CYTOGENETIC STUDIES IN CHRONIC MYELOGENOUS LEUKEMIA: A PRELIMINARY REPORT

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SYNOPSIS

Between December 1978 and December 1986, cytogenetic studies were done on 75 chronic myelogenous leukemia (CML) patients. 47 patients were male (63%) and 28 were female (37%). Their ages range from six years to 81 years. Philadelphia (Ph^1) chromosome was present in 54 patients (72%) and absent in 21 patients (28%). Successful banding studies on seven patients with the Ph^1 chromosome revealed that five patients had the standard translocation t(9;22) and two had variant translocation. One patient with the variant translocation had a complex translocation involving three chromosomes, i.e. 46, XY, t(4;9;22) while the other patient had two translocations, i.e. 46, XY, t(9;22), ?t(5;15). In the unbanded cases, one Ph^1-positive patient was found to have Ph^1-negative and polyploid cells, and in another Ph^1-positive patient an additional Ph^1 chromosome was present in some of the hyperdiploid cells.

The significance of these chromosome abnormalities on the prognosis is discussed, with reference to the literature. Problems encountered in our cytogenetic studies will also be discussed.

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INTRODUCTION

Chronic myelogenous leukemia (CML) is a disease of marrow stem cells. Cytogenetic studies revealed that about 85–90% of the patients with CML have the Philadelphia (Ph^1) chromosome(1,2). About 90% of the Ph^1 chromosome is due to the standard translocation between chromosome 9 and 22, i.e. t(9;22) (q34; q11) while 10% of the Ph^1 chromosome is due to variant translocation (or "non-standard" Ph^1 chromosome). There are two types of variant translocation, one is a simple translocation in which the deleted segment of chromosome 22 is apparently translocated onto a chromosome other than chromosome 9 and the other is a complex translocation involving three or more different chromosomes.

In this preliminary study, we present here the various types of chromosome abnormalities found in CML patients whose bone marrow aspirate was sent to our Cytogenetic Laboratory and those who came personally to our Hematology Department for cytogenetic studies.

MATERIALS AND METHODS

Between December 1978 and December 1986 cytogenetic studies were done on 75 CML patients. Bone marrow aspirate was taken from CML patients in the hospitals in the Kuala Lumpur area and then sent immediately to our Cytogenetic Laboratory for chromosome studies. Some of the patients referred came personally to our Hematology Department for the study.

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1–2 cc of bone marrow aspirate was collected into a sterile heparin tube containing 5 ml of medium TC 199. The specimen collected was processed immediately (direct method) by dividing them equally and dispensing into 2–3 plain sterile tubes containing medium TC 199 and incubated with 0.1 ml of 0.08 mg/ml of colcemid for 30 minutes at 37°C. The bone marrow aspirate was then harvested by adding 0.075M potassium chloride solution for 10 minutes as hypotonic treatment, fixed three times in methanol-acetic acid fixative and dropped on to cold slides. The slides were air dried and aged for about 3–4 days or longer. The slides were then G-banded according to the technique of Seabright (3) and stained with Giemsa.

Various problems were encountered. The chromosomes in most of the cases were fuzzy, open armed and showed breakages; and hence could not be banded as they get "spoilt" easily after banding. Most of the chromosome spreads appeared to be clumped in nature and in some cases the metaphase spreads were few in number (less than 10 spreads). The usual procedure in our Laboratory is to screen for 30 metaphase spreads and in cases of mosaicism, 50 or more spreads are analysed. The type of chromosome abnormalities if present were karyotyped from those cases that could be banded.

The "problem" cases were stained with Giemsa and screened for the presence or absence of the Ph^1 chromosome. The number of metaphase spreads analysed is usually recorded. Unsuccessful cases, that is those without any chromosome spreads or the spreads were clumped until they could not be analysed, were excluded from this report. They constitute 30% of all cases seen over the period.

RESULTS

54 (72%) of the 75 CML patients were Ph^1-positive while 21 (28%) of them were Ph^1-negative. Among the 54 Ph^1-positive patients, successful G-banding studies in seven of them revealed that five have the standard t(9;22) translocation (Fig. 1), and two had complex variant translocation. One male patient with the variant translocation had a complex translocation involving three chromosomes; chromosomes 4, 9 and 22; i.e. 46, XY, t (4;9;22) (Fig. 2) while the other patient also a

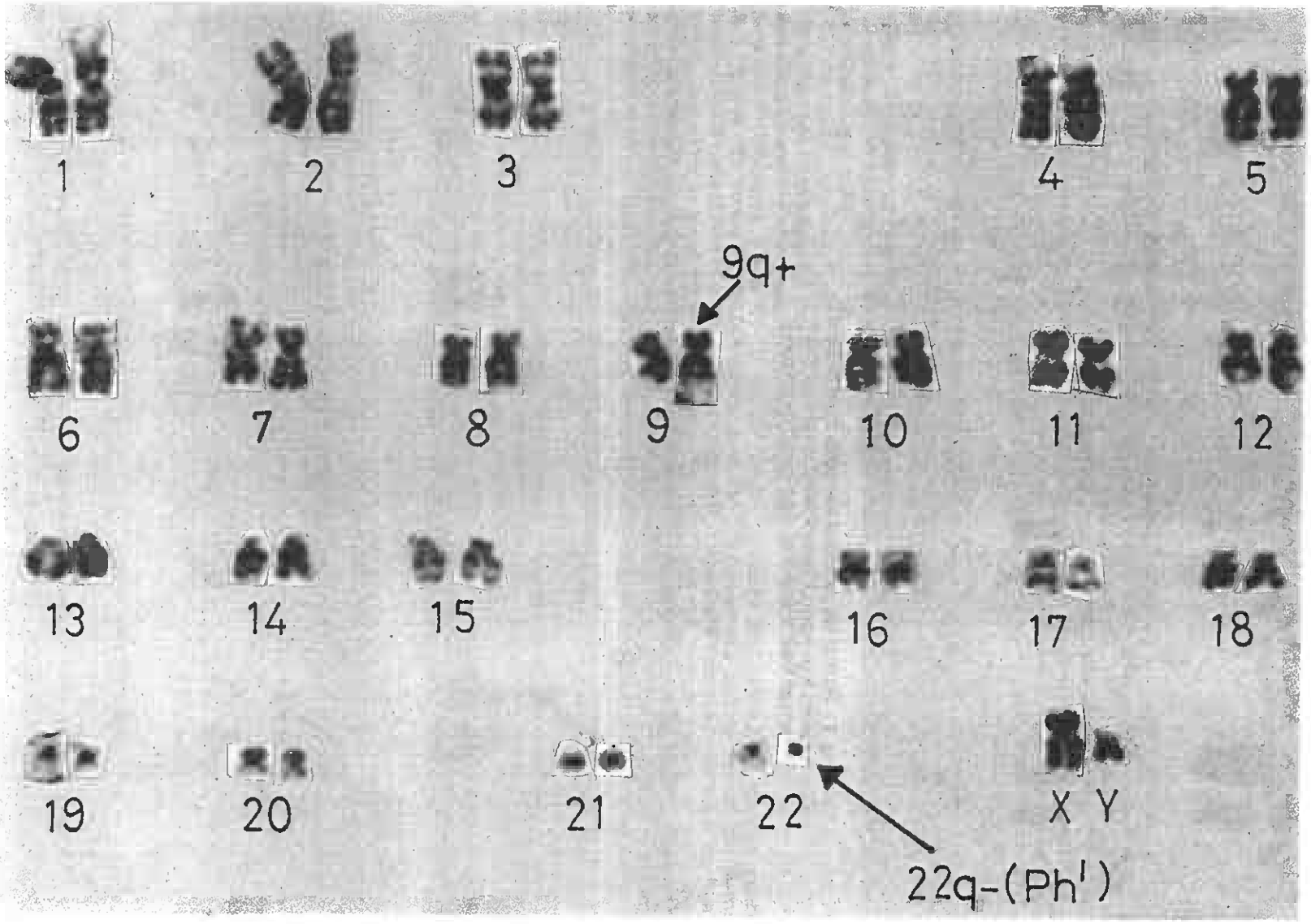

Fig. 1 Karyotype of a cell from a male chronic myelogenous leukemia patient showing the Philadelphia (Ph') chromosome due to a translocation between chromosomes 9 and 22 (Indicated by arrows)

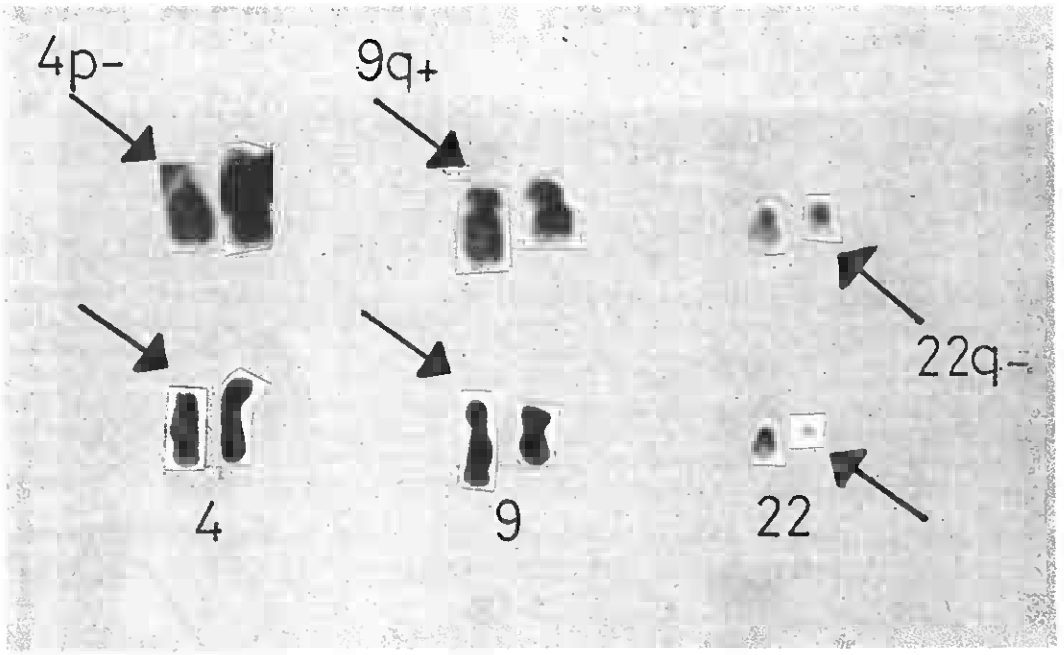

Fig. 2 Partial karyotype of two cells from patient D showing a complex translocation involving three chromosomes; 4, 9 and 22. (Indicated by arrows)

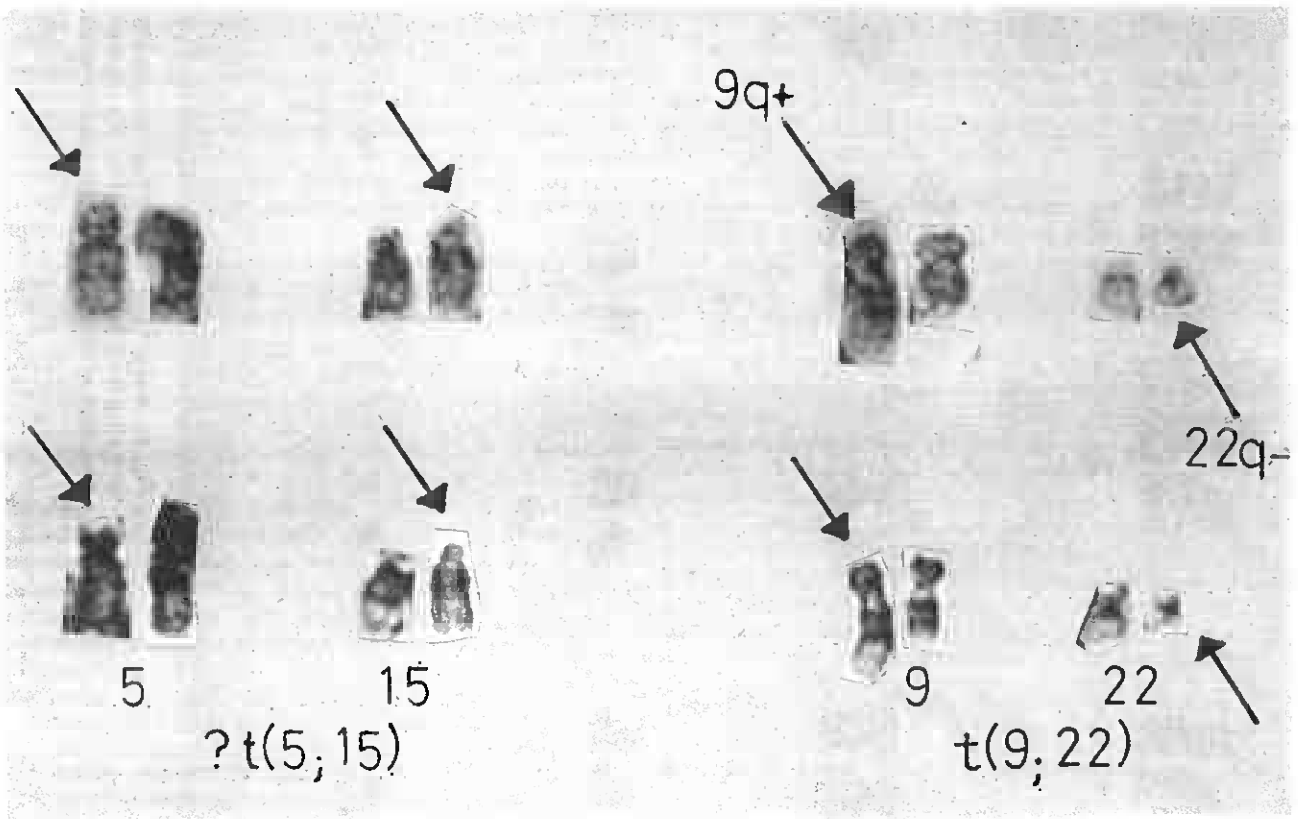

Fig. 3 Partial karyotype of two cells from patient A showing two translocations, one between chromosomes 9 and 22 t(9;22) and the other translocation probably between chromosomes 5 and 15 ?t(5;15) (Indicated by arrows)

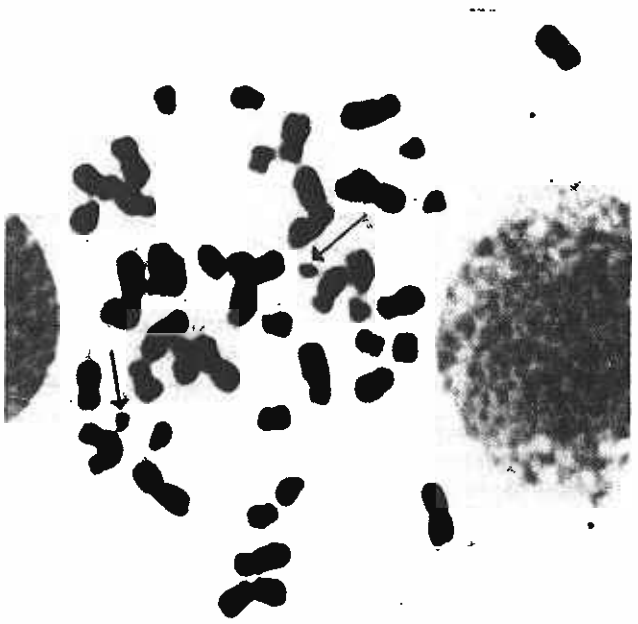

Fig. 4 Karyotype of a hyperdiploid cell from patient B showing two Philadelphia (Ph) chromosome. (Indicated by arrows)

male, had two translocations; one the standard t(9;22) translocation and the other translocation was probably between chromosome 5 and 15, i.e. 46, XY, t(9;22), ?t(5;15) (Fig. 3). In the unbanded cases, one Ph¹-positive male patient was found to have an additional Ph¹ chromosome in some hyperdiploid cells (Fig. 4) while another Ph-positive patient had Ph¹-negative and polyploid cells as well. The cytogenetic findings of the 4 patients with additional chromosomal abnormalities and complex translocations are summarised in Table 1.

Out of 47 male CML patients, 35 (74%) were Ph¹-positive and 12 (26%) were Ph¹-negative. The age range for the Ph¹-positive males was from six to 61 years and this was almost similar to that of the Ph¹-negative males i.e. from 10 to 60 years. 19 (68%) of the female CML patients were Ph¹-positive while nine (32%) of them were Ph¹-negative. The age range for female CML Ph¹-positive and Ph¹-negative patients was also almost identical; from nine to 81 years for the Ph¹-positive group and ten to 81 years for the Ph¹-negative group. The age range for the Ph¹-positive and Ph¹-negative patients with respect to their sex are summarised in Table 2(a) and 2(b). In the female CML patients the age range appears to be wider than that of the male CML patients.

TABLE 2(a)
AGE RANGE OF MALE PH¹-POSITIVE AND PH¹-NEGATIVE CML PATIENTS

	Nos. of Male CML Patients	Age Range (years)
Ph¹-positive	35 (74%)	6 – 61
Ph¹-negative	12 (26%)	10 – 60

TABLE 2(b)
AGE RANGE OF FEMALE PH¹-POSITIVE AND PH¹-NEGATIVE CML PATIENTS

	Nos. of Female CML Patients	Age Range (years)
Ph¹-positive	19 (68%)	9 – 81
Ph¹-negative	9 (32%)	10 – 81

DISCUSSION

About 80% of the bone marrow aspirate samples for cytogenetic studies sent to our Hematology Department are from the hospitals in the Kuala Lumpur area and the clinical history of the patients supplied is usually inadequate. The information supplied was that the patients have CML; most of the time there was no mention of whether the patient was in chronic or acute phase or on treatment, although this information is requested. Cytogenetic studies were performed usually once only in these patients and there were no follow up studies in most of them. Hence, it was difficult to correlate the survival of these patients with their chromosomal abnormalities.

The Ph¹ chromosome is not only found in CML, but has been reported to occur also in 33% of adult acute lymphoblastic leukemia (ALL) (4), and 10% of childhood ALL (5) and 2–3% of acute myeloid leukemia (4,6). In our study, 72% of the CML patients are Ph¹ positive, which is rather low compared to 85–90%(1,2) reported in the literature. This could be due to the fact that our sample size was small in number. 28% of our CML patients are Ph-negative. Pugh et al, 1985(7) found that most patients initially diagnosed as having CML

TABLE 1
CYTOGENETIC FINDINGS IN 4 MALE CML PATIENTS WITH ADDITIONAL CHROMOSOME ABERRATIONS OR COMPLEX TRANSLOCATIONS

Patient	Age (years)	Cytogenetic Studies
A	21	2n = 46, XY, t(9;22), ?t(5;15) Hyperdiploid cells present.
B	24	Ph¹-positive. 18 of the 30 cells (60%) observed were hyperdiploid. Two Ph¹ chromosomes were observed in some of the hyperdiploid cells.
C	25	Ph¹-positive: 56 cells (37%) Ph¹-negative: 82 cells (55%) Polyploidy (about 4n): 12 cells (8%)
D	38	2n = 46, XY, t(4; 9; 22)

but are Ph¹-negative did not have CML but have some type of myelodysplasia such as chronic myelomonocytic leukemia or refractory anemia with excess of blasts. Ph¹-negative CML patients appear to have a shorter life expectancy than Ph¹-positive patients and they show a poor response to chemotherapy. The presence of Ph¹-negative marrow cells in Ph¹-positive CML has been claimed not to be associated with any survival advantage(8).

During the chronic phase of CML, 30% of the Ph¹-positive patients have additional chromosomal abnormalities such as Trisomy 8, isochromosome for the long arm of chromosome 17, duplication or triplication of the Ph¹ chromosome and the loss of the Y chromosome.(9) When patients enter the terminal acute phase, 20% of the Ph¹-positive patients will retain the Ph¹ chromosome alone while 80% will have additional chromosome aberrations(2) which are almost similar to those in the chronic phase. These additional chromosome aberrations were found to preceed the clinical signs of the blast crisis by 2–4 months in most of the cases.(2) The survival advantage of additional chromosomal changes at the blastic crisis is not clear.

Sandberg, 1980(10) found that the survival of patients with CML having variant translocation do not differ from that of patients with the standard Ph¹ translocation. In a study by Potter et al 1981(11), it was found that the benign phase was significantly shorter for the non-standard Ph¹ patients (median 20 months) than for those with the standard Ph¹ (median 43 months).

The role of oncogenes in the pathogenesis of CML have recently been reviewed.(12) In the t(9;22) translocation, the Abelson oncogene *(c-abl)* from chromosome 9 has been translocated to the Ph¹ chromosome. Simple variant translocations were thought to involve chromosome 22 and another chromosome other than 9. Detailed analysis in several cases indicate that probably such simple variant translocations do not occur; and at the molecular level the *c-abl* gene is still translocated to chromosome 22. Other oncogenes such as the *N-ras* and *c-myc* oncogenes have also been implicated in CML. Studies are being currently done to elucidate the role of the oncogenes, through their gene products, in the pathogenesis of CML.

Thus cytogenetic studies are potentially beneficial in CML. Ph¹-positive patients in the chronic phase developing additional chromosome aberrations in the progress of the disease may be undergoing transformation
to the blastic crisis; hence clinicians will be able to prognostigate the course of CML in follow up cytogenetic studies.

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