

in the age group 40-49. Regarding the control group, the age range was between 24-71 years and the mean was 49.60 ± 14.17 years, with the largest number of cases falling in the 50-59 years age group.

Regarding Immunohistochemical staining results, we depended on Digital Labelling Index (DLI) parameters in considering what is positive and what is negative; 11 out of the 16 CML cases were positive for WT1 DLI, while none of the

control cases were positive; regarding CD31, 15 cases were positive in CML, while none of the control cases were positive. WT1 DLI was significantly higher in CML than controls 4.17 ± 3.67 ($P = 0.003$).

WT1 DLI was significantly higher in blastic and accelerated phases of CML than Chronic phase (6.46 ± 4.34 , 9.49 ± 1.47 respectively versus 2.27 ± 2.81 in chronic phase, $P = 0.032$ and 0.001 respectively) as seen in Table 1.

Table 1: Comparison of WT1 DLI between CML phases Subclasses

Parameter	CML phase	Mean (DLI) \pm SD	Vs	CML phase	Mean \pm SD	P	SE
WT1	CML chronic	2.27 ± 2.81	Vs	CML blastic	6.46 ± 4.34	0.032	1.53
				CML accelerated	9.49 ± 1.49	0.001	1.69
	CML blastic	6.46 ± 4.34	Vs	CML accelerated	8.49 ± 2.49	0.154	1.94

WT1 DLI was not significantly correlated with age and gender of the patient. WT1 DLI was significantly positively correlated with blast % BMA in CML ($r = 0.619$, $p = 0.011$), while it was not significantly correlated with PCV, WBC count or Platelets count; on the other hand. Angiogenesis parameter used in this study, CD31 DLI, was significantly higher in CML than in controls (8.38 ± 2.51 versus 0.1 ± 0.01 , $P = 0.004$). There was no significant correlation between WT1 DLI with CD31 DLI in CML.

Discussion

The concrete role of WT1 in hematopoiesis and leukemogenesis remains unclear. Studies on the oncogenic activity of WT1 have led to conflicting results demonstrating cell proliferation in some and cell growth arrest in others ⁽¹⁰⁾. In the presented cross sectional case control study, WT1 DLI was positive in 11 CML cases (68.75%) while none of the control cases were positive for WT1. These results were in accordance to other studies such as Rosenfeld et al ⁽¹¹⁾, who found, using Real Time PCR technique, that WT1 gene was overexpressed in all cases of CML; while Huang et al ⁽¹²⁾ found, using conventional nested PCR not QR-PCR, that 17 out of 37 CML showed WT1 expression.

Interestingly, we have found that WT1 protein expression level was significantly higher in CML accelerated and CML blastic phases than CML chronic phase, while there was no significant difference in WT1 expression level between CML accelerated and CML blastic phases. This goes with Kreuzer et al study ⁽¹³⁾, which showed WT1 overexpression, using Real Time- PCR, in all CML patients studied, but revealed differences in WT1 expression levels within this patient population; similarly Huang et al ⁽¹²⁾ showed that 5/18 (27.7%) CML blastic crisis patients, 1/5 (20%) CML patients in accelerated phase, and 1/10 (10%) CML patients in chronic phase have had high WT1 expression level; on the other hand, using conventional PCR, Menssen ⁽¹⁴⁾ revealed overexpression of WT1 in all blast crisis cases but not in chronic phase cases. These data support the notion that increased levels of WT1 expression are indeed specific to leukemic blasts with respect to normal hematopoietic progenitors and not a simple consequence of the differentiation degree.

In this study, WT1 protein expression in CML was not significantly associated with gender and age of the patients, and WT1 protein expression was not significantly associated with various hematological parameters (WBC count, platelets count, PCV level, and peripheral blood blast %)