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INTERNATIONAL SCALE–NORMALIZED COPY NUMBER VALUES OF MAJOR BCR-ABL1 FUSION GENE (P210 TRANSCRIPT), HAEMATOLOGICAL PARAMETERS AND LACTATE DEHYDROGENASE ACTIVITY IN CHRONIC MYELOID LEUKAEMIA PATIENTS: A COMPARATIVE STUDY FROM WESTERN INDIA

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ABSTRACT

Chronic Myeloid Leukaemia patients (CML) with presence of Breakpoint Cluster Region–Abelson (BCR-ABL) gene rearrangement [t (9; 22) (q34; q11)] predicts response to targeted therapy and survival. Hence, this study intends to explore clinical relevance of Major BCR-ABL fusion gene (MBCR-ABL) expression in CML patients and validate %NCN (Normalized copy number) with %IS-NCN (Normalized copy number on international scale). Fifty haematologically & cytogenetically confirmed CML patients were enrolled. Total RNA was extracted from blood samples and was reverse transcribed to cDNA followed by quantification of MBCR-ABL1 transcript by real time PCR. Statistical analysis was done by SPSS software. Fifty CML patients, included 15 untreated (UT) patients, 15 treatment non-responders (TNRs) and 20 treatment responders (TRs). MBCR-ABL1 expression decreased across groups, with TRs showing the lowest averages, suggesting progression towards major molecular response. CML patients with abnormal haematological parameters trended higher expression levels of MBCR-ABL1 across all groups. Combining MBCR-ABL1 fusion gene analysis with traditional cytogenetic and haematological assessments, enhances disease monitoring and more timely interventions towards improved outcomes in managing CML. Moreover, the %NCN correspond to the IS-NCN values of M-BCR-ABL fusion gene, haematological parameters and Lactate dehydrogenase (LDH) values, which are useful in diagnosis and monitoring treatment response and management of CML patients during follow up.

Keywords: MBCR-ABL1 fusion, CML, NCN, IS-NCN, real time RTqPCR, LDH

1. INTRODUCTION

According to American Institute of Cancer Research, chronic myeloid leukaemia (CML) accounted for 0.7 to 1.5 cases per 100,000 populations in 2021. Similarly, in India, reports from national cancer registry programme, it accounted for 0.6 to 2.2 cases per 100,000 populations in 2020. A defining feature of CML is the presence of the Philadelphia chromosome (Ph+) in over 95% of cases [1]. This genetic anomaly arises from the fusion of parts of chromosomes 9 and 22, resulting in the creation of the BCR-ABL1 fusion gene. The BCR-ABL1 fusion protein, particularly the P210 variant, contains distinct domains governing its interactions and functions. Dysregulated tyrosine kinase activity within this fusion protein is essential for its oncogenic potential [1-3].

Laboratory investigating for CML include complete blood count, peripheral blood smear, and bone marrow examinations. Typically, the finding in CML patients show low haemoglobin levels, total white blood cell (WBC) count and variable platelet counts, which can be low, normal, or elevated. Peripheral blood smears often reveal an increased number of mature and immature granulocytes, predominantly myelocytes. Untreated bone marrow in CML cases typically appears hypercellular due to excessive granulocyte proliferation, with myelocytes being the predominant cell type [4,5].

"NCN" refers to "Normalized Copy Number," a measure of BCR-ABL1 transcript levels in CML. "IS" stands for "International Scale," providing a standardized method for reporting these levels. IS-NCN provides a globally accepted standard for reporting BCR-ABL1 transcript levels. Validating NCN measurements against this international standard scale ensures consistency and comparability of results across different laboratories and methods. Validating NCN measurements with the IS-NCN in CML is important for standardization, accuracy, clinical decision-making about treatment strategies and quality assurance of assay [6].

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Thus, in an effort to gain further insight into validation of NCN and IS-NCN, this study aims to explore clinical relevance of Major BCR-ABL (MBCR-ABL) fusion gene expression by reverse transcriptase real time polymerase chain reaction (RT-qPCR) analysis in CML patients and validate NCN and IS-NCN of assay.

2. MATERIALS AND METHODS

2.1 Sample collection and Selection criteria:

Total 50 haematologically and cytogenetically confirmed patients of CML were enrolled in this study. The study was approved by the Institutional Review Committee of the GCRI. 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 c-DNA synthesis

Total RNA was extracted from blood sample using QIAamp RNA Blood Mini Kit (Qiagen). The extracted RNA was quantified using Qubit fluorimeter 3.0 (Invitrogen). RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcriptase kit (Life Technologies, USA). The conversion step was performed on ProFlex PCR system (Applied Biosystems®, USA). The samples were then stored at −80˚C until further use.

2.3 Detection of MBCR-ABL1 fusion gene by quantitative real time PCR (qPCR):

BCR-ABL1 p210 b2a2 or b3a2 transcripts (MBCR-ABL1 fusion transcript) were detected using ipsogen BCR-ABL1 Mbcr RGQ RT-PCR Kit, relative to ABL1 control gene expression. qPCR was performed using QuantStudio5® Real-Time PCR system (Applied Biosystems®, USA). Primers and probe sets provided in the kit are designed according to Europe Against Cancer (EAC) recommendations. The kit also includes an IS-MMR calibrator allowing conversion of results to the International Scale for accurate measurement of molecular respons. The IS-MMR calibrator was included in every run to prevent intra- and inter-lab variation.

The ABL1 and MBCR-ABL1 standard curves were established and used to transform raw CT values for the unknown samples into ABL1 and MBCR-ABL1 copy numbers (ABL1CN or BCR-ABL1 MbcrCN). The ratio of these copy number values gives the normalized copy number (NCN). The experimental IS-MMR calibrator NCN result (NCNcal), and its assigned value (IS-Cal value) indicated in the certificate of analysis, were used to calculate the normalized copy number on the international scale (IS-NCNsample).

Statistical analysis was performed using SPSS statistical software version 20.0 (SPSS Inc., USA) by independent sample t-test. p-value ≤ 0.05 was considered to be significant.

3. RESULTS

The 50 CML patients enrolled in the study were divided into two groups: 15 untreated (UT) patients and 35 treated patients. Further, CML patients in treated group were subdivided as treatment non responders (TNRs) (15 patients) and treatment responders (TRs) (20 patients) (Figure 1).

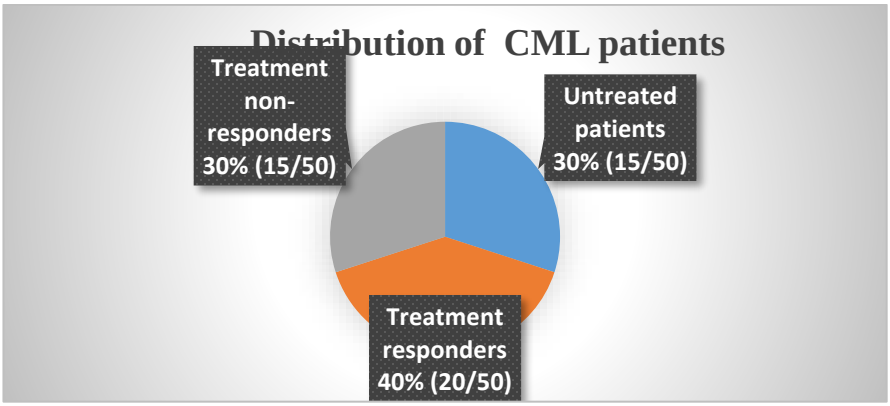

Figure 1: Distribution of CML patients as Untreated (UT), Treatment non responders (TNRs) and Treatment responders (TRs)

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Table 1: Clinical and haematological characteristics of CML patients

	N(%)	Groups		
CML Patients		**UT(%)**	**TNR(%)**	**TR(%)**
Total	50(100)	15(30)	15(30)	20(40)
Gender				
Male	31(62)	8(16)	7(14)	16(32)
Female	19(38)	7(14)	8(16)	4(8)
Age				
≤37	26(52)	9(18)	5(10)	12(24)
>37	24(48)	6(12)	10(20)	8(16)
RBC				
Within Normal RBC count	7(14)	2(4)	2(4)	3(6)
Abnormal RBC count	43(86)	13(26)	13(26)	17(34)
WBC				
Within Normal WBC count	16(32)	0(0)	2(4)	14(28)
Abnormal WBC count	34(68)	15(30)	13(26)	6(12)
Platelet				
Normal platelet count	23(46)	5(10)	5(10)	13(26)
Abnormal platelet count	27(54)	10(20)	10(20)	7(14)
Haemoglobin (Hb)				
Within Normal Hb level	5(10)	0(0)	2(4)	3(6)
Abnormal Hb level	45(90)	15(30)	13(26)	17(34)
Haematocrit (HCT)				
Within Normal HCT level	7(14)	1(2)	3(6)	3(6)
Abnormal HCT level	43(86)	14(28)	12(24)	17(34)

UT – Untreated, TNR - Treatment Non-responders, TR-Treatment Responders

The clinical and haematological characteristics of the enrolled CML patients are summarized in Table 1. Among them, 62% were male and 38% female. Median age was 37 years, dividing patients into, younger age group with ≤ 37 years age (52%) and older age group with >37 years age (48%). All patients showed t (9; 22) positivity. Additionally, majority of the CML patients showed abnormal RBC count (43/50, 86%), abnormal WBC count (34/50, 68%), platelets (27/50, 54%), haemoglobin (Hb) level (45/50, 90%) and haematocrit (HCT) level (43/50, 86%).

In the study, quantitative analysis of MBCR-ABL1 fusion gene by RT-qPCR method showed, higher MBCR-ABL1 fusion copies in UT group of patients followed by TNRs and TRs. There was a decreasing trend in average fusion copies, with the lowest average observed in the TR group. (Figure 2a). Accordingly, the average %NCN and % IS-NCN levels displayed a progressive decrease, indicating a move towards major molecular response, in the TR group when compared to the other two groups (UT and TNR groups). (Figure 2b).

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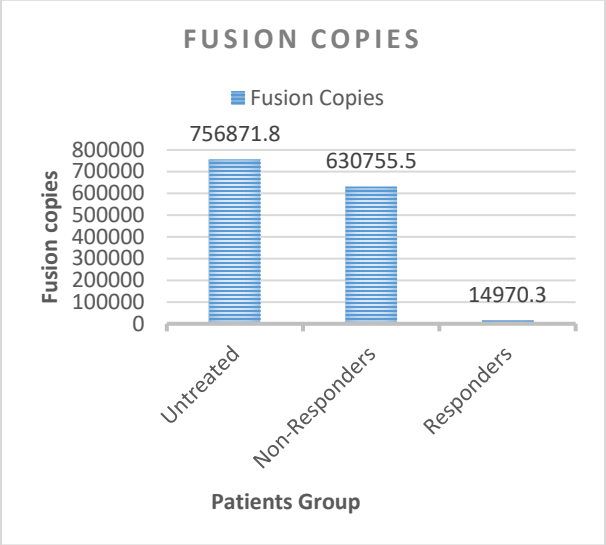

Figure 2(a): Fusion copies among different patient groups

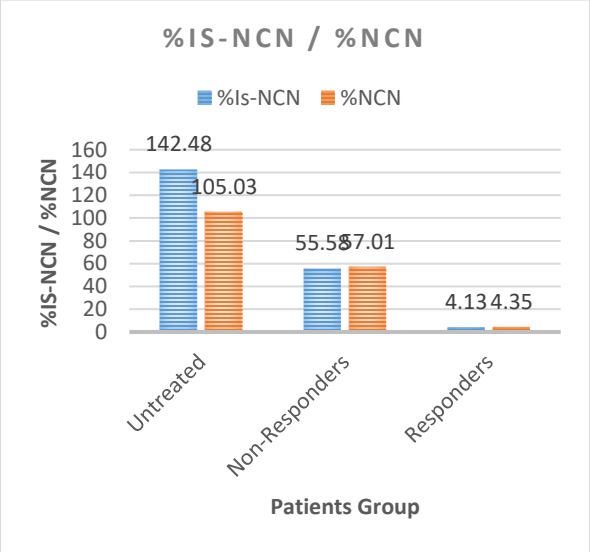

Figure 2(b): %IS-NCN and %NCN among different patient groups

Table 2 depicts mean values of various haematological parameters among different groups of patients. It reveals that TRs had significantly lower WBC counts (p=0.05) and platelet counts (p=0.005) as compared to UT patients and TNRs. However, no significant correlation of any other haematological parameters were observed between these subgroups of CML patients. Further, Lactate dehydrogenase (LDH) levels were markedly lower in the TR group compared to the UT group and TNR group, which demonstrates a highly significant difference (p= 0.0001).

Table 2. Correlation of haematological parameters and LDH levels among different patient groups

Haematological Parameters	UT	TNR	TR	*p-value*
RBC (g/dl)	3.65	4.02	3.92	0.89
WBC (x10^3 cells/µl)	180.43	130.84	22.54	**0.05**
Platelets (x10^3 cells/µl)	536.53	502.2	218.1	**0.005**
Hb (g/dl)	9.86	9.93	11.16	0.91
HCT (%)	30.06	30.71	33.96	0.15
LDH (U/L)	932.466	752.933	254.45	**0.0001**

3.1 Correlation of MBCR-ABL1 with various haematological parameters among UT, TNR and TR groups of CML patients

CML patients with abnormal WBC counts consistently exhibited higher expression of MBCR-ABL1 fusion copies and %IS-NCN in UT and TNRs (Figure 3). On the other hand, CML patients with abnormal platelet counts had higher levels of % IS-NCN of MBCR-ABL1 in UT and TNRs (Figure 4).

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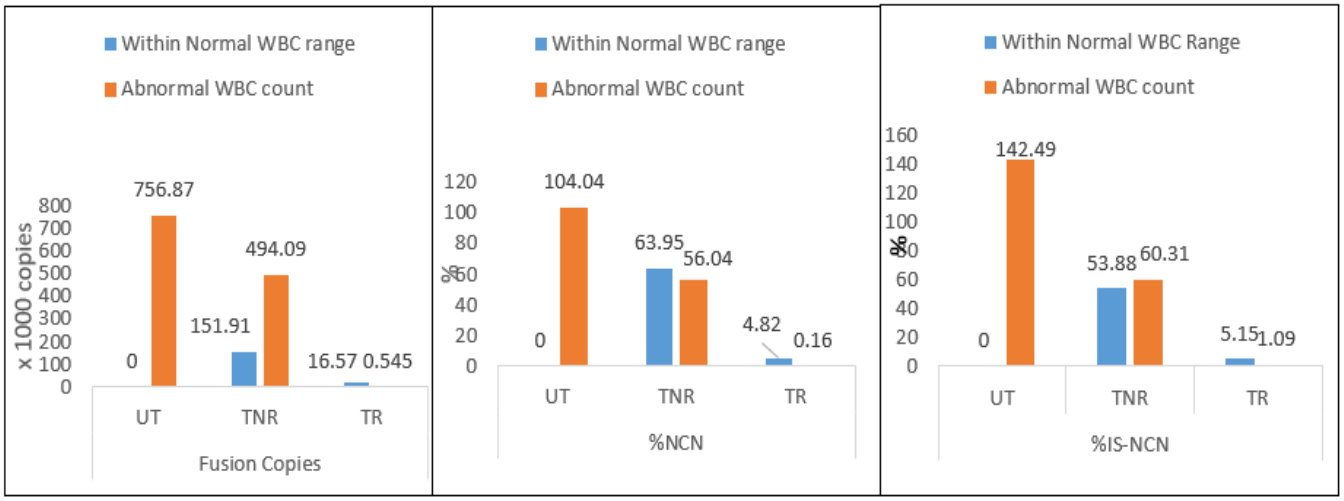
Figure 3: Correlation of fusion copies, % NCN levels and %IS-NCN of MBCR-ABL1 with WBC count in UT, TNR and TR groups of CML patients



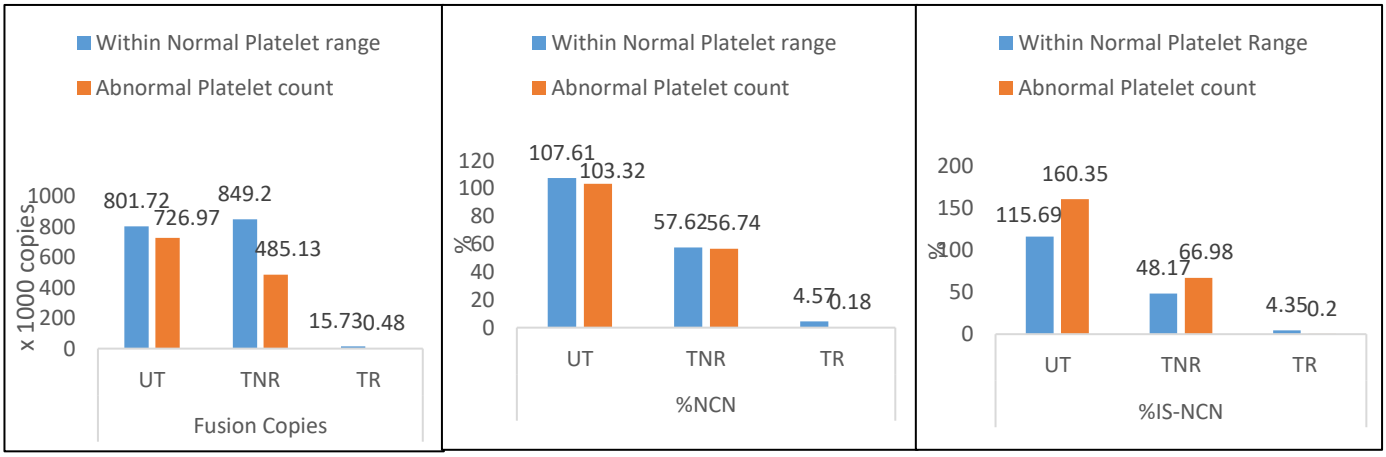


Figure 4: Correlation of fusion copies, % NCN levels and %IS-NCN of MBCR-ABL1 with platelet count in UT, TNR and TR groups of CML patients

3.2 Correlation of MBCR-ABL1 with LDH levels among UT, TNR and TR groups of CML patients

In all groups, abnormal LDH levels were observed to be elevated with MBCR-ABL1 expression. Additionally, UT patients with abnormal LDH levels showed higher MBCR-ABL1 fusion copies, %NCN and %IS-NCN, while TRs with abnormal LDH levels exhibited lower MBCR-ABL1 expression compared to TNRs (Figure 5).

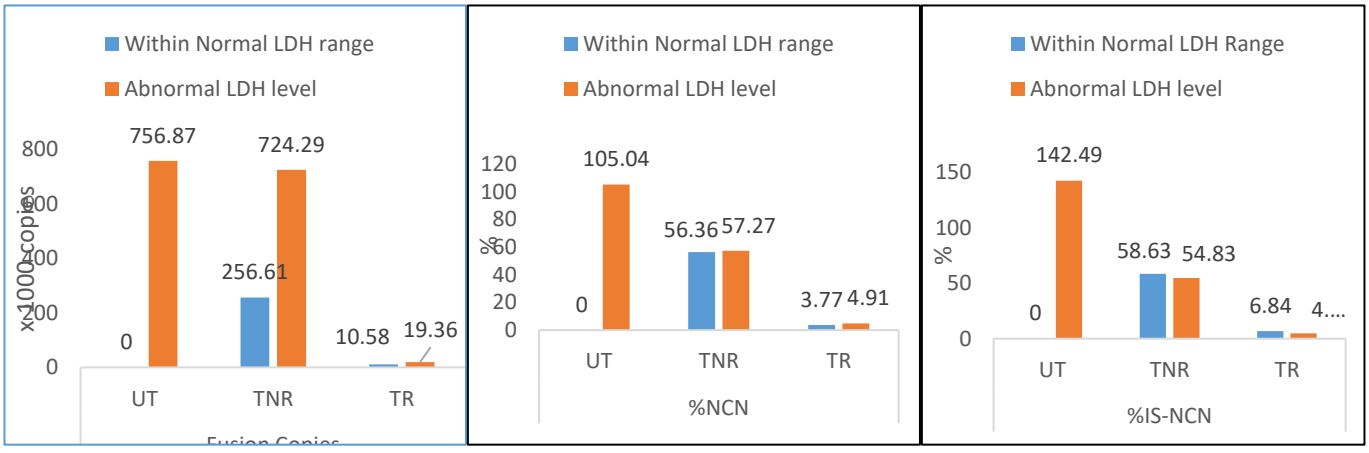


Figure 5: Correlation of fusion copies, % NCN levels and %IS-NCN of MBCR-ABL1 with LDH levels in UT, TNR and TR groups of CML patients

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4. DISCUSSION

Chronic Myeloid Leukaemia (CML) typically presents with nonspecific symptoms such as fatigue, malaise, and weight loss. Additionally, patients may exhibit splenomegaly and in advanced stages, signs of leucostasis such as headache, blurred vision, or priapism. Differential diagnosis includes other causes of leucocytosis and splenomegaly, necessitating a comprehensive clinical assessment [7]. CML staging involves assessing the disease's phase, which includes chronic phase (CP), accelerated phase (AP), and blast phase (BP), based on peripheral blood and bone marrow findings, as well as clinical features. Additionally, risk stratification tools such as the Sokal, Hasford, and EUTOS scores aid in predicting prognosis and guiding treatment decisions [8].

Laboratory investigations reveal characteristic peripheral blood abnormalities in CML, including leucocytosis with a left-shifted myeloid series, thrombocytosis, and anaemia. Bone marrow examination typically shows hypercellularity with increased myeloid precursors and a marked increase in the granulocyte-to-erythroid ratio. These findings, coupled with the presence of the Philadelphia chromosome (Ph) or its molecular equivalent, MBCR-ABL1 fusion gene, confirm the diagnosis of CML [9]. Cytogenetic analysis, such as conventional karyotyping or fluorescence in situ hybridization (FISH) plays a crucial role in detecting the Philadelphia chromosome or BCR-ABL1 fusion gene. Moreover, molecular techniques like quantitative polymerase chain reaction (qPCR) or reverse transcription-polymerase chain reaction (RT-PCR) provide quantitative assessment of MBCR-ABL1 transcript levels, aiding in disease monitoring and treatment response evaluation [10].

In the present study, the median age of total 50 patients was 37 years. The median age at diagnosis in Asia and Africa is also less than 50 years, which again reflects the lower median age of the population diagnosed as CML [11]. Similarly, prior studies by Amin et al (2021), Arsalan et al (2023), Radhi et al (2021) and Karaosmanoglu et al (2023) reported similar age groups for CML ranging from 37 to 42 years [12-15]. Moreover, in current study, among 50 patients, 31 (62%) were males and 19 (38%) were females, indicating a male dominance in CML incidence. This observation aligns with previous findings by Amin et al (2021), Radhi et al (2023) and Kumar et al (2019) [12,14,16]. The male predominance may be attributed to the higher prevalence of haematological neoplasms in males, potentially influenced by genetic and hormonal disparities.

On assessing %NCN and %IS-NCN counts among three patient groups, untreated patients exhibit the highest levels of both measures, with averages of 105.03 and 142.48, respectively. This is followed by treatment non-responders and treatment responders, with significantly lower averages of 57.01/55.58 and 4.35/4.13, respectively. These findings strongly indicate the impact of treatment response on %NCN and %IS-NCN levels, showcasing an inverse correlation; as treatment response decreases, the levels of %NCN and %IS-NCN increase. Hence, lower treatment response corresponds to higher %NCN and %IS-NCN levels, emphasizing the significance of treatment efficacy in managing patients.

The comparison across untreated, treatment non-responders, and treatment responder groups reveals distinct averages for various haematological parameters. Specifically, the untreated group exhibits an average RBC count of 3.65 x 103 cells/µl, while the treatment non-responders and responder groups show averages of 4.2 x 103 cells/µl and 3.92 x 103 cells/µl, respectively. WBC counts are measured at 180.43 x 103 cells/µl, 130.84 x 103 cells/µl, and 22.54 x 103 cells/µl for UT, TNR and TR groups, respectively. Platelet counts follow a similar trend with values of 536.53 x 103 cells/µl, 502.2 x 103 cells/µl, and 218.1 x 103 cells/µl in UT, TNR and TR groups, respectively. Haemoglobin levels are recorded at 9.86 g/dl, 9.93 g/dl, and 11.16 g/dl, while HCT levels are measured at 30.06%, 30.71%, and 33.96% in UT, TNR and TR groups, respectively. Statistical analysis indicates significant differences in WBC count ($p=0.05$) and platelet count ($p=0.005$) between treatment TNRs and TRs, while RBC count ($p=0.89$), Hb level ($p=0.910$), and HCT level ($p=0.150$) do not exhibit significant variations among the groups.

Analysing the haematological parameters like RBCs, WBCs, platelets, haemoglobin count, and haematocrit count, this study aligns with previous research. Specifically, studies by Pinto et al (2021), Ting et al (2017), Karaosmanoglu et al (2023) and Kumar et al (2019) found no significant difference in median RBC levels between patient groups [15-18]. Regarding WBC levels, present results correspond with those of Amin et al (2021), Arsalan et al (2023), Karaosmanoglu et al (2023), Kumar et al (2019) and Bhatti et al (2012), which showed a high significant difference between patients [12,13,15,16,19]. Similarly, studies by Amin et al (2021), Kumar et al (2019) and Bhatti et al (2012) revealed significant disparities in platelet counts between patient and control groups, which parallels the observation of current study [12,16,19]. However, concerning Haemoglobin level, current results contradict those of Kumar et al. (2019), Bhatti et al (2012) and Savage et al (1997) which demonstrated a significant difference between patient and control groups [16,19,7]. Lastly, present study aligns with previous

research by Pinto et al (2021), Ting et al (2017), Karaosmanoglu et al (2023) and Kumar et al (2019) indicating no significant difference in haematocrit levels between patient groups [15-18].

The UT group demonstrates an average LDH level of 932.47 U/L, whereas the TNR and TR groups exhibit averages of 752.94 U/L and 254.45 U/L, respectively. Statistical analysis reveals highly significant differences in LDH levels ($p=0.0001$) among patient groups. Previous studies by Kumar et al (2019), Tatakihara et al (2010), and Patel et al (1991) have reported similar findings, demonstrating significant distinctions between patient groups ($p\leq0.0001$), consistent with present results [16,20,21].

5. CONCLUSION

The findings of this study in population from Western India, suggest that MBCR-ABL1 fusion gene expression and LDH levels are valuable indicators for diagnosing and monitoring CML, with significant correlations observed between haematological parameters and treatment response status, particularly in WBC count, platelet count, LDH levels, %IS-NCN and %NCN. The integration of RT-qPCR-based monitoring of MBCR-ABL1 alongside conventional haematological, cytogenetic and LDH assessments provides comprehensive insights into treatment responses and disease dynamics in CML patients. It will enhance the accuracy of disease monitoring and facilitate timely therapeutic interventions for improved patient outcomes in CML management. Moreover, the %NCN values of the studied patients correspond to the International Scale – NCN values of MBCR-ABL1 fusion gene and are useful not only in diagnosis but more specifically in monitoring treatment response and management of CML patients during follow up.

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