Philadelphia Chromosome In Acute Lymphoblastic Leukemia

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Abstract

Background. Acute lymphoblastic leukemia (ALL) is a malignant disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow. Philadelphia chromosome (Ph)-positive ALL, a high-risk cytogenetic subset, accounts for 25-30% of adult ALL cases but occurs in less than 5% of children. We aimed with this study to detect Ph chromosome in acute lymphoblastic leukemia patients, using (FISH), and to assess their relation with other standard prognostic factors and therapeutic response.

Patients and methods. This study was carried out on 39 newly diagnosed ALL patients. All patients were subjected to; History, clinical examination and Laboratory investigations, which included CBC (Complete Blood Count), P.BL.(Peripheral Blood) smear and BM(Bone Marrow) examination, immunophenotyping and Fluorescence in situ hybridization to detect Ph chromosome.

Results. This study was carried out on 39 newly diagnosed ALL patients show: Statistical analysis of patients' t(9;22) with other factors revealed significant association ($p<0.05$) of t(9;22) with patients outcome, age >35 years, hepatosplenomegaly, absence of lymphadenopathy, TLC $\geq 50 \times 10^9/L$, absolute P.Bl blasts $\geq 4.4 \times 10^9/L$ and immunophenotyping.

Conclusion. Ph chromosome expression serve as a powerful prognostic marker in adulthood ALL, As ph +ve adult acute lymphoblastic leukemia has poor prognosis and can be used as prognostic indicators for therapeutic response.

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant (clonal) disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow **(1)**.

Adult and childhood ALL differ markedly in the prevalence of various cytogenetic abnormalities. Ph positive ALL, a high-risk cytogenetic subset, accounts for 25-30% of adult ALL cases but occurs in less than 5% of children. **(2).**

Ph chromosome results from a reciprocal translocation that fuses the Abelson tyrosine kinase (ABL1) from chromosome 9 to the breakpoint cluster region (BCR) on chromosome 22. The unique biology of Ph +ve acute lymphoblastic leukemia (ALL) is attributable to the constitutive expression of oncoprotein BCR/ABL1 with tyrosine kinase activity**. (3).**

Fluorescence in situ hybridization technology represents an important advancement in cytogenetics. FISH is a marriage of classical cytogenetics and molecular technologies and has a large number of applications. FISH techniques have replaced special stains in many laboratories *(4).*

These powerful techniques allow us to detect and physically map on interphase nuclei, chromatin fibers, or metaphase chromosomes probes derived from single copy genes to repetitive DNA sequences. *(5).*

Aim of the work*:* This work aims to detect Ph chromosome in acute lymphoblastic leukemia patients, using fluorescence in situ hybridization (FISH), and to assess their relation with other standard prognostic factors and therapeutic response.

Patients and methods: This study was carried out on 39 acute lymphoblastic leukemia (ALL) patients who were attending the hematology oncology clinics. All patients were subjected to the following: History, clinical examination and laboratory investigations, which included: CBC using LH750 (Beckman Coulter), Examination of Leishman stained P.BL. , B.M.A and examination of Leishman stained smears. Immunophenotyping on BM or P.BL. samples, performed on EPICS XL Coulter Flow cytometer and Fluorescence in situ hybridization for detection of t(9;22)(q34; q11)

Statistical Analysis Methods : IBM SPSS statistics (V. 22.0, IBM Corp., USA, 2013) was used for data analysis. The probability of error at 0.05 was considered significant, while at 0.01 and 0.001 are highly significant.

Results

Clinical findings: The current study was carried out on 39 newly diagnosed adult ALL patients. Out of all patients; 24 (61.5%) were males and 15 (38.5%) were females with male to female ratio of (1.6:1). 19 (48.7%) patients presented with hepatomegaly , 20 (51.3%) patients presented with splenomegaly, 24 (61.5%) patients presented with lymphadenopathy and 2 (5.1%) patients presented with CNS infiltration.

Laboratory findings: 1-Hemoglobin level (Hb) ranged from 4.6 to 10.1g/dl with a mean value of (7.35±1.6) g/dl. 2-Total leucocytic count (TLC): ranged from 2.6 to 101x109/L with a median value of (51.8±30.2)x109/L. 3-Platelets count: ranged from 33 to 128x109/L with a mean value of (80.5±26.7)x109/L. 4-Absolute peripheral blood blast: ranged from 2 to 61x109/L with a mean value of (31.5)x109/L. 5-B.M.E: According to WHO classification, The absolute BM blast ranged from 24 to 98x10^9/L with a mean value of (66±21)x10^9/L. 6-Immunophenotyping (IPT): 35 of patients were expressing CD10. Among them CD13 or 33 were positive in 6 patients .

Fluorescence in Situ Hybridization Analysis:

   Metaphase and/or interphase FISH analysis were successfully performed on 39 BM and/or P.BL. samples and revealed the following:

Structural aberrations: Positive results for t(9;22) was encountered in 11 (28.2%) patients, 11(q23) was detected in 2 (5.1%) patients, t(1;19) was detected in 1 (2.5%) patient.

Results of ALL patients't(9;22) in relation to different prognostic factors: Showed significant association (p<0.05) of ph chromosome +ve cases with age >35 years, hepatosplenomegaly, absence of lymphadenopathy, TLC $\geq$50X10^9/L, absolute P.Bl blasts $\geq$4.4X10^9/L and immunophenotyping. On the other hand, gender, CNS infilteration, Hb and platelet count showed non significant statistical difference (p>0.05) **(Table 1).**

Table(1): Results of ALL patients't(9;22) in relation to different prognostic factors:

Parameter	Groups	No.	%	t(9;22) (total No.=11)				P	Significance
				+ve		-ve			
				No.	%	No.	%		
Age(Years)	≥35 <35	18 21	46.2 53.8	9 2	81.8 18.2	9 19	32.1 67.9	0.005	HS
Sex ♂: ♀1.6:1	Male Female	24 15	61.5 38.5	7 4	63.6 36.4	17 11	60.7 39.3	0.866	NS
Hepatomegaly	Yes No	19 20	48.7 51.3	11 0	100 0	8 20	28.6 71.4	0.000	HS
Splenomegaly	Yes No	20 19	51.3 48.7	11 0	100 0	9 19	32.1 67.9	0.000	HS
Lymphadenopathy	Yes No	24 15	61.5 38.5	1 10	9.1 90.9	23 5	82.1 17.9	0.000	HS
CNS Infilteration	Yes No	2 37	5.1 94.9	1 10	9.1 90.9	1 27	3.6 96.4	0.482	NS
Hb	<10g/dl ≥10g/dl	36 3	92.3 7.7	9 2	81.8 18.2	27 1	96.4 3.6	0.123	NS
TLC(x10^9/L)	<50 ≥50	26 13	66.7 33.3	3 8	27.3 72.7	23 5	82.1 17.9	0.001	HS
Platelet count(x10^9/L)	<100 ≥100	33 6	84.6 15.4	11 0	100 0	22 6	78.6 21.4	0.095	NS
Absolute PB Blasts	<4.4 ≥4.4	19 20	48.7 51.3	1 10	9.1 90.9	18 10	64.3 35.7	0.002	HS
IPT	CD10: Positive Negative	35 4	89.7 10.3	11 0	100 0	24 4	85.7 14.3	0.314	NS
	CD13/33: Positive Negative	6 33	15.4 84.6	5 6	45.5 54.5	1 27	3.6 96.4	0.000	HS

P: Prevelance HS: Highly Significant, S: Significant, NS: None Significant, Hb: hemoglobin, TLC: total leucocytic count,
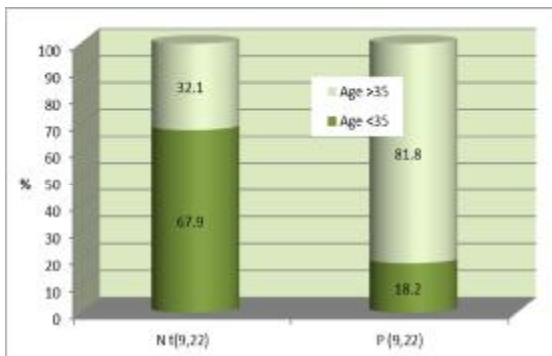
IPT; Immunophenotyping.

Figure (1): Bar chart of age in relation to ALL patients't(9;22).
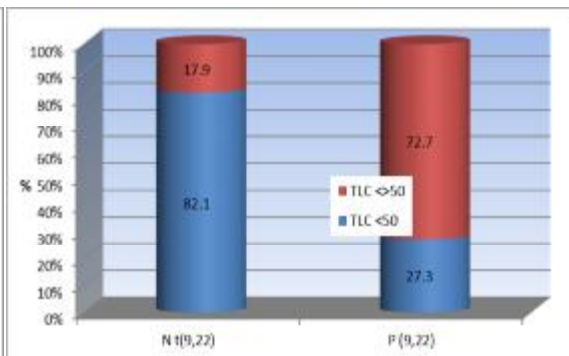 81.8% of patients with t(9;22) had age> 35 yrs old.

Figure (2): Bar chart of TLC in relation to ALL patients't(9;22).
  72.7% of patients with t(9;22) had TLC > 50X109/L.

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Discussion

The current study was carried out on thirty nine diagnosed patients suffering from acute lymphoblastic leukemia.

In the present work ph chromosome present in 11 patients with a frequency of 28.2% (11/39 cases). This is in concordance with (**Ghazavi F et al., 2015**) who reported that ph chromosome is occurs with an incidence in adult (30%) but slightly higher than (**Noreen et al.,2012**) who reported that BCR/ABL fusion gene is occurs with an incidence (20.3%) .

Moreover MLL (11q23) gene rearrangements present in 2 patients with a frequency of 5.1% which lesser than (**Schafer et al., 2015**) who reported MLL gene rearrangement with 10% in adult ALL and 8% of pediatric ALL with about 80% of them in infants. the t(1;19) present in one patient with a frequency of 2.56% which is in concordance with (**Al Ustwania et al., 2016**) that reported t(1;19) 3% in adult ALL.

According to t(9;22) in relation to different prognostic factors. It showed that most of ph +ve ALL patients were presented with age > 35 years old so high significant relation (p=0.005) was detected between the patients age and ph +ve ALL patients. As regards clinical findings in this work, all of ph +ve ALL patients had hepatosplenomegaly with high significant relation (p=0.000) between them. While 9.1% of ph +ve ALL patients had lymphadenopathy with high significant, negative relation (p=0.000) between lymphadenopathy and ph +ve ALL patients. But 9.1% had CNS infilteration with no significant association (p=0.482) to ph +ve, but these results differ from (*Ilana de Franc et al.,2014*) that showed no statistically significant differences between BCR-

ABL positive and negative patients in respect to the clinical variables.

As regards the haematological findings, There was high significant statistical association could be detected between t(9;22) and TLC $\geq 50X10^9$/L with (p=0.001) and with absolute P.Bl blasts $\geq 4.4X10^9$/L with (p=0.001) This finding is concordant with the previously published reports by (*Cetin et al.,2012*).

No significant statistical association could be detected between t(9;22) and CNS infiltration, Hb level<10g/dl, platelets <100x10^9/L and CD10 . This finding is concordant with the previously published reports by (*Cetin et al.,2012*).

Immunophenotypic patterns of ph+ve ALL patients in this work including CD10 and aberrant expression of CD13 or 33, show no significant (P=0.314) statistical association was detected between CD10 and t(9;22). Similary in, (*Sanam et al.,2015*) reported that CD10 expression had no statistical relationship with t(9;22). On the other hand, There was high negative significant association (p=0.000) was detected between t(9;22) and CD13 or 33 positive expression.

There was high significant, negative correlation between outcome and positive ph chromosome t(9;22). among 11 cases with ph +ve only two cases had CR but nine cases had IR. these results are in agreement with those reported by (*Aldoss et al.,2015*)

Conclusion

ph chromosome expression may serve as a powerful prognostic marker in adulthood ALL, As ph +ve adult acute lymphoblastic leukemia has poor prognosis. and can be used as prognostic indicators for therapeutic response.

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