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ADVANCED MONITORING OF B-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA IN WESTERN INDIA: CORRELATION OF MINOR BCR-ABL1 FUSION GENE WITH HAEMATOLOGICAL PARAMETERS AND LACTATE DEHYDROGENASE ACTIVITY

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ABSTRACT

B-Cell Acute Lymphoblastic Leukaemia is generally characterized by presence of Philadelphia chromosome and minor BCR-ABL1 (mBCR-ABL1) fusion gene is a molecular aberration in these patients that influences treatment management and prognosis. Hence, it was aimed to explore clinical relevance of mBCR-ABL1 fusion gene expression in B-ALL patients from western India. Fifty B-ALL patients were enrolled: 14 Pre-treated (PT) patients, 11 treatment non responders (TNR) and 25 treatment responders (TR). Amongst three subgroups of B-ALL patients, mBCR-ABL1 fusion gene copies and %NCN were significantly lower in TR as compared to PT and TNR. Among haematological parameters, PT had significantly higher WBC counts ($p<0.001$) and lower platelet counts ($p<0.001$) than TNR and TR groups. Moreover, TR with abnormal WBC counts and platelet counts had lower levels for mBCR-ABL1 fusion copies and %NCN as compared to other groups. Further, Lactate Dehydrogenase activity was found to be significantly higher in PT group in comparison with TR and TNR groups. Moreover, TNR patients having abnormal LDH levels had higher expression of mBCR-ABL1 fusion copies and %NCN as compared to PT and TR subgroups. Therefore, correlation of mBCR-ABL1 fusion gene expression with haematological parameters and LDH activity would be beneficial in treatment monitoring of B-ALL patients.

Keywords: minor BCR-ABL1 fusion, %NCN, haematological parameters, LDH activity, B-ALL

1. INTRODUCTION

Leukemias constitute the most common diagnostic group of childhood and adult cancers worldwide as well as in India (1). In India, leukemias ranks 8th among all malignancy with estimated 48,419 new cases per year, according to Globocan 2020 (2). Acute Lymphoblastic Leukemia (ALL) is a malignancy of B or T lymphoblast characterized by uncontrolled proliferation of abnormal, immature lymphocytes and their progenitors (3). B-ALL is a malignant transformation and differentiation of a clonal population of B-lineage precursor cells within the bone marrow, blood, and extra medullary sites (4). B-cell precursor ALL is a heterogeneous disease in which risk assessment is based on clinical information at diagnosis, response to therapy and cytogenetic aberrations (5). Philadelphia chromosome-positive (Ph^+) ALL is defined by the t (9; 22) (q34; q11) that produces BCR-ABL1, and causing BCR-ABL1 chimeric attaching gene (fraction point e1a2) which is commonly known as minor BCR-ABL1 fusion gene (6) and it creates a 190kD protein with tyrosine kinase activation that is able to change the various signalling pathways with contribution in tumor growth and proliferation (7).

The Ph^+ chromosome, or its molecular counterpart, the BCR-ABL1 fusion gene, a common molecular defect in childhood and adult ALL, is a valuable tumor marker whose detection influences prognosis and clinical management decisions (8). The incidence of BCR/ABL1-like ALL increases with age, accounting for 10.0–15.0% in children, 21.0% in adolescents, 27.0–27.9% in young adults (21–39 years), 20.4% in adults (40–59 years), and 24.0% in older adults (>60 years) (9). In B- ALL, risk stratification has been based on clinical factors such as age, White Blood Cell (WBC) count and response to chemotherapy; however, the identification of recurrent genetic alterations has helped refine individual prognosis and guide management (10). Establishing BCR/ABL1-like diagnostic algorithm remains of clinical significance in view of prognostic relevance and the advent of clinical trials of targeted therapy (9). Present study aimed

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to investigate the expression of mBCR-ABL1 fusion gene expression in the peripheral blood of B-ALL patients from western India and correlate with various haematological parameters as well as LDH activity.

2. MATERIALS AND METHODS

2.1 Sample collection

Fifty haematologically and cytogenetically confirmed B-ALL patients were enrolled in this study and blood samples were collected. The study was approved by the Institutional Review Committee of The Gujarat Cancer & Research Institute. General consent was taken from all these patients. While sera positive patients (HIV/ HBsAg/ HCV) were excluded from the study.

2.2 RNA isolation and quantification

Total RNA was extracted from blood samples using QIAamp RNA Blood Mini kit (Qaigen) and RNA quantification was performed by using Qubit fluorimeter 3.0 (Invitrogen).

2.3 Reverse transcription (RT) and qPCR for mBCR-ABL1 fusion gene

Minor BCR-ABL1 expression was detected by commercially available minor BCR-ABL1 quantitative kit in a QuantStudio5 detection system, according to the manufacture's instruction. Thermal cycler conditions were 94°C for 10 minutes (1 cycle) followed by 45 cycles of amplification (94°C for 15 seconds and 60°C for 1 minute). Standard curves for ABL1 and BCR-ABL1 mbcr were established and utilized to convert raw CT values of unknown samples into ABL1CN and BCR-ABL1 mbcr CN copy numbers. The ratio of these copy number values yields the NCN. Copy number of fusion gene (BCR-ABL1) and control gene (ABL1) were recorded and Normalized Copy Number (%NCN) was calculated according to the following formula.

%NCN = Fusion CN / Control CN x 100

2.4 Statistical Analysis

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc., USA). Student's t-test was performed to correlate compare the means of two different groups. P values ≤ 0.05 were considered to be statistically significant.

3. RESULTS

Fifty patients diagnosed with B- ALL enrolled in the study were divided into two groups: 14 Pre-treated (PT) patients and 36 treated patients. Further, B- ALL patients in treated group were subdivided as treatment non responders (TNR) (11 patients) and treatment responders (TR) (25 patients) (Figure 1).

The clinical and haematological characteristics of the enrolled B-ALL patients are summarized in Table 1. Among them, 62% were males and 38% females. Median age was 24 years, dividing patients into ≤24 years with younger age group (52%) and with elder age group >24 years age (48%). Additionally, majority of the B-ALL patients showed abnormal RBC counts (46/50, 92%), WBC counts (36/50, 72%), platelets (35/50, 70%), haemoglobin (43/50, 86%) and Haematocrit (HCT) (45/50, 90%). Majority of the patients (34/50, 68%) showed presence of the Philadelphia chromosome showing FISH t (9; 22) positivity in 13 PT, 10 TNR and 11 TR patients.

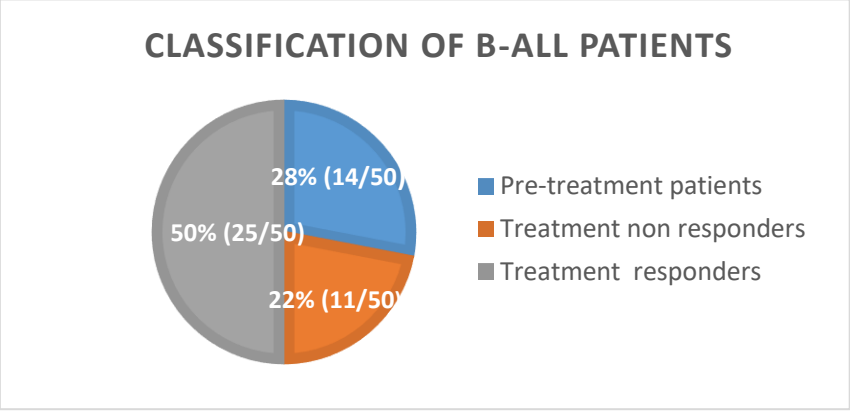

Figure 1: Classification of ALL patients

Table 1: Clinical and Haematological Characteristics of B-ALL Patients

	N (%)	Patients with B-ALL		
		PT (%)	TNR (%)	TR (%)
Total	50(100)	14(28)	11(22)	25(50)

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Gender				
Male	31(62)	10(20)	09(18)	12(24)
Female	19(38)	04(08)	02(04)	13(26)
Age				
≤24 years	26(52)	2(4)	7(14)	17(34)
>24 years	24(48)	12(24)	04(08)	08(16)
RBC				
Within normal RBC counts	04(08)	01(02)	00(00)	03(06)
Abnormal RBC counts	46(92)	13(26)	11(22)	22(44)
WBC				
Within normal WBC counts	14(28)	02(04)	01(02)	11(22)
Abnormal WBC counts	36(72)	12(24)	10(20)	14(28)
Platelet				
Within normal Platelet counts	15(30)	00(00)	04(08)	11(22)
Abnormal Platelet counts	35(70)	14(28)	07(14)	14(28)
Haemoglobin				
Within normal Hb level	07(14)	02(04)	01(02)	04(08)
Abnormal Hb level	43(86)	12(24)	10(20)	21(42)
HCT				
Within normal HCT level	05(10)	01(02)	01(02)	03(06)
Abnormal HCT level	45(90)	13(26)	10(20)	12(44)
FISH				
Negative	16 (32)	01 (02)	01 (02)	14 (28)
Positive	34 (68)	13 (26)	10 (20)	11 (22)

PT - Pre-treatment, TNR - Treatment Non-responders, TR- Treatment Responders

3.1 Quantitative analysis of minor BCR-ABL1 Fusion transcript by RT-qPCR Method

In this study, quantitative analysis of mBCR-ABL1 fusion gene by RT-qPCR method showed a significantly lower average of mBCR-ABL1 fusion copies (P<0.001) in TR group as compared to PT and TNR groups. (Figure 2a). Similarly, TR had significantly decreased average %NCN (P<0.001) versus PT patients and TNRs. (Figure b).

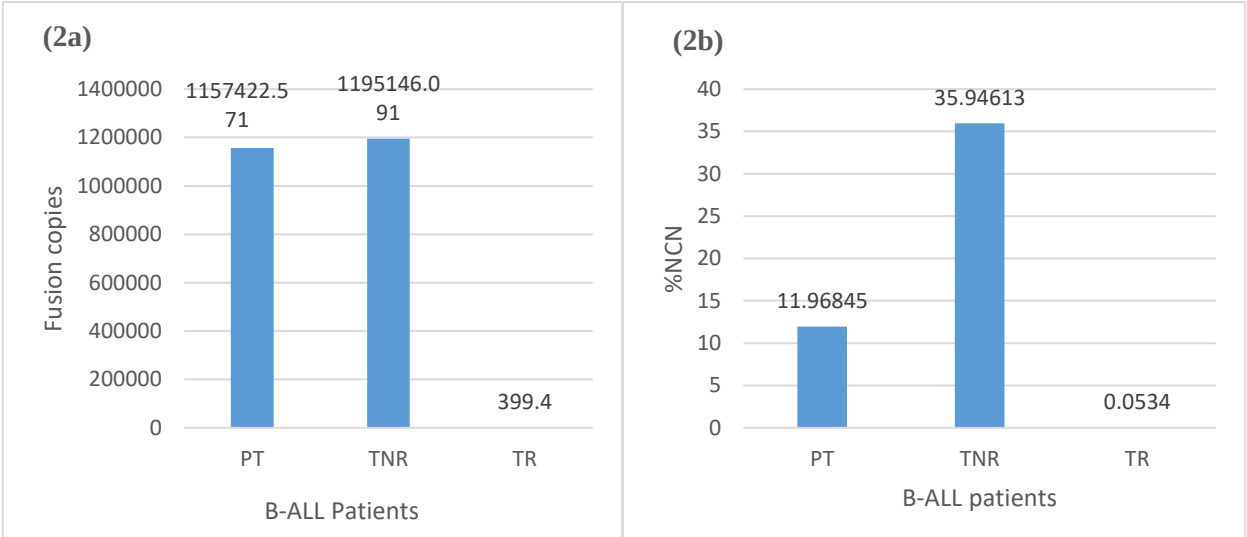

Figure 2 a & 2b: (2a) Mean fusion copies (2b) mean %NCN levels in PT, TNR and TR subgroups of B-ALL

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3.2 Correlation of haematological parameters and LDH levels among PT, TNR and TR subgroups

When correlated with haematological parameters, PT patients had significantly higher WBC counts ($p<0.001$) and lower platelet counts ($p<0.001$) than TNR and TR groups. No significant association was observed with RBC count, haemoglobin levels and HCT% among the 3 subgroups. Moreover, mean LDH levels were found to be significantly higher in PT group as compared to TR and TNR ($P=0.008$) (Table 2).

Table 2: Correlation of Haematological Parameters and LDH levels among PT, TNR and TR subgroups of B-ALL Patients

Haematological parameters	PT	TNR	TR	P value
Mean RBC (g/dl)	2.92	3.36	3.25	0.61
Mean WBC (x10^3cells/µl)	57.6	22.56	4.11	<0.001
Mean Platelets (x10^3cells/µl)	27.57	125.8	190.04	<0.001
Mean Haemoglobin (g/dl)	12.64	9.0	10.86	0.11
Mean HCT (%)	25.2	28.1	26.88	0.61
Mean LDH levels (U/L)	682	362.83	306.40	0.008

3.3 Correlation of minor BCR-ABL1 with haematological parameters and LDH levels among PT, TNR and TR subgroups

TR with abnormal WBC counts (Figure 3) and platelet counts (Figure 4) had lower expression levels for average mBCR-ABL1 fusion copies and average %NCN in comparison with PT and TNR subgroups. Whereas TNR group with abnormal WBC counts and platelet counts had the highest levels of mBCR-ABL1 fusion copies and %NCN.

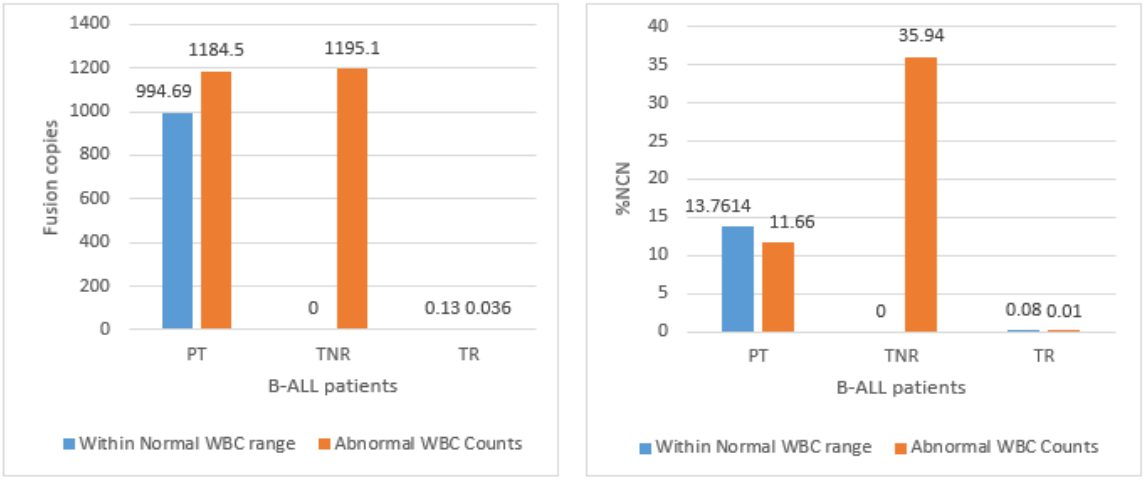

Figure: 3 Correlation of WBC with fusion copies and % NCN levels in PT, TNR and TR subgroups of B- ALL Patients

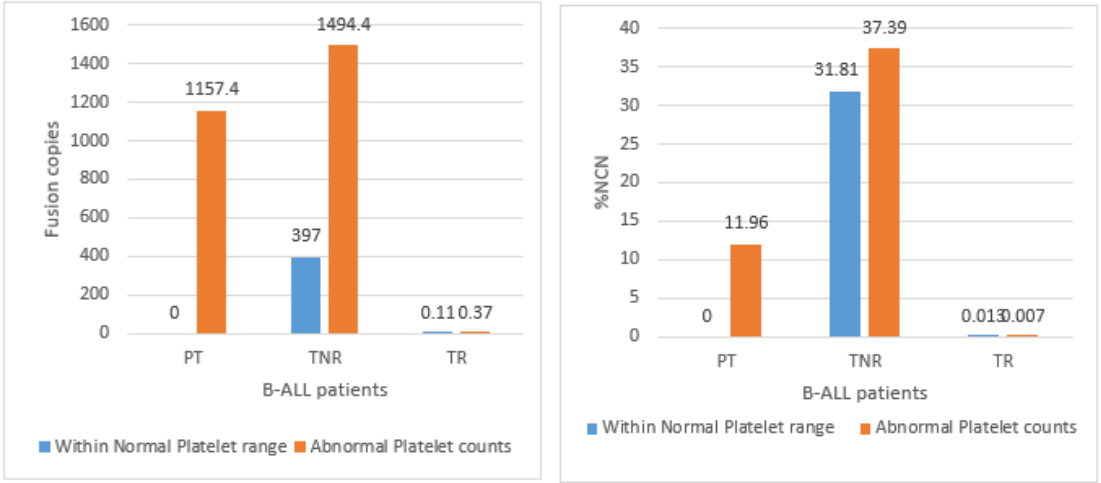

Figure 4: Correlation of Platelets with fusion copies and % NCN levels in PT, TNR and TR subgroups of B-ALL Patients

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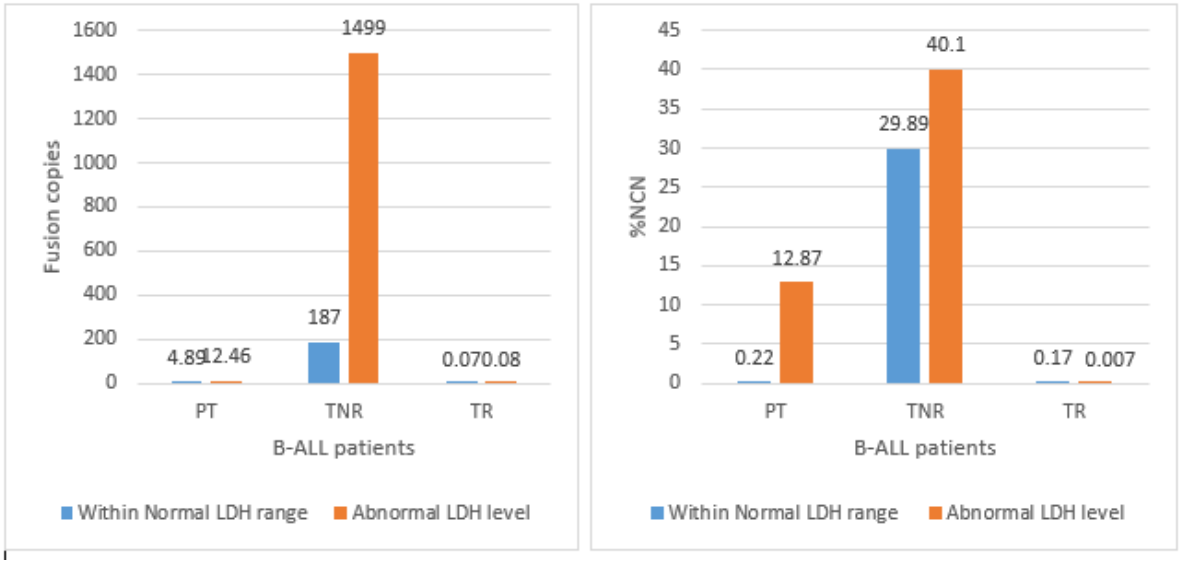

Figure: 5 Correlation of LDH with fusion copies and % NCN levels in PT, TNR and TR subgroups of B-ALL patients

Furthermore, TNR with abnormal LDH levels had higher average fusion copies as well as %NCN (Figure 5) as compared to PT and TR patients.

4. DISCUSSION

ALL is a malignant disease characterized by distinct morphologic, immunophenotypic, cytogenetic, and molecular features, some of which have important clinical implications for diagnosis or prediction of response to a particular treatment (11, 12). Philadelphia chromosome (Ph) is generated due to reciprocal translocation, t (9; 22) (q34; q11.2), resulting in BCR-ABL1 fusion, a constitutively active tyrosine kinase. BCR-ABL1 fusion is seen essentially in all cases of CML and among 25% of the adults with ALL, while only in 3-5% of paediatric ALL (13). There are three breakpoint cluster regions (bcrs) within BCR, when the gene is rearranged with ABL1. Out of these, the upstream minor (m)-bcr, which generates the p190 BCR-ABL1 protein, is primarily associated with Ph+ ALL (14).

B-ALL, a most common subtype of ALL, occurs when the bone marrow produces too many abnormal B-lymphocytes (15). Tyrosine Kinase Inhibitor (TKI) therapy is less effective in B-ALL patients due to primary or secondary resistance, resulting in low overall survival (16). Moreover, the presence of BCR-ABL1 translocation in ALL is associated with aggressive disease and has been shown to have a poor prognosis (17). Hence, investigating mBCR-ABL1 fusion might give insights for prognostic evaluation in ALL patients. Present study examined mBCR-ABL1 fusion transcripts by RT-qPCR method in B-ALL patients and further its correlation with various haematological parameters and LDH activity was evaluated.

Out of 50 enrolled B-ALL patients, the mean age was 24 years, and majority of the patients (N=31) were males. Our study is in accordance with several other reports having male predominance (18, 19). Similar to the current study, the median age in ALL patients was 20 years (20). However, Tabatabei et al (2019) showed no significant difference between prevalence of mBCR-ABL1 and age, gender, and ethnicity in children with ALL due to low positive population (21). Present results and previous observations suggest increased incidence of haematological malignancies in males and younger patients might be influenced by changing life-style factors as well as deformities at genetic level (22).

Present study divided B-ALL patients into 3 subgroups: PT, TNR and TR. On examining the fusion copies and %NCN of mBCR-ABL1 among the three groups, the TNR subgroups exhibits the highest levels in both measures with averages of 1195146.09 fusion copies and 35.946 %NCN. This was followed by the PT group with averages of 1157422.57 fusion copies and 11.968 %. NCN. The TR group exhibits the lowest levels of both factors with averages of 399.4 fusion copies and 0.053 %NCN, suggesting decrease in mBCR-ABL1 copies due to treatment response.

Further, correlation of various haematological parameters among PT, TNR and TR groups was performed. PT had significantly higher WBC counts ($p<0.001$) and lower platelet counts ($p<0.001$) than TNR and TR groups. In accordance to this, high WBC counts and low platelet counts showed a trend towards poor survival in adult ALL patients (23). Seenaa et al (2015) described that leukemic cells associated with lack of normal blood growth results in the lack of normal WBC (increasing the risk of infection), the lack of RBC (resulting in fatigue, weakness, and anemia-low Hb and PCV) and also the lack of normal platelet production (increasing the risk of bleeding) (24). Additionally, present results revealed that TR with abnormal WBC counts and platelet counts had lower levels for average mBCR-ABL1 fusion copies and %NCN. Previous study demonstrated significantly higher median WBC counts in BCR-ABL positive ALL

patients as compared to BCR-ABL1negative patients (96 000/mL vs. 23 000//mL, P = 0.002). Additionally, complete remission was reported in 74% BCR-ABL1negative patients and 35% BCR-ABL1 positive patients (P = 0.001) (20). These results suggest that mBCR-ABL1 transcript can be used as a marker for determining the diagnosis and prognosis of ALL patients. However, further studies with large cohort are required for a definite conclusion.

LDH is one of the most common prognostic indicator in cancer patients. It has an important role in germ cell tumors and in association with chorionic gonadotropin, can predict response to therapy and the prospects of remission. It is also a valuable prognostic marker in lymphoma, leukemia, and in colonic cancer (25). Hence, present study investigated LDH levels in the 3 subgroups of B-ALL patients. When mean LDH levels were compared, it was found to be significantly higher in PT patients compared to TNR and TR (P=0.008). Higher LDH levels in pre-treated patients demonstrates increased cellular LDH activity in ALL when they are untretaed, which reflects a shift towards anaerobic metabolism and increased glycolysis in the cytoplasm of malignant cells accompanied by high cellular turnover rate (26). In accordance with present study, elevated LDH was found in patients with ALL and related with increased cell proliferation and turnover. Further, ALL patients had a significant increase (P<0.0001) in level activity of LDH (721.3±329.3 U/L, ALL patients versus 177± 17 U/L controls) compared to healthy children (27).

In addition, when LDH levels were associated with mBCR-ABL1 transcript levels among B-ALL subgroups, TNR group having abnormal LDH levels exhibited highest average fusion copies and %NCN followed by PT and TR group. On the other side, TRs had the lowest LDH levels and also had lower mBCR-ABL1 expression as compared to TNRs and PT group. Hence, decreased LDH levels in TR subgroup associated with treatment response.

In accordance with present results, previous study described a significantly reduced level of LDH in ALL only after induction of chemotherapy (28). Moreover, recent report in childhood ALL showed that mean value of serum LDH was significantly lower at 29th days of induction of remission compared to the value before chemotherapy (432.40±310.52 U/L vs. 829.10±345.58 U/L, p<0.05) (29). Hafiz et al (2007) reported that the level of serum LDH was significantly high in ALL cases than control (p<0.001) on admission (30). Further, total WBC count was significantly decreased along with serum LDH level at day 14 and day 29 of induction (p<0.001) and a significant rise of platelet count was observed at day 29 of induction in relation to significant decrease of serum LDH level (p<0.001) (31). Present study also found higher LDH levels in pre-treated patients. However, treatment responders had significantly lower WBC counts and higher platelet counts as well as decreased mean LDH levels. Thus, patient with highest LDH level (greater than 1000 U/l) is most likely to be non-responsive to treatment, whereas those with lowest level (less than 300 IU/l) have the minimum risk of failure of treatment (32). Following induction, serum LDH level decreases and when tumor growth resumes as the patient relapse from therapy serum LDH level rises again. Therefore, estimation of serum LDH may be helpful in evaluating the response to therapy. (30).

The classification of B-ALL patients on the basis of treatment, facilitated analysis of mBCR-ABL1 fusion gene expression dynamics with different haematological parameters and LDH activity, providing valuable insights into the management of ALL.

5. CONCLUSION

In the view of outcomes discussed, the quantitative analysis of minor BCR-ABL1 fusion gene copies and %NCN holds significant clinical relevance in understanding and managing B-ALL patients. The correlation between treatment responses among mBCR-ABL1 fusion gene copies and %NCN with haematological parameters as well as LDH activity, underscores their potential utility in monitoring disease progression and response to therapy in B-ALL patients. Among treatment non responders, elevated levels of mBCR-ABL1 fusion gene copies and %NCN are indicative of severe diseased condition, reflected in significant abnormal WBC counts and platelets counts along with increased LDH activity.

Thus, integrating quantitative analysis of minor BCR-ABL1 fusion gene copies and %NCN with traditional haematological parameters can enhance the precision of prognostic evaluations and therapeutic decision-making, ultimately contributing to improved patient outcomes in B-ALL patients from western India.

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