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COMPARISON BETWEEN PHILADELPHIA POSITIVE AND PHILADELPHIA NEGATIVE ADULT ACUTE LEUKEMIAS

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ABSTRACT

This was a retrospective study designed to evaluate the frequency and clinical significance of Philadelphia (Ph) chromosome in acute leukaemia. The place and duration of this work was Armed forces Institute of Pathology Rawalpindi from April 1988 to January 1990 and a private medical center from June 1999 to July 2002. A total of 50 cases of acute leukaemia were included in the study. Thirteen cases presenting de-novo ALL and thirty two cases as denovo ANLL (AML and AMML). Two patients were diagnosed as blast transformation phase of CGL with ALL phenotype whereas 03 cases presented with acute leukaemia transformation from MDS with AML / AMML phenotype. The samples received were either peripheral blood or bone marrow aspirate. Chromosomal analysis was performed using culture, banding and staining technique. Morphology, clinical findings, therapeutic response and survival were compared in patients with and without the Ph chromosome. Ph chromosome was found to be +ve in 02 newly diagnosed patients presenting with ALL. Ph chromosome in association with additional chromosomal abnormalities persisted in 02 cases transformed into ALL from CGL, and it was found in 05 cases of de novo AML. The study failed to reveal any consistent chromosomal translocation involving chromosome 9 and 22 in 03 AML cases transformed from MDS. Patients with Ph+ ALL differed from those with Ph-ALL in being older, in having more frequent lymphadenopathy and splenomegaly and in demonstrating higher initial leucocyte count and more peripheral blasts. Complete remission was obtained in 09 patients with Ph-ve ALL but in only 2 of 4 with Ph +ve ALL. Adults with Ph –ve ALL also survived significantly longer. Five adults with ANLL (AML / AMML) who were Ph +ve did not respond to treatment and survived significantly shorter than adults with Ph –ve AML. No clinical or morphological features indicated which patients with acute leukemia would have Ph chromosome. The Philadelphia chromosome has been considered relatively specific for chronic granulocytic leukaemia. However patients with acute leukaemia (ALL and AML) can also present with positive Philadelphia chromosome. In our study, we have described 09 cases with positive Philadelphia chromosome. Comparison was made with the remaining 41 cases who were Ph negative. Thus it can be concluded that the presence of Ph chromosome in adult acute leukaemia may have biological and clinical significance.

INTRODUCTION

The Philadelphia (Ph) chromosome was identified as the first consistent chromosomal abnormality in CML. It was initially thought to be a deletion of chromosome 22 but was later on shown to be a translocation involving chromosome 9 and 22 [t (9 : 22) (q 34: q 11.2)]. The t (9 : 22) occurs in a pluripotent stem cell that gives rise to both lymphoid and myeloid lineage cells. The genetic consequence is the movement of portion of Abelson (ABL) proto-oncogene on chromosome 9 adjacent to a portion of the BCR gene on chromosome 22.[1] The gene product (BCR/ABL protein) is located on the cytoplasmic surface of the cell membrane and acquires a novel function in transmitting growth regularity signals from cell surface receptors to the nucleus via the *ras* signal transduction pathway.[2]

Patients with CML who transform into acute blast phase (ALL or AML) show karyotypic evolution with the appearance of new chromosomal abnormalities in addition to the Ph chromosome. This change in the karyotype is considered to be a grave prognostic sign.[3,4]

The incidence of t (9 : 22) is about 30% in denovo adult ALL and about 1% in denovo AML. It has been found that additional chromosomal abnormalities frequently coexist with t (9 : 22), the most common being monosomy[7]. Distinguishing patients with Ph+ve acute leukaemia from those with Ph-ve acute leukaemia has important therapeutic and prognostic significance.[5,6]

(The BCR-ABL oncogene on the Ph chromosome and the reciprocal ABL-BCR on the derivative $9q^+$ chromosome. In classic CML, BCR-ABL

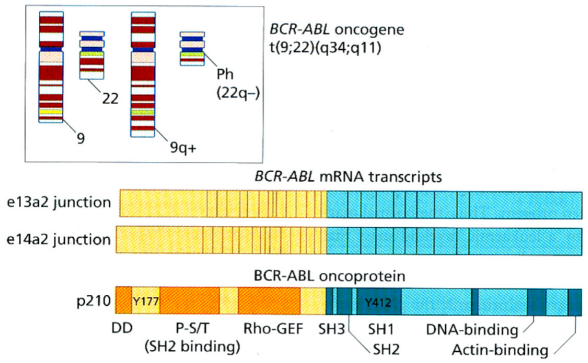

Fig. 1: *Philadelphia chromosome t (9 : 22).*

is transcribed into mRNA molecules with e13a2 or e14a2 junctions, which are then translated into the p210$^{BCR-ABL}$ oncoprotein).

PATIENTS AND METHODS

The patient population of this study consisted of 30 individuals referred to Armed Forces Institute of Pathology, Rawalpindi, mainly from civil and military hospitals between April 1988 and Jan 1990 and 20 patients referred a private medical center between June 1999 and July 2002. Patients of both sexes were included in the study. Preliminary investigations included presenting complaints, the present and past history, therapeutic history and history of blood transfusion. GPE and systemic examination were carried out for anaemia, haemorrhagic manifestations, involvement of gums, lymphadenopathy and organomegaly. In addition skeletal system was also examined to find out bone tenderness.

The specimen obtained from each patient included: peripheral blood collected in EDTA and heparin anticoagulants: Initial pretreatment blood and bone marrow aspirates were studied in all patients. On the basis of the presenting cytology and cytochemistry using criteria provisionally described, patients were divided into three groups. AML 31 patients; acute MML 04 and ALL 15 patients. The presenting symptoms, physical findings and laboratory data were evaluated in all patients. Response to treatment was evaluated using acute leukaemia group B criteria for complete remission in acute leukaemia. Survival was tabulated from the date of diagnosis.

RESULTS

Adult acute lymphoblastic leukaemia:

Chromosomal studies: During the retrospective 04 years and 10 months cytogenetic study utilizing peripheral blood and bone marrow aspirate, 13 untreated adults with ALL and 02 treated cases of CGL with ALL blast transformation were examined. A total of 04 cases were found to be Ph+ve. Hyperdiploidy was also seen in association with Ph chromosome in 03 cases (02 cases transformed from CGL and 01 in denovo ALL).[3,7] Normal metaphases without the Philadelphia chromosome were demonstrated in rest of the 11 cases. In addition marker chromosome was seen in one patient and hypodiploidy in another patient without the Ph chromosome.

Serial chromosome studies were further performed in 02 Ph+ve patients and in 06 Ph-ve ALL patients. One Ph+ve ALL patient was studied at diagnosis and at relapse after partial remission. Similar abnormalities were found in both studies. One Ph+ve ALL patient was studied in relapse and in remission. In relapse out of 08 metaphases analysed, 05 revealed more than 46 chromosomes. Chromosomal analysis was further carried out during the remission phase. Surprisingly, normal metaphase without the Ph+ chromosome was seen. Similarly 02 Ph-ve ALL patients who demonstrated marker chromosome and hypodiploidy respectively at the time of diagnosis have had normal metaphases during remission. Correlation of

Table 1: *Chromosomal study in patients with acute lymphoblastic leukaemia.*

Pt. No	Cytology	No: Cells analyzed	Chromosome number							
			Ph+ cells	Ph+ <46	Ph+ 46	Ph+ 47	Ph+ 48	Ph+ 49	>50	Ph- cells
1.	ALL	10	08		03		4	1		02
2.	ALL	17	17		11	2	2	2		0
3.	ALL	11	11		03	8				
4.	ALL	16	12		12					04
5.	ALL	22	0	02	20					22
6.	ALL	18	0		X					18

Key: x : Marker chromosome

Table 2: *Acute lymphoblastic leukaemia: Comparison of presenting symptoms, physical findings and laboratory parameters of Ph+ve with Ph-ve patients.*

	Ph+		Ph-			Ph+		Ph-	
Symptoms	**No.**	**%**	**No.**	**%**	**Physical findings**	**No.**	**%**	**No.**	**%**
Fever	4	100	10	90	Lymphadenopathy	3	75	6	54.5
Weakness	4	100	11	100	Hepatomegaly	2	50	4	36.3
Haemorrhage	3	75	7	63.6	Splenomegaly	3	75	7	63.3
Infection	3	75	9	81.8	CNS involvement	1	22	1	9
Bone pain	4	100	5	45.4					

Table 3:

Laboratory parameters	Median value Ph+	Median value Ph-
Hb (g/dL)	8	9
PLTs (x109 /L)	20	65
WBCs (x109 /L)	90.0	4.5
Blasts in blood (%)	70	10
Blasts in marrow (%)	90	70

cells with Ph+ve and the morphological pattern of ALL showed a more significant ALL cytological pattern (strong positivity) than patients presenting with Ph-ve acute leukaemia.[4]

Symptoms and Physical findings:
Ph+ve ALL patients were slightly older at diagnosis (median 26.5 years, range 14-62) than Ph-ve ALL patients (median 19 years, range 14-61). Among the Ph+ve ALL patients, 03 were males and one female; whereas of the Ph-ve ALL patients 07 were females and 04 were males. Bone pain was a significant finding in 03 out of 04 Ph+ve ALL patients compared with only 2 out of 11 with Ph-ve ALL. Lymphadenopathy was the most frequent abnormality in adults with both Ph+ve and Ph-ve ALL. Hepatosplenomegaly was more frequent in Ph+ve patients. In addition, gum hypertrophy and skin ecchymotic spots were found to be more frequent in Ph+ve ALL patients.[8-11]

Lab findings:
No significant difference in the presenting haemoglobin and platelet count was observed at the time of diagnosis in Ph+ve and Ph-ve ALL. However the median leukocyte count was significantly higher in patients presenting with Ph+ve ALL. These patients also had higher initial percentage of blasts in the peripheral blood.[11,12]

Response to Treatment and survival:
All 15 patients with ALL were placed on induction treatment with prednisolone, vincristine and daunorubicin. Complete remission was achieved in 11 patients with Ph -ve ALL compared to only 02 patients with Ph+ ALL (50%). Complete remission lasted for 5–10 months in Ph+ve patients compared to 3 to 40 months in patients with Ph-ve ALL. The median duration of remission in Ph-ve adults on this regimen was 24 months. It was also found that 01 of the 02 Ph+ve ALL patients who obtained complete remission but did not receive prophylactic intrathecal methotrexate developed CNS leukaemia concurrent with haematological relapse. Of the 5 out of 11 Ph-ve ALL patients who did not receive prophylactic CNS therapy, one developed CNS relapse at 06 months. Survival of patients with Ph +ve ALL was significantly shorter than patients with Ph-ve ALL.[13-15]

Adult acute non-lymphoblastic leukaemia:
During the retrospective 4 years and 10 months, peripheral blood and bone marrow chromosomal study, 35 previously untreated adults with AML or

Table 4: *Acute non-lymphoblastic leukaemia (AML / AMML).*

No	Cytology	No cells analyzed	Ph+ Cells	Ph+ < 46	Ph+ 46	Ph+ 47	Ph+ 48	Ph+ 49	Ph+ >50	Ph- cells
1.	AML	30	29	6	22				1	1
2.	AML	30	30	3	24				3	
3.	AML	24	22	2	20					2
4.	AMML	14	14	5	9					0
5.	AMML	15	13	03	10					02

AMML were evaluated. Ph chromosome was found in 05 patients.

Morphology:
In a total of 27 patients who demonstrated the cytological pattern of AML (M1/M2), 3 were Ph +ve. In 8 patients who had AMML 2 were positive for Philadelphia chromosome.

Symptoms and physical findings: Ph+ve ANLL patients ranged in age from 29 to 74 years (median 47.5 years). Ph-ve ANLL patients ranged from 18 to 80 years (median 56 years). All 05 Ph+ve patients were males. Twenty (66.6%) of the Ph-ve patients were males while 10 patients (33.3%) were females. The presenting complaints in both groups (Ph-ve or Ph+ve) were fever and weakness. It was found that lymphadenopathy and hepatosplenomegaly occurred more frequently in Ph+ve patients. Disseminated intravascular coagulation was present in 2 out of the 5 Ph+ve patients but in only 16 out of the 30 Ph-ve patients. CNS involvement was also demonstrated in 1 Ph+ve patient but in none of 30 Ph-ve ANLL patients.[4,5,16,17]

Lab findings:
Ph+ve patients presented with higher initial platelet and leukocyte count and lower initial percentage of blasts in the blood and bone marrow.[7,11]

Table 5: ***ANLL:*** *Comparison of presenting symptoms, physical findings and lab parameters of 5 Ph+ve patients with 30 Ph-ve patients.*

Total Ph+ve patients: 05		Total Ph-ve patients: 30		
Symptoms	**No.**	**%**	**No.**	**%**
Fever	3	60	10	33.3
Weakness	5	100	20	66
Haemorrhage	3	60	10	33.3
Infection	3	60	12	40
Bone pain	2	40	5	17

Response to treatment and survival:
Among 5 patients with Ph+ve ANLL, 3 could receive treatment with standard protocol (cytosine arabinoside and daunorubicin). Two patients developed DIC and died due to intracerebral haemorrhage. One patient with Ph+ve AML (M2) went into partial remission, he left the hospital against medical advice and could not be followed up post-chemotherapy. Twenty five patients with Ph-ve ANLL were treated with aggressive chemotherapy regimen consisting of daunorubicin, cytosine arabinocide and 6 – thioguanine, among them 49% obtained complete remission. Survival for Ph+ve patients was significantly shorter than for Ph-ve ANLL patients.[5]

Table 6: *Comparison of physical findings in the two groups.*

Total Ph+ve patients: 05		Total Ph-ve patients: 30		
Physical findings	**No.**	**%**	**No.**	**%**
Lymphadenopathy	3	60	06	20
Hepatomegaly	2	40	10	33
Splenomegaly	3	60	13	43
Gum hypertrophy	1	20	5	17
CNS involvement	1	20	0	0
DIC	2	40	05	17

Table 7: *Laboratory parameters in the two groups.*

Median values:	05 Ph+ve patients	30 Ph-ve patients
Laboratory parameters	**Ph+ve**	**Ph-ve**
Hb g/dL	8.0	9.0
Platelets (x109/L)	100	60
WBC (x109/L)	150	30
Blasts peripheral blood %	45	65
Blasts bone marrow %	42	74

DISCUSSION

Retrospective study of peripheral blood and bone marrow chromosomes in previously untreated patients presenting with acute lymphoblastic leukaemia has demonstrated an unexpectedly large number of patients (23%) who possess Ph chromosome.[6]

Patients presenting with Ph+ve ALL differed from those with Ph-ve ALL being somewhat older at diagnosis, and having more frequent lymphadenopathy, splenomegaly and in demonstrated higher initial leukocyte counts and more blasts in the peripheral blood.[10,18] In our study no clear cut correlation was established between Ph+ve ALL and clinical or haematological parameters. Recognition of patients with Ph chromosome appear to be important for therapeutic reasons. among the 4 patients with Ph+ve ALL available for treatment, only 2 patients went into complete remission after receiving standard protocol regimen. From the present study, it appears that Ph+ve ALL patients require more aggressive chemotherapy combined with optimum supportive measures.[13,18-20] The relations-

hip of patients presenting with de novo ALL and positive Ph chromosome and patients with known Ph+ve CGL who enter blast crisis is unclear.[6]

The present retrospective study of the peripheral blood and bone marrow in adults with AML or AMML has revealed only 5 patients (17%) with the Ph chromosome. These results are similar to those reported in other series.[5,7,16,21] The total number of patients with Ph+ve ANLL is too small (5 out of 35 patients) to draw definite conclusions about which patients presenting with AML or AMML are likely to have Ph chromosome. As evident from the therapeutic protocol for ANLL, survival to date have been significantly less for Ph+ve than for Ph-ve ANLL patients.[5,17,18,22] The present study also indicates that increased number of patients presenting with ALL without any prior history to suggest CGL have positive chromosomal analysis for Ph chromosome.[3,8,10,20]

We **conclude** that demonstration of Ph chromosome in acute leukaemias has important therapeutic and prognostic significance.[5,10,11,18,19,23] In all patients who present with acute leukaemias, the peripheral blood and bone marrow aspirated samples should be analysed for Ph chromosome.[6,15,24,17,18]

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