Original Research Article

Profile and outcome of children with Acute Lymphoblastic Leukemia: A study from a tertiary care centre from North India

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most common type of childhood malignancy. The study of prognostic indicators that can predict the survival of these patients is necessary to identify the factors associated with the rate of relapse in any set of patients. Therefore, the present study aimed to evaluate the clinical characteristics, prognostic factors and the treatment outcome measurements of pediatric ALL patients treated in a tertiary care center in North India. **Material and methods:** This hospital-based observational study was conducted on 180 pediatric patients aged between 1 year to 18 years who had visited the Department of Medical Oncology at Sher-I-Kashmir institute of Medical science, Srinagar Jammu and Kashmir between the January 2015 to December 2019 were included. **Result:** A total of 180 children with ALL were included in this study. 57.8% were males and 42.2% were females with a mean age of 9.2 (±3.5) years and median of 8 years. Majority of patients (68%) were below 10 years. 57.8% were males and 42.2% were females. The male: female ratio was 1.3:1. Central nervous system disease at diagnosis was seen in 7.8% patients. The mean and the median TLC were 56000/µl and 12000/µl respectively. The TLC was more than 20000/µl in 63.3% patients. The immune phenotyping analysis revealed Pre B ALL in 77.8%, B cell in 9.4% and T cell ALL in 12.8% patients. Conventional cytogenetic revealed normal cytogenetics in 87.6%, hyperdiploidy in 8.5% and hypodiploidy in 3.9%. FISH for TEL AML, MLL, BCR-ABL gene rearrangement analysis was positive in 23.3%, 4.4%, 11.1% patients respectively. No statistical significant association was observed between the age of the patients, gender, TLC count, BCR-ABL or TEL-AML assays. The presence of CNS disease and survival of the patients showed a significant statistical correlation (P=0.038). The MLL-gene rearrangement analysis had significant statistical correlation (p-value=00). The overall survival rate was 78%. The EFS rate was 70.5%. There were a total of 45 relapses, among the relapse cases, 55.5% had bone marrow relapse, 11.1% had CNS relapse, 26.7% had combined CNS and bone marrow, and 6.7% had a testicular relapse. **Conclusion:** The study results show that our results are comparable to some other centres in India. Identification of prognostic risk factors and risk stratification helps to achieve a higher survival rate childhood ALL patients.

Keywords: Pediatric, ALL, survival, risk stratification, prognosis, remission.

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of malignancy found in children.It accounts for 25% of all childhood

cancers and approximately 75% of all cases of childhood leukemia.The pathogenesis of ALL is heterogeneous, thus different subtypes, ALL shows different treatment responsiveness, rate of relapse and are associated with different survival rates[1]. The incidence of ALL is higher in children who are aged between 2 years and 5 years and a male predominance has also been reported in many cases. The survival rate of childhood ALL cases has significantly improved in the past decade, owing to development in the treatment regimen and improvement in risk-stratification strategy. The therapy modification based on the pharmacogenomics of individual patients also has helped in the care of ALL patients [2].

Identification of various risk factors based on an array of biological, social and genetic variables such as age, sex, immune-phenotyping, cytogenetics study including both convention and molecular, presence of CNS diseases, white blood cell (WBC) count and level of minimal residual disease (MRD) plays a significant role in selecting the proper therapy and also predicting the survival rate. On the other hand, the level of minimal residual disease or MRD after the end of the induction also proves to be an important prognostic factor for patients suffering from ALL [3].

The outcome result from different studies reported from India showed a lower survival rate compared to the western countries. They have also reported a higher incidence on of risk factors among the Indian population[4]. The study of the risk factors is also important for choosing the therapy. Patients with the highest risk of relapse should undergo more intensive therapies. This, in turn, can help all the patients to achieve the same cure in spite of the difference associated with the rate of relapse[5].

Therefore it is necessary to study the prognostic risk factors as well as the survival data to identify the factors associated with the rate of relapse in any set of patients. In spite of several studies conducted around the world, there is still lacunae in the existing literature on stratification of risk factors, survival and relapse disease among children suffering from ALL in India. Hence, this study was undertaken to identify the various prognostic factors along with their impacts on survival among patients treated for ALL in our institute. The present study was aimed to evaluate the clinical characteristics and the treatment outcome measurements of pediatric ALL patients treated at a tertiary care center.

Material and Method

The study was a hospital based, observational study conducted between January 2015 to December 2019 in the pediatric unit of the Department of Medical Oncology at Sher-I -Kashmir institute of Medical science, Srinagar Jammu and Kashmir. The study included analysis of data of children in the age group of 1 to 18 years, diagnosed as acute lymphoblastic leukaemia (ALL), who visited the department during the study period for their treatment. All the cases of ALL below age of 1 year (infant ALL) and those who had already relapsed at the time they presented to our centre were excluded from the study. The data was collected from 180 patients regarding their age, gender, laboratory indices like hemoglobulin, leukocyte count, platelet count, CSF cytology (positive or negative for blasts), Immunophenotypic data (Pre B ALL, B cell ALL, T cell ALL) by flowcytometry, Cytogenetics data (hyperdiploidy, normal, hypodiploidy) by conventional karyotyping method and Fluorescent In Situ Hybridization (F.I.S.H) technique was used to determine presence or absence of BCR-ABL, TEL AML, MLL gene rearrangements. In the 180 patients the status of blasts in peripheral blood at day 8 and bone marrow status at day 33 was assessed to determine the response to therapy. The outcome in terms of survivor and non-survivor was determined. The last point of contact in survivors and non-survivors was recorded. All the patients were risk stratified and the treatment was modified accordingly. Treatment protocol used was BFM 95 protocol, and treatment was risk adopted, either standard, moderate or high risk.

For the calculation of prognostic variables, descriptive statistics were used. The patients were divided into 2 groups, the survivors and non-survivors. Qualitative variables were compared using the Chi-square test. A univariate analysis was carried out to identify the variables with a significant association with relapse or death. These were then subjected to further analysis to identify the significant predictors for adverse outcomes. The odds ratios with 95% confidence interval were calculated. A "p" value of < 0.05 was taken as significant.

All the data were collected and tabulated in an excel sheet for further statistical analysis. The SPSS software (version 16) for Windows was used for statistical analysis. A "p" value of < 0.05 was taken as significant. Analysis of disease outcome was examined as overall survival (OS) and event-free survival (EFS). OS was measured from the date of initial diagnosis of ALL to date of death from any cause or date of the last contact using the Kaplan-Meier method which is a nonparametric (actuarial) technique for estimating time-related events (the survivorship function).

Result

This study was observational in nature. The first patient was enrolled in January 2015 and the last patient was enrolled in December 2019. The data of 180 patients with ALL who visited our Centre during the study period revealed the following results. The age range of the study population varied between 1.5 to 18 years.The various patient characteristics and its impact on survival is mentioned in table 1. The mean and the median age were 9.2 (±3.5) years and 8 years respectively. Majority of patients (68%) were below 10 years . There were 57.8% boys and 42.2% girls. The male: female ratio was 1.3:1.The common clinical manifestations at admission were fever (80%), pallor (70%), bleeding manifestations (40%) and musculoskeletal aches and pain (20%). The clinical examination revealed hepatomegaly (70%), splenomegaly (50%), lymphadenopathy (45%) and testicular enlargement (1%) patients. Central nervous system disease at diagnosis was seen in 7.8% patients. The Total leukocyte count (TLC/µl) ranged between 100/µl to 11 lakhs/µl. The mean and the median TLC were 56000 /µl and 12000 /µl respectively. The TLC was more than 20000 /µl in 63.3% patients. The platelet count at presentation was less than 30.0×10^9/L in 38% patients, more than 30.0×10^9/L to 50.0×10^9/L in 40% patients and greater than 50×10^9/L in 32% patients. Risk stratification of the patients has been done in this study into high risk, moderate risk, standard risk and treatment was modified accordingly, giving more intense therapy to the high risk patients. Patients having any one of the following criteria leukemic cells < 1000micromol/L in the peripheral blood on day 8 after 7-day prednisone pre-phase, WBC <20 000 micromole/L, age >1 <6 years, a complete remission on day 33 (M1-marrow), no translocation t(9;22) or BCR/ABL recombination, no translocation t(4;11) or MLL/AF4 recombination, no T cell phenotype were considered as standard risk. The Medium risk group constituted of leukemic cells < 1000micromol/L in the peripheral blood on day 8 after 7 day prednisone pre-phase, complete remission on day 33 (M1-marrow), no translocation t(9;22) or BCR/ABL recombination, no translocation t(4;11) or MLL/AF4 recombination. All 4 criteria must be met as well as at least one of the following leukocytes > 20000 micromol/, age < 1 year, age > 6 years. The high risk group constituted 1000micromol/L leukemic cells in the peripheral blood on day 8, no complete remission on day 33, translocation t(9;22) or BCR/ABL recombination, translocation t(4;11) or MLL/AF4 recombination (Each criterion alone qualifies as high risk regardless of age and WBC).

The Immunophenotyping analysis revealed Pre B ALL in 77.8%, B cell in 9.4%, and T cell ALL in 12.8% patients. Conventional cytogenetic revealed normal cytogenetics in 87.6%, hyperdiploidy in 8.5%,hypodiploidy in 3.9%. FISH for TEL AML, MLL, BCR-ABL gene rearrangement analysis was positive in 23.3%, 4.4%, 11.1% patients respectively.

The peripheral blood status at day 8 showed complete disappearance of blasts in 96% of patients. The bone marrow status at day 33 revealed complete remission in 87% patients. The outcome of 180 patients revealed 127 (70.5%) survivors and 53 (29.5%) non survivors (figure 1). Among the 53 non survivors, 15 patients had relapsed but were alive, 29 patients had relapsed and died due to treatment, 9

patients died due to treatment related toxicity. Among the 45 relapses, 25 had isolated bone marrow relapse (55.5%), 5 had isolated CNS relapse (11.1%), 12 had combined CNS-BM relapse (26.7%) and the remaining 3 had isolated testicular relapses (6.7%). Bone marrow was the commonest site of relapse(Figure 2). So in our study the overall survival at median of 2.5 years was 78% and the event free survival (EFS) rate was 70.5%.

No statistical significant association was observed between the age of the patients, gender, TLC count. The difference in the median survival time for patients with Pre B ALL, B cell ALL and T cell ALL was not statistically significant(Table 1). The median survival time in patients, who had normal karyotype, hyperdiploidy and hypodiploidy were similar and this difference was statistically not significant. The median survival was not statistically significant with respect to TEL AML, BCR-ABL however MLL gene rearrangement was statistically significant (Figure 3) clearly indicating that all patients with MLL positivity eventually relapse and die.

Table 1:Patient characteristics and their impact on survival

AGE	**LESS THAN 10 YEASRS= 103 MORE THAN 10 YEARS =77**	**P VALUE 0.313**
GENDER	MALES=104 FEMALES=76	P VALUE 0.014
CNS STATUS	POSITIVE =14 NEGATIVE=166	P VALUE 0.03
TLC	<20000=114 20000-50000=39 >50000=27	P VALUE 0.317
IMMUNOPHENOTYPE	PRE B CELL=140 B CELL=17 T CELL =24	P VALUE 0.317
CYNOGENETICS	NORMAL=109 HYPER DIPLOID=9 HYPODIOLOID=5 N/A=57	P VALUE=0.56
TEL- AML	POSITIVE=42 NEGATIVE=104 N/A =34	P VALUE 0.76
BCR-ABL	POSITIVE=20 NEGATIVE=138 N/A =22	P VALUE=0.15
MLL STATUS	POSITIVE=8 NEGATIVE=159 N/A =13	P VALUE=0.00
PREDNISOLONE RESPONSE	GOOD PREDNISOLONE RESPONSE=173 POOR PREDNISOLONE RESPONSE=7	P VALUE=0.150
DAY 33 MARROW	IN REMISSION=157 NOT IN REMISSION=23	P VALUE=0.000

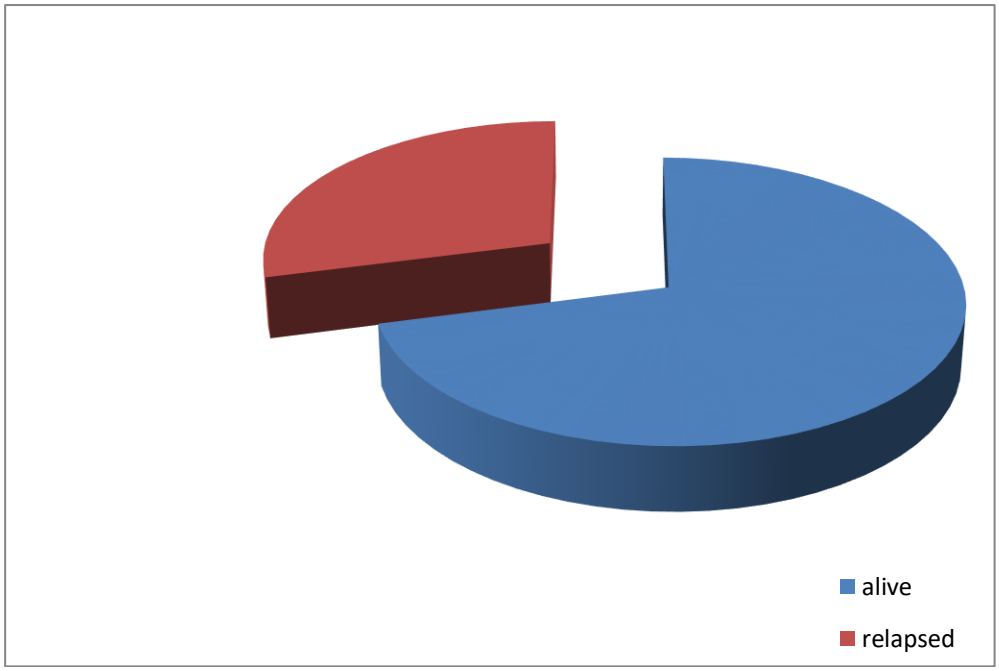

Fig. 1:Overall status of patients.

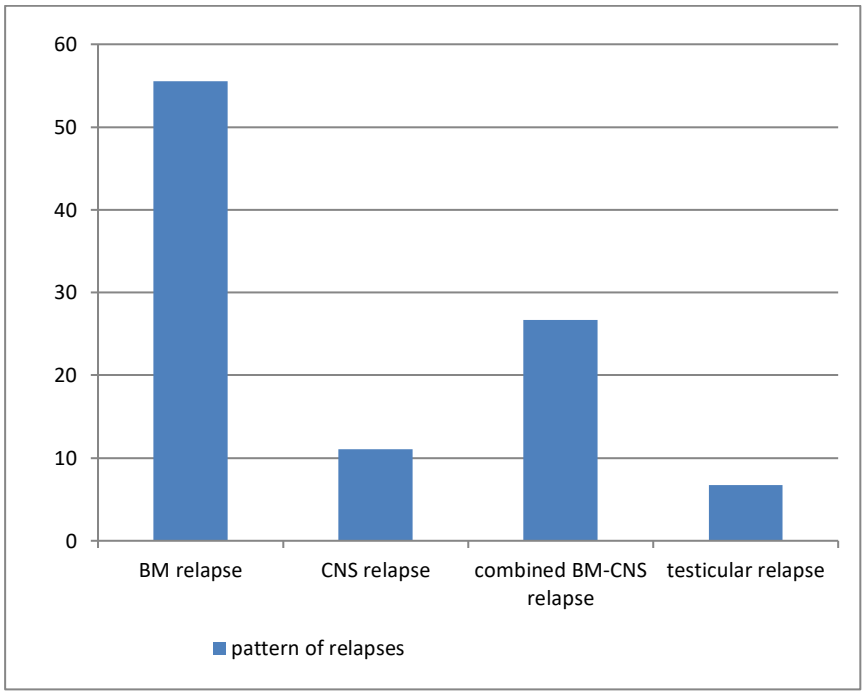

Fig. 2:Pattern of relapses

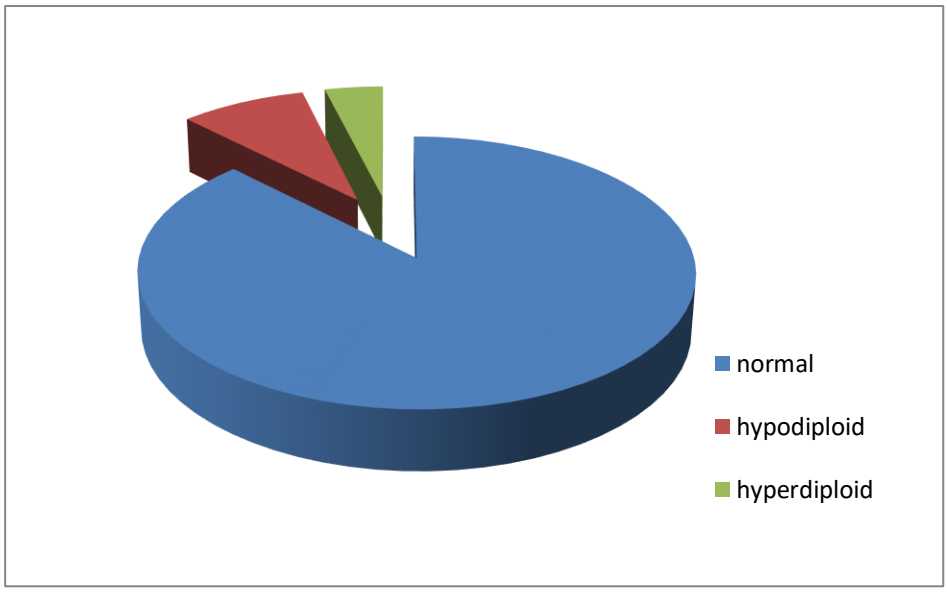

Fig 3:Cytogenetics (karyotyping)

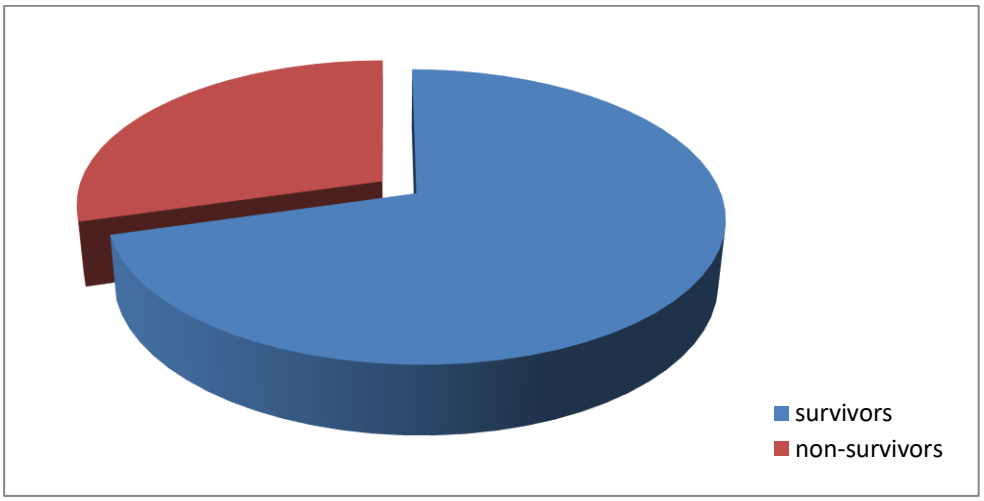

Fig 4:outcomes

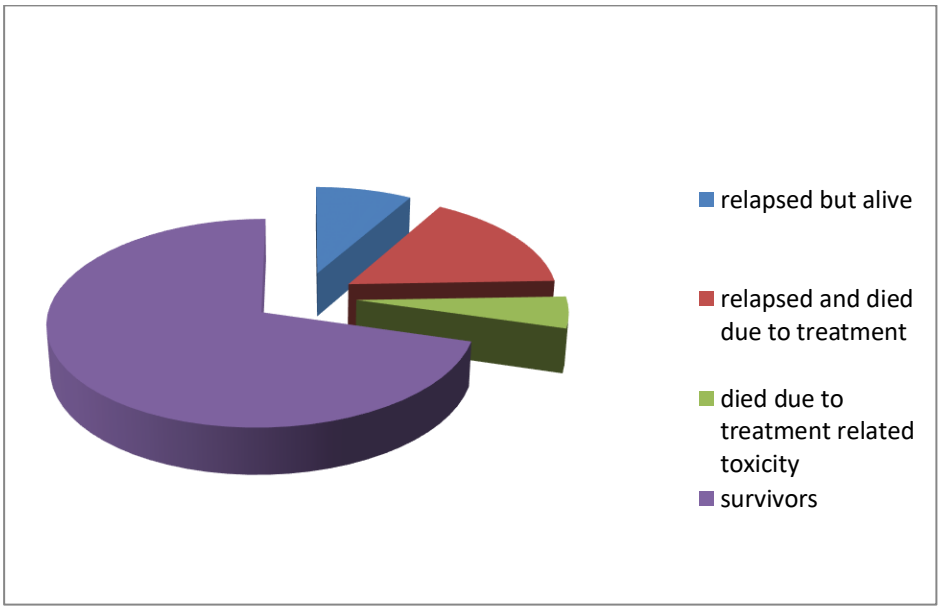

Fig. 5 -outcomes

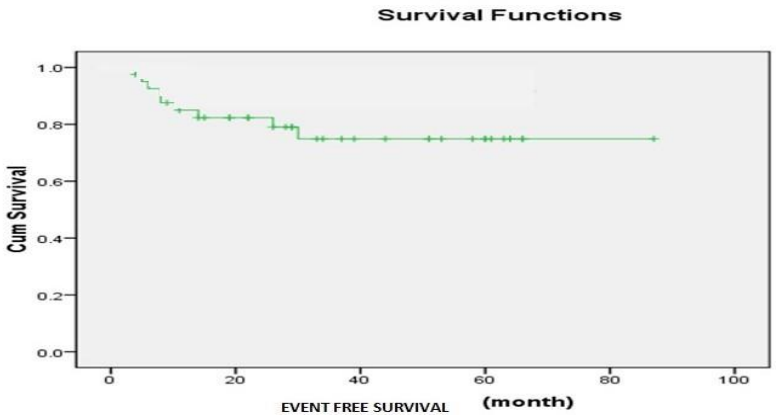

Fig 6:Kalpan-Meier EFS Curve

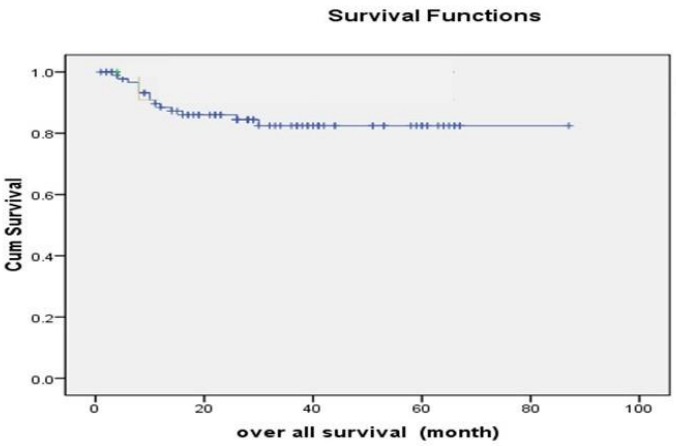

Fig. 7 – Kaplan-Meier OS curve

Discussion

There is limited data from India available on the survival of the children suffering from ALL. Moreover there is no data on the childhood ALL from Jammu and Kashmir. The present study was undertaken to shed light on the incidence of various prognostic factors and the survival analysis of patients who were treated for childhood ALL at our study center. The survival analysis of our study population showed an overall survival rate of 78% and event free survival rate of 70.5%. This result is in accordance with the previous results found by the ***Khalid et.al***.[6]. The present study reported that in the relapse group maximum patients reported a bone marrow relapse (25/180). The survival analysis revealed that patients were alive for a period of 27.75 ± 17.709 months with a median of 24.50 months. In the study population it was found that most of the pre B ALL patients were alive during the study period. However, no difference in outcome analysis was observed between the B Cell ALL and T cell ALL patients. In a previous study it was reported that T-ALL and pre-B ALL had no difference in their prognostic values[7]. In contrast, in a study by ***Chessells et al*** it was reported that T-ALL children had a early relapse rate[8]. In another study that has reported a higher event free survival rate (61.4±2.1%) showed that T-cell ALL patients had a less favorable prognostic value compared to the pre-B ALL patients[9]. This result is in contrast to the previous studies that have shown that in B-ALL patients the survival rate is higher compared with the T-ALL patients[10]. This difference can be explained in terms of genotypic differences among the study pool from the previous study with the present study pool. This study reported that indicating that female patients had a better survival rate compared with male patients. However, this result was not statistically significant as observed from the Pearson's chi-square test. Moreover, it was also seen that the survival rate was higher among patients below 10 years of age (81.6%) compared to the patients who were more than 10 years of age (75.3%). In previous studies, it was reported that young adults have a better survival rate when they are treated according to the pediatric protocols rather than the adult treatment regimen. The remission rates of these adolescents also were higher in these patients[11].The presence of CNS disease and survival of the patients showed a significant statistical correlation (P=0.038). Among the patients who were alive, 80.7% of patients had no symptoms of CNS diseases. It was a general belief that childhood ALL is positively associated with a high incidence of CNS disease and poor prognosis significantly related to the higher incidence of CNS complications[12].

In previous studies it was stressed that total leucocyte count (TLC) higher than 50,000 have a worst outcome. Patients with a high WBC count at diagnosis can be categorized as higher risk group and have a significant role in prognosis of the patients suffering from ALL[13]. In the present study maximum of the patient had a lower TLC count at diagnosis. Among patients who survived, 77.2% had a TLC less than 20000 compared to 22.8% dead patients. A difference of TLC count was observed between the alive and dead patients however on further analysis no statistical correlation was found between the TLC count and survival analysis.

In the previous study results it that has compared the difference of prognostic factors between the developing countries has reported that the proportions of immunophenotypic data were higher in the developing countries compared to the developed countries[14].In the present study Total 109 patients who were alive at the time of analysis showed normal cytogenetics data, 9 showed hyperploidy and 5 showed hypoploidy after the cytogenetics analysis. Among the dead patients of the study it was also shown that maximum patients had a normal cytogenetisc data. The difference between the cytogenetics between the alive and dead patients no statistical correlation was observed. Another study conducted in a tertiary care hospital among children suffering from ALL showed that in conventional cytogenetics analysis 78% had a normal cytogenetics profile[15].

Studies have also indicated that MLL- gene rearrangements were statistically correlated with the survival of the patients. Among the patients who survived only 1 patient showed a positive result for MLL-gene rearrangement data. Among the dead patients, 7 patients were positive for MLL gene analysis. In a similar study, ***Alkhayat et al***., have found that in childhood ALL hypoploidy and MLL gene rearrangements were the sign of bad prognosis with short survival and high rate relapse as compared with the standard risk group (P=0.031) and the event-free survival was found to be statistically significant (P=0.04)[16]. In another study by Mi et al it was reported that prognosis in patients suffering from ALL mainly depends on the genetic abnormalities[17].In the present study no statistical correlation was observed between the survival of the patients and the BCR-ABL or TEL-AML assays results. Tsang et al in a previous study have shown that in patients with positive TEL-AML gene rearrangements had a complete remission and better induction. Moreover, they have also suggested that patients who had a positive TEL-AML data can opt for less aggressive treatment regimen[18].In another study conducted in Southern India, 8.3% patients showed positive results for BCR-ABL. This study has also pointed out that BCR-ABL translocations indicates a poor prognosis in patients suffering from ALL[19].

Early bone marrow response was reported to have a good prognostic feature. This response is usually evaluated to check the prognosis of

the patients after the treatment started. In a previous study it was reported that Day 15 marrow provides a superior prognostic marker. This study thus recommended adding bone marrow response as a prognosis stratification criterion in ALL patients[20].

Present study also showed that a god bone marrow response at day 33 is associated with better outcomes. After statistical analysis, a significant correlation was observed (p-value=00). The study also showed that 55.5% had bone marrow relapse, 11.1% had CNS, 26.7% had both CNS and bone marrow, and 6.7% had a testicular relapse.

Conclusion

The present study results show that significant improvement has taken place in ALL outcomes. It shows that a higher survival rate can be achieved in childhood ALL patients with an intensive treatment regimen. Moreover, identification of prognostic risk factors can also help to identify the relapse rate and choosing the correct treatment regimen.

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