

CYTOGENETIC STUDIES IN CHRONIC MYELOID LEUKEMIA PATIENTS

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SUMMARY : *Cytogenetic aberrations were studied using bone marrow (BM) and peripheral blood (PB) samples from seven patients who were thought to have chronic myeloid leukemia (CML) in GATA Medical Genetics Department between January 1993 and July 1993. Four of the seven patients were at "initial diagnosis phase" (ID) during the study, whereas three were already diagnosed as CML and were in remission. Four of the seven patients were male and three were female.*

Simultaneous peripheral blood and bone marrow chromosomal studies were performed in four out of seven patients. In two of them only BM were available whereas in one only PB could be studied because of failure in BM aspiration. Philadelphia Chromosome (Ph 1) were found in five out of seven patients (% 71). Three of these Ph 1 positive cases were at ID phase, two in remission. In both of the "remission cases" Ph 1 chromosome were seen in the BM analysis.

Key Words : *Chronic Myeloid Leukemia, Philadelphia Chromosome, Cytogenetics.*

INTRODUCTION

CML is a clonal panmyelopathy involving all of the hemic lineages and at least some of the lymphoid lines. It is characterized by myeloid hyperplasia of the bone marrow, extramedullary hematopoiesis, expansion of the total body granulocyte pool, elevation of the leukocyte count with the appearance of complete range of granulocyte precursor cells in the peripheral blood, and a specific cytogenetic marker, the Ph 1 chromosome (Fig 1) (1).

It is primarily a disease of middle age; the peak incidence is seen in the fourth and fifth decades. There is no significant racial and sexual predilection and no demonstrable hereditary component. The only environmental factor implicated in the aetio-

logy of CML is ionizing radiation (13).

CML starts with the acquisition of the Ph 1 chromosome translocations which generates a chimeric tyrosine specific protein kinase gene [t (9 ; 22) (q34 ; q11)] (Fig 1). With juxtaposition of c-abl to the bcr region, a novel fusion gene (bcr / abl) is produced (6, 9). The protein product of this gene, the p 210 protein, differs from normal p 145 in at least two respects; augmented tyrosin kinase activity and high ability to autophosphorylate (8). This makes the cells' growth independent of extracellular growth factor stimulation which probably results in the rescue of cells from programmed cell death (i.e. apoptosis) (13).

The Ph 1 chromosome was initially believed to be specific for CML. However studies thereafter

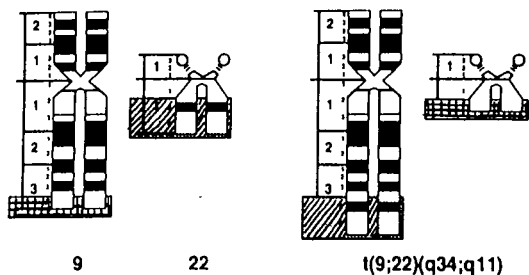


Fig - 1 : The philadelphia chromosome translocation (Ph 1) [t(9;22)(q34;q11)].

have shown that acute lymphoblastic leukemias as well as chronic myelocytic leukemias also expressed Ph 1 chromosomes. Cytogenetically the Ph 1 chromosome of the Ph 1 positive acute leukemias is indistinguishable from that of CML. At the molecular level however the hybrid protein for Ph 1 positive acute lymphoma cases, p 190 is different from the hybrid protein present in CML (p 210). Thus it is confirmed that the breakpoints relevant to t(9;22) are different in acute leukemia than in CML (2).

In recent studies, the Ph 1 chromosome is found in 90 % of all patients with CML (1, 13). Approximately 5 % to 10 % of patients with otherwise typical CML do not manifest the Ph 1 chromosome (7, 12). Secondary chromosomal aberrations in addition to the Ph 1 chromosome are noticed in 22 % of all patients. These appear as especially trisomy 8, 17 p-, -22, trisomy 19, and trisomy 21 (3, 5). For the majority of patients with the Ph 1 chromosome posi-

ve CML, the disease usually undergoes transformation within a mean of three years after diagnosis (12). Here we report cytogenetic studies from seven patients who were thought to have CML or were diagnosed as CML in GATA Medical Genetics Department between January 1993 and July 1993. Results are correlated with the clinical and morphological findings.

MATERIALS AND METHODS

Peripheral Blood (PB) and Bone Marrow (BM) samples from seven patients with a clinical diagnosis or suspicion of CML were sent to our laboratory for cytogenetic evaluation between January 1993 and July 1993 (Table 1). There were four males and three females, the median age being 35.7 + 14.5 SD years, with in a range of 19-64 years.

Cytogenetic analysis were done from heparinized BM aspirate cells (Fig 2). BM cells were studied after one hour and 24 hours incubation of cells. Methotrexate synchronization (High Resolution) also were applied to all of them (14). In case that blastic crises existed, the PB cells were treated as bone marrow cells (Table 2). Chromosomal preparations were GTG banded. Metaphases were karyotyped according to the International System for Human Cytogenetic Nomenclature (4).

RESULTS

Ph 1 chromosomes were found in five out of seven patients (% 71) (Fig 3). Three were at "INITIAL DIAGNOSIS" and two were in "REMISSION" phase (Table 1, case 1, 2, 4, 6, 7). In four of them we analysed the Ph 1 chromosome by BM sample karyotyping (Table 1, case 1, 2, 6, 7). One case, who was at ID phase and had a leucocyte count of 80.000, was analysed by only PB karyotyping (Table 1 case 4). Two Ph 1 chromosome positive

No	Disease Phase (*)	Source of Chromosome	Leukocyte count	Ph. Chr.	Patient Outcome
1	ID	PB / BM	73.000	+ / +	Survives in remission
2	ID	PB / BM	60.000	+ / +	Survives in remission
3	ID	0 / BM	36.800	0 / -	Survives in remission
4	ID	PB / 0	80.000	+ / 0	Survives in remission
5	Remission	PB / BM	6.000	- / -	Survives in remission
6	Remission	PB / BM	6.000	- / +	Survives in remission
7	Remission	0 / BM	18.000	0 / +	Died in blast crises

Table 1 : Clinical staging, leukocyte count, patient outcome and cytogenetic studies of 7 CML patients.

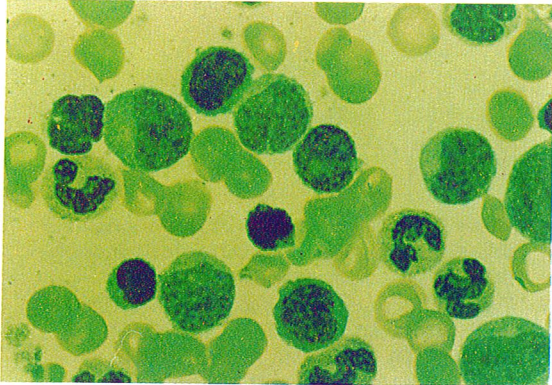


Fig - 2 : Bone marrow cells of a patient.

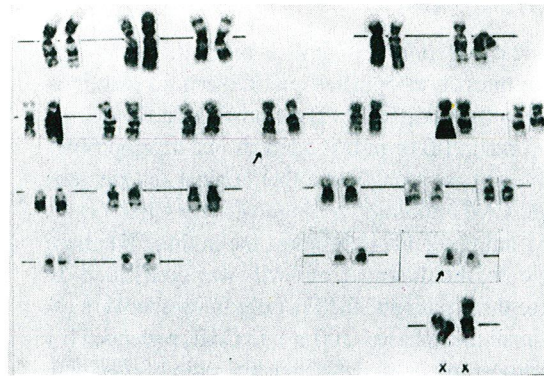


Fig - 3b : Ph 1 chromosome GTH banded.

Case No	PB Sample				BM Sample		
	1 h inc	24 h inc	MTX HR	72 h inc	1 h inc	24 h inc	MTX HR
1	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+
3				+	+	+	+
4	+	+	+	+			
5				+	+		+
6				+	+		+
7				+	+	+	+

inc. : incubation

MTX HR : methotrexate high resolution

Table 2 : BM and PB cytogenetic methods applied to 7 cases.

cases, who were at ID phase and had a leucocyte count of 73.000 and of 60.000 respectively were analysed by PB and BM karyotyping simultaneously. In these two cases the Ph 1 chromosome were shown both in PB and in BM samples together (Table 1 case 1, 2).

In two CML patients, who were at ID phase the Ph 1 chromosome were detected in all metaphases (100 %) both in PB and in BM karyotypes (Table 1). In two CML patients who were in remission phase, the Ph 1 chromosome were found in all metaphases (100 %) of BM karyotyping (Table 1 case 6, 7).

In one case, who was in ID phase and had a leucocyte count of 36.000, we studied only BM sample and in the other case, who was in remission phase and had a leucocyte count of 6.000, we studied PB and BM samples together (Table 1 case 3, 5). In these two cases we were not able to show the Ph 1 chro-



Fig - 3a : Ph 1 chromosome, giemsa staining.

mosome.

No other chromosomal abnormalities were found in these seven cases.

DISCUSSION

The differential diagnosis of CML from other bone marrow neoplasias and leukomoid reactions is sometimes difficult. The finding of a Ph 1 chromosome in PB or in BM analysis in a myeloproliferative disease along with other clinical and hematologic CML findings, is diagnostic for CML (1, 4). By finding the Ph 1 chromosome in three ID phase patients, the diagnose of CML was confirmed. In these three patients, the Ph 1 chromosome were seen in all metaphases (100 %). In CML patients, Ph 1 chromosome ratio, defined as the ratio of metaphases which include Ph 1 chromosome, can be high before the therapy.

The Ph 1 chromosome was found to be positive in two CML patients who were grouped as in remission on clinical and hematological grounds. In one of these patients the Ph 1 chromosome was observed in all metaphases in BM karyotyping (Table 1 case 6); this patient is in still remission clinically, six months later than the cytogenetic analysis. The other patient, who was in clinical remission phase and had a leukocyte count of 18.000, had Ph 1 chromosome also in 100 % of the metaphases analysed (Table 1 case 7). This patient died of blast crises ten months after the initial diagnosis and five months after the karyotyping.

Recent studies prove Ph 1 chromosome negative cases to have poorer prognosis (4, 11, 13). We failed to find any Ph 1 chromosome in case 3 who was at ID phase. She was diagnosed and treated as CML on clinical and hematologic basis (Table 1). She has been in remission for the last four months.

The acquisition of additional chromosomal abnormalities such as extra Ph 1 chromosome, trisomy 18, 17p-, or -22 decreases the capability of maturation which terminates in blastic crises (1, 10). In this study we were not able to show any chromosomal aberrations other than Ph 1 chromosome. This may be due to the small size of the study group.

As is clearly seen from the report, the cytogenetic analysis of CML patients is very important in every stage for the initial diagnosis, treatment and long term follow up.

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