STUDY THE VARIATIONS OF LYMPHOCYTES IN PERIPHERAL BLOOD AND BONE MARROW IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS UNDERGOING THE INDUCTION PHASE OF THE CHEMOTHERAPY

N.V. Warnakulasuriya[1], D.N. Wanigasinghe[1], R. Tudugala[2], P. Herath[3], D.U. Kottahachchi[1]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Rathmalana, Sri Lanka.
[2]Department of Radiography and Radiotherapy, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Rathmalana, Sri Lanka.
[3]Department of Hematology, Apeksha Hospital, Maharagama, Sri Lanka

Background: leukemia (ALL) is the second most common acute leukemia in adults. It has two main categories; B ALL and T ALL. The current method for monitoring ALL is through bone marrow aspiration, which can be difficult to perform and time-consuming. Therefore, finding an alternative method to detect ALL is absolutely required.

Objective: To study the variations of Lymphocytes in Peripheral Blood (PB) and Bone Marrow (BM) in different chemotherapy phases of B & T ALL.

Methods: A total of 105 newly diagnosed ALL patients in ages 5-50 years; 75 with B ALL and 30 with T ALL, attended the Haematology Clinic at Apeksha Hospital, Maharagama was selected for the study. Laboratory investigations; Lymphocyte percentage in Peripheral Blood (L%_PB) was obtained from analyzer reports and verified through a manual Differential Count, Lymphocytes in Bone Marrow (L%_BM) and, Blast cell percentage in Bone Marrow (BL%_BM) from myelogram reports. Blast-to-Lymphocytes-Ratio in the BM (BLR_BM) was calculated. The patients were followed throughout the Initial (D0), Induction I & 2 (D8 & D29) chemotherapy phases. Statistical analysis was performed using IBM SPSS v26. First, the data were tested for normalization, followed by the Wilcoxon Signed Ranks Test considering two groups at a time.

Results: Since the data did not follow the normal distribution non-parametric tests were used. In B ALL, Wilcoxon Signed Ranks Test results revealed that the L%_PB showed increased mean values while D8-D29 showed mild decreased mean values with a statistical significance (p=0.000). The L%_BM too followed a similar pattern with a significance (p=0.000) in all the phases of D0 to D29. BLR_BM followed the opposite pattern of the L%_BM with a significance (p=0.000) in all the phases. In T ALL the results are similar and, the L%_PB was only in D0-D8, L%_BM in D0-D8 & D8-D29, and BLR_BM in D0-D8 & D0-D29.

Conclusion: Initial findings revealed that the chemotherapy has induced the Lymphocytes of PB and BM by regulating the Lymphoblasts in the BM as the mean values of BLR decreased at the end of the induction phase and, to be validated by increasing the number of patients.

Keywords: *Acute Lymphoblastic Leukemia, Induction Chemotherapy, Blast-to-Lymphocyte-ratio, Bone marrow lymphoblasts.*

*nethmiviranya98@gmail.com

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N.V. Warnakulasuriya[1], D.N. Wanigasinghe[1], R. Tudugala[2], S. Suresh[3], D.U. Kottahachchi[1]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Rathmalana, Sri Lanka.
[2]Department of Radiography and Radiotherapy, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Rathmalana, Sri Lanka.
[3]Department of Hematology, Apeksha Hospital, Maharagama, Sri Lanka

Introduction: Acute Lymphoblastic Leukemia (ALL) is a neoplasm resulting from malignant transformation and unusual proliferation of lymphoid precursor cells in bone marrow, blood, and extra medullary sites (Gallegos et al.,2013; Terwilliger and Abdul, 2017). ALL is the most common childhood malignancy among all pediatric malignancies. It has two main categories; B ALL and T ALL. Out of those the B ALL is predominance and most common in children (Hoffbrand and Moss, 2015). Currently, the medical staff often use bone marrow biopsies, and chromosomal analysis to identify and monitor ALL (Chiaretti et al., 2014; Hoelzer et al.,2016). However, these investigations are not easy to conduct, time-consuming, and require experienced personnel, and finding cost-effective methods is challenging.

Methodology: A total of 105 newly diagnosed ALL patients; 75 with B ALL and 30 with T ALL, who attended the Hematology Clinic at Apeksha Hospital, Maharagama was selected for the study. Laboratory investigations; Lymphocytes% in Peripheral Blood (L%_PB) was obtained using 5-part Mindray BC6800 automated hematological analyzer reports. The Lymphocytes% in Bone Marrow (L%_BM) and Blast cells in Bone Marrow (BL%_BM) were obtained from myelogram reports. Blasts to Lymphocytes Ratio in the BM (BLR_BM) was calculated. The whole induction therapy phase was targeted and all the aforesaid counts were used in the study for the initial (D0), induction phase I (D8), and induction phase II (D29). Results were tabulated in XL sheets and were analyzed using Microsoft Excel 2013.12 SPSS version 23 (Released 2015, IBM statistics for Windows version 23, IBM Corp., Armonk, NY) software. Initially, the data in all subgroups (D0, D8 & D29) were checked separately for normalization, followed by the Wilcoxon Signed Ranks Test considering two groups at a time.

RESULTS AND DISCUSSION:

Normality testing of the data pertaining to L%_PB, L%_BM, BLR_BM of B ALL of B ALL are shown in Table 1.

Table 1: Data normalization using SPSS (Descriptive Statistics Explore) in B ALL.

Kolmogorov-Smirnova	
Parameter	**Sig.**
L_PB_D0	.200*
L_PB_D8	.070
L_PB_D29	.004
L_BM_D0	.000
L_BM_D8	.001
L_BM_D29	.000
BLR_BM_D0	.000
BLR_BM_D29	.000

Since the sample size of B ALL is n>=50 Kolmogorov-Smirnov test is used and other than L%_PB D0, none of the grouped data was in a normal distribution.

Normality testing of the data pertaining to L%_PB, L%_BM, BLR_BM of T ALL are shown in Table

Table 2: Data normalization using SPSS (Descriptive Statistics Explore) in T ALL.

Shapiro-Wilk	
Parameter	**Sig.**
L_PB_D0	.001
L_PB_D8	.308
L_PB_D29	.624
L_BM_D0	.003
L_BM_D8	.163
L_BM_D29	.002
BLR_BM_D0	.000
BLR_BM_D29	.000

Since the sample size of T ALL is n<50 Shapiro-Wilk test is used and none of the grouped data was in a normal distribution.

For B ALL – Statistical analysis,

2 Related samples (Wilcoxon Signed Ranks Test) were performed considering two L%_PB, L%_BM, and BLR_BM groups at a time, are shown in Tables 3, 4 & 5.

Table 3: Data normalization Wilcoxon Signed Ranks Test for L%_PB in B ALL.

	L_PB_D8- L_PB_D0	L_PB_D29 - L_PB_D8	L_PB_D29-L_PB_D0
Z	-6.152[b]	-5.778[c]	-1.600[b]
Sig. (2-tailed)	.000	.000	.110

Table 4: Wilcoxon Signed Ranks Test for L%_BM in B ALL

	L_PB_D8- L_PB_D0	L_PB_D29 - L_PB_D8	L_PB_D29-L_PB_D0
Z	-7.024b	-6.193c	-3.535b
Sig. (2-tailed)	.000	.000	.000

Table 5: Wilcoxon Signed Ranks Test for BLR_BM in B ALL.

	L_PB_D8- L_PB_D0	L_PB_D29 - L_PB_D8	L_PB_D29-L_PB_D0
Z	-7.525b	-4.157c	-7.525b
Sig. (2-tailed)	.000	.000	.000

For T ALL – Statistical analysis,

The 2 Related samples (Wilcoxon Signed Ranks Test) were performed considering two L%_PB, L%_BM, and BLR_BM groups at a time, are shown in Tables 6, 7 & 8.

Table 6: Wilcoxon Signed Ranks Test for L%_PB in T ALL.

	L_PB_D8- L_PB_D0	L_PB_D29 - L_PB_D8	L_PB_D29-L_PB_D0
Z	-2.483b	-1.662c	-1.270b

Sig. (2-tailed)	.013	.097	.204

Table 7: Wilcoxon Signed Ranks Test for L%_BM in T ALL.

	L_PB_D8- L_PB_D0	L_PB_D29 - L_PB_D8	L_PB_D29-L_PB_D0
Z	-3.681b	-3.771c	-.343c
Sig. (2-tailed)	.000	.000	.732

Table 8: Wilcoxon Signed Ranks Test for BLR_BM in T ALL.

	L_PB_D8- L_PB_D0	L_PB_D29 - L_PB_D8	L_PB_D29-L_PB_D0
Z	-3.920b	-1.000b	-3.920b
Sig. (2-tailed)	.000	.317	.000

Since the data did not follow the normal distribution non non-parametric tests were used. In B ALL, Wilcoxon Signed Ranks Test results revealed that the L%_PB showed increased mean values while D8-D29 showed mild decreased mean values with a statistical significance (p=0.000). The L%_BM too followed a similar pattern in their mean values with a significance (p=0.000) in all the phases of D0-D8, D8-D29, and D0-D29. BLR_BM followed the opposite pattern of the L%_BM in their means with a significance (p=0.000) in all the phases. In T ALL, the Wilcoxon Signed Ranks Test results revealed that the L%_PB showed identical patterns in their but significant (p-0,013) only in D0-D8. The L%_BM too followed a similar pattern in their mean values with a significance (p=0.000) in D0-D8, and D8-D29. As in B ALL, the BLR_BM too followed the opposite pattern of the L%_BM in their means with a significance (p=0.000) in D0-D8 & D0-D29.

DISCUSSION: In general, following induction chemotherapy, there was a decrease in BL%_BM and BLR_BM, while the L%_PB increased. Previous research studies have consistently demonstrated that a reduction in blast cell count in the peripheral blood, or the restoration of normal haematopoiesis, is a highly significant prognostic indicator for the outcome of childhood acute lymphoblastic leukemia (ALL) (Dai et al.,2021; Donadieu et al.,2000).

An extensive review of research previously reported that a rapid decline in peripheral circulating BL%_PB on Day 8 of induction is a favourable prognostic factor for B-cell ALL. In this current study, we have also observed this rapid decline in the majority of B-cell ALL and T-cell ALL cases (Conter et al.,2000). However, only a limited number of research studies have been published and investigated the impact of these parameters on the prognosis of ALL.

CONCLUSIONS: These results clearly stated that the chemotherapy has induced the Lymphocytes of PB and BM by regulating the Lymphoblasts in the BM as the mean values of BLR were decreasing at the end of the induction phase. However, these findings should be validated by increasing the number of patients in all the phases.

RECOMMENDATIONS:

- Further research could be conducted on isolating bone marrow Lymphocytes in order to gain more comprehensive insights into the genetic rearrangement, translocations, and molecular background.
- Conduct further research to study the bone marrow behavior from peripheral blood up to consolidation and maintenance chemotherapy in order to identify the bone marrow relapse cases.

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ACKNOWLEDGMENTS

We acknowledge the staff of Department of Haematology, Apeksha Hospital- Maharagama for providing facilities for this research.