%) were females with a mean age of 15.63 years. There were 24 (23.53%) patients above 20 years. The mean TLC at time of diagnosis was 64.65x10^3/µL and median TLC was 15.05 x $10^{3/}$µl. The median blast percentage in peripheral blood was 55%. Hyperleucocytosis (TLC >50X10^9/L) was seen in 27 (26.47%) patients and peripheral smear blasts >50% was noticed in 52 (50.98%) patients. Immunophenotyping revealed 14 (13.73%) cases of T-ALL and the remaining were B-ALL (83.33%). Philadelphia chromosome, an adverse cytogenetic aberration was identified in 16 (15.69%) cases at the time of diagnosis. Of them 8 had t (9,22) and the remaining 8 had variant (9,22) translocation.

The median time of bone marrow complete remission was 1 month (range, 1-9 months). Of 102 ALL patients, 34 (33.33%) had isolated CNS relapse (Group1) and 68 (66.67%) had concurrent BMR (Group 2). The median time interval from the time of CNS relapse was 1.25 years. Isolated CNS relapse was common in patients older than 20 years (11/34, 32.35%) and combined relapse was seen more often in patients less than 5 years (23/68, 33.83%).

Involvement of other sites in group 1 and group 2 patients are as in Figure (1).

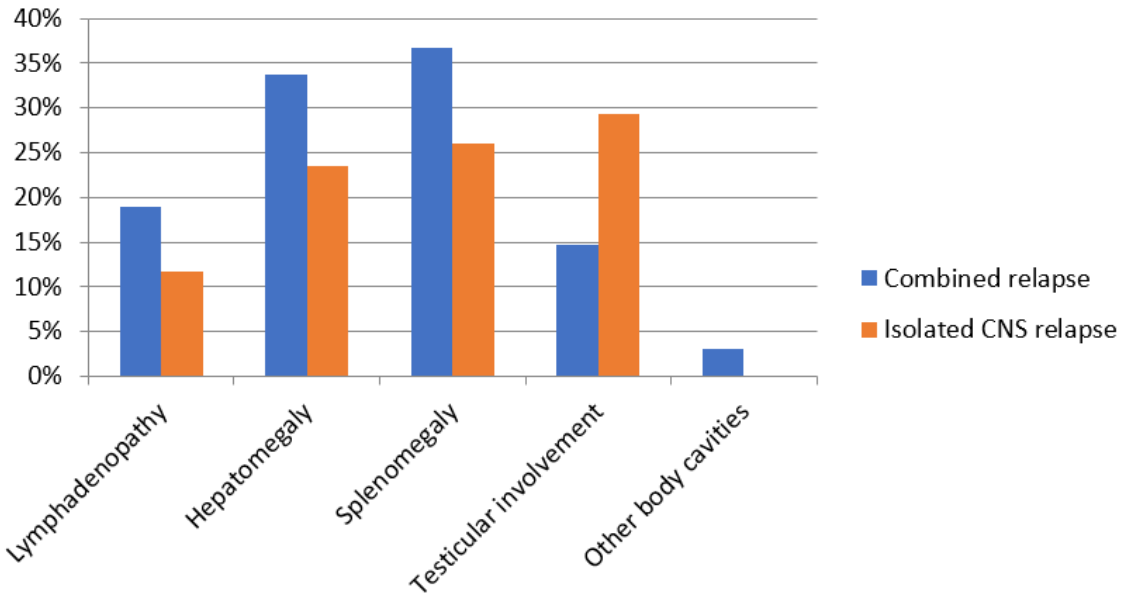


Fig.1: Involvement of other sites in group 1 and group 2 patients.

Testicular involvement was seen more often (10/34, 29.41%) in group1 patients, whereas splenic involvement (25/68, 36.77%) was frequently in group 2 patients. Mediastinal and other body cavity involvement was seen in group 2 patients accounting for two cases each.

Among 102 patients with CNS relapse, 18 (17.65%) patients experienced multiple CNS relapses at different points of time. Out of 18 cases with multiple CNS relapses, 3 patients were positive for Philadelphia chromosome. The shortest interval between two relapses noted was 2 months with range being 2 to 37 months. Hyperleucocytosis (TLC >50X10^9/L) was seen 31.8% of group 2 and 17.6% of group1 patients. Peripheral smear blasts >50% was noted in 56.9% of group 2 and 44.1% of group1 patients. (Fig. 2).

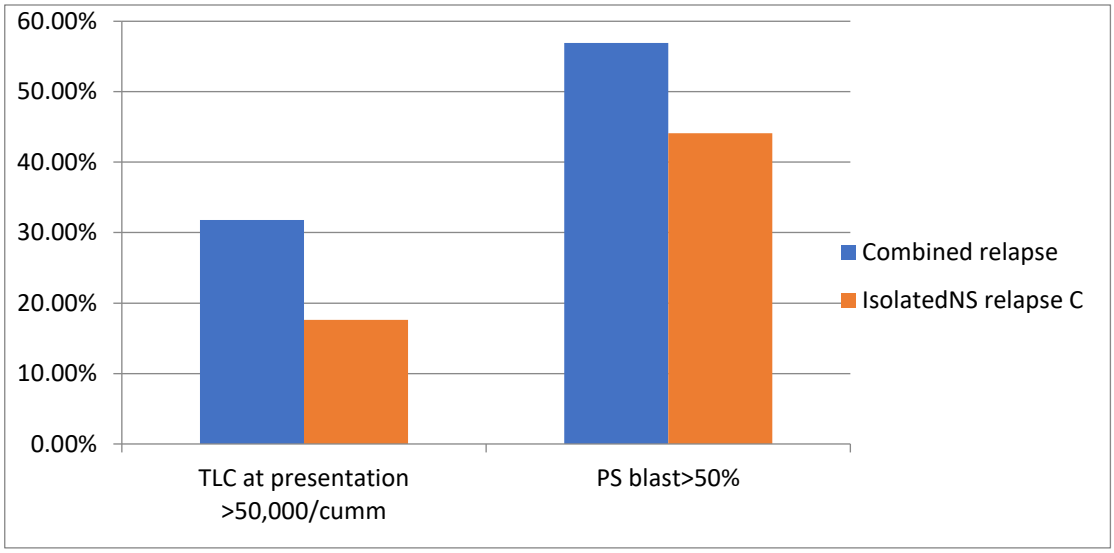


Fig.2: TLC and blast in group 1 and group 2 patients

The incidence of CNS relapse in our two- year retrospective study was 14.17%. Two- year cumulative incidence of isolated CNS relapse was 33.33%. The comparison of characteristics between two groups is shown in Table 1. In our study, hyperleucocytosis was more often seen in group 2 (31.8%) compared to group 1 (17.6%) and blasts more than 50% was frequently seen in group 2 (57.4%) compared to group 1 (44.2). Hepatomegaly and splenomegaly were observed most often in group 2. Mediastinal mass and body cavity infiltration was seen in group 2 even though less in number. The median duration between initial diagnosis of ALL and CNS relapse in group 1 and group 2 was 1.25 years and 1.3 years respectively.

Our study showed that there was significant correlation between TLC at relapse and with CNS relapse with concurrent BM involvement (p-0.009). There was also significant correlation between number of CNS relapse (p-0.014) and hyperleucocytosis (p-0.002) with isolated CNS relapse and concurrent BM involvement. There was no significant correlation between age, sex, CSF involvement at time of diagnosis, phenotype of ALL, cytogenetics, involvement of other sites and CNS relapse.

Table 1: characteristics of 102 ALL patients with CNS relapse.

Characteristics	Group 1 (N=34)	Group 2 (N=68)
Age (yrs), n (%)		
<10	14 (41.2)	37 (54.4)
11-20	09 (26.5)	18 (26.5)
>20	11 (32.4)	13 (19.2)
Sex, n, (%)		
Male	09 (26.5)	21 (30.9)
Female	25 (73.5)	47 (69.1)
Phenotype, n (%)		
B-ALL	31 (91.2)	57 (83.8)
T-ALL	03 (8.8)	11 (16.2)
TLC at diagnosis, n, (%)		
>50X10^9/L	6 (17.6)	22 (31.8)
PS blast % at diagnosis, n, (%)		
>50%	15 (44.2)	39 (57.4)
BM remission (months), n (%)		
>2	3 (8.8)	7 (10.3)
TLC at relapse, n (%)		
<4x10^9/L	09 (26.5)	13 (19.2)
4-10X 10^9/L	06 (17.6)	33 (48.5)
>10X10^9/L	19 (55.8)	22 (31.8)
Philadelphia chromosome, n (%)	08/29(27.6)	08/60 (13.3)
Hepatomegaly, n (%)	07 (20.6)	23 (33.8)
Splenomegaly,n (%)	10(29.4)	25 (36.8)
Mediastinal mass,n (%)	00 (00)	02 (2.9)
Lymphadenopathy, n (%)	04 (11.8)	13 (19.2)
Body cavity infiltration, n (%)	00 (00)	02 (2.9)
Involvement of other sites/organs, n (%)	00(00)	04 (5.9)
CSF relapse (No of times), n (%)		
Once	23 (67.6)	60 (88.2)
More than once	11 (32.4)	08 (11.8)
Median time of relapse (Years)	1.25	1.3

DISCUSSION

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, accounting for 25% of overall childhood malignancies and approximately 75% of leukemias in children. representing a significant portion of pediatric cancers. Complete remission (CR) rates have improved over the years, reaching around 70-80% (Badr *et.al.,* 2013). At the time of diagnosis, CNS involvement is relatively rare, occurring in less than 5% of children and 10% of adults with ALL. However, when CNS disease is present, it poses a substantial risk to both relapse-free survival and overall survival rates.[2] Historically, before the implementation of CNS therapy and prophylaxis, CNS involvement presented a major challenge, contributing to a significant portion of relapses (Cortes *et.al.,* 1995).

In our study 14.17% of patients experienced CNS relapse, that varied compared to other studies (C. S. Cancela *et.al.*, 2012 and Tariq Abadi Al-Shujairi *et.al.,* 2008). The variance could be attributed to differences in the intensity of regimens used for CNS-directed therapy. We observed a significant association between hyperleucocytosis and isolated or combined CNS relapse, consistent with findings in other studies (Tariq Abadi Al-Shujairi *et.al.,* 2008 and Frishman-Levy L *et.al.,* 2017). Hyperleucocytosis has been recognized

as a risk factor for CNS relapse in ALL which was similar to other studies (Munch V, *et al.*,2017 and Pieters R, &Carroll WL,2010). Our study contradicted previous findings where age served as an independent predictor of outcome in ALL (Davis IR, Westerman DA.2014).Contrary to our study t(9;22) translocation causing the BCR-ABL1 fusion are well known to be associated with a higher incidence of CNS replase (Alsadeq *et.al.*, 2017).

In ALL, CNS involvement is an important concern and molecular mechanisms responsible for CNS entry of leukemic cells need to be understood for prophylactic and therapeutic intervention. CNS leukemia is a leptomeningeal disease and ALL cells that have entered the CNS were found to highly express vascular endothelial growth factor (VEGF). The proliferation and survival of ALL cells was independent of VEGF but their transendothelial migration through CNS was mediated by VEGF (Munch et.al., 2017). The rate of diagnosis of CNS leukemia can be improved by cytological assessment using flow cytometry technology (Bromberg JE et.al., 2007). As many as 50-75% of patients may develop CNS relapse in absence of CNS prophylactic therapy but in those who receive systematic CNS prophylaxsis 2-10% of patients develop CNS relapse (Pui et.al., 2006). Factors like hyperleucocytosis, T- cell immunophenotype, genetic aberrations like Philadelphia chromosome and t (4; 11) are associated with enhanced risk of CNS relapse. Recent studies have suggested that gene polymorphisms in those involved in pharmacodynamics of antileukemic drugs are associated with CNS relapse (Pui *et.al.*, 2008).

Previously it was considered that leukemic cell dissemination into CNS was via blood. But recently is has been discovered that dural lymphatics provide a better route for cells to enter or leave CNS. This hypothesis is important in the context of CNS relapse as leukemic cells may reconquer systemic circulation and this could be why isolated CNS relapse patients have BM MRD and require systemic therapy (Pui CH *et.al.*, 2008). CNS involvement is under-diagnosed most often. Besides clinical evaluation, three independent methods like CNS radiology, CSF cytology and flow cytometry can be used for diagnosis (Chamberlain *et.al.*, 2009). Thus, early diagnosis and proper systemic and CNS prophylactic therapy is essential for overall survival of ALL patients.

Conclusion:

In our study we found that hyperleucocytosis and TLC at time of relapse were significantly associated with CNS relapse. Early diagnosis using proper technique, timely intervention in the form systemic and CNS prophylactic therapy and ideal follow up are necessary for appropriate treatment of ALL patients to improve their overall survival. Further studies on large numbers will help to establish the risk factors associated with CNS relapse.

Declarations:

Ethics Approval: Ethics: Institutional approval was taken. (IRC/2021/P-47 dated July 20th 2021**).** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards

Conflict of Interest: The authors declare no conflict of interest.

Author contribution: All authors contributed equally, and have read and agreed to the published version of the manuscript.

Data Availability Statement: The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request**.**

Funding Information: The study received no funding from any sources.

Acknowledgment: We acknowledge the support rendered by the institute where the study was carried out. We also thank the technicians who helped in collecting data retrieval.

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