EFFECT OF CHEMOTHERAPY ON PHILADELPHIA CHROMOSOME IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

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ABSTRACT

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder as a result of neoplastic transformation of the primitive hemopoetic cells. It is well known that the Philadelphia chromosome (ph) is a specific abnormality found in 90% of CML patients. It has been reported that interferon has better effect on disease control and prognosis. Cytogenetic analysis of ph chromosome plays very important role in the prognosis and monitoring of therapy. In this present study 35 diagnosed patients of CML were considered, which included untreated patients of various age groups (2-62yrs). The cases were referred from haematology clinic of All India Institute of Medical Sciences (AIIMS). Out of 35 patients only 13 patients were available after six month of therapy for follow-up cytogenetic analysis. Out of 13 patient, 2 were ph negative, 8 were 100% ph positive and 3 were ph positive mosaic before therapy. Of the 3 mosaic patients, 2 remained unchanged after therapy and one patient became 100% ph negative. Though in general significant reduction in ph% by interferon therapy was seen but minority patients showed complete cytogenetic remission.

Key Words: *Chronic myeloid leukemia, chemotherapy, Philadelphia chromosome.*

INTRODUCTION

Chronic myeloid leukemia is a clonal myeloproliferative disorder as a result of neoplastic transformation of the primitive haemopoietic stem cell. It accounts for about 7-15% of all leukemias.¹ Males are more commonly affected than female (14:1).²

The Philadelphia (ph) chromosome in the malignant cells is found in more than 90% of patients with CML. Nowell and Hungerford³ reported a very small chromosome that appeared to be a partially deleted copy of one of the two smallest autosome pairs. This small element became known as the Philadelphia chromosome.⁴

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The genetics of CML plays very important role in the prognosis of patient's condition and for the monitoring of therapy, which are consistently associated with chromosome abnormality. Cytogenetic molecular analysis of the Philadelphia chromosome provide important information to the physician that are relevant to the leukemic burden of the patients.

It was planned to study chromosome in clinically diagnosed CML patients before and on chemotherapy.

MATERIAL AND METHODS

Thirty- five diagnosed cases of chronic myeloid leukemia were considered for this study, which included untreated patients of various age groups (2-62years). The diagnosis of CML was made on the basis of hematologic investigations, and cases were referred from hematology clinic of AIIMS.

SAMPLE COLLECTION

Bone marrow (BM) aspiration was done by the haematologist from the posterior superior iliac spine using heparinised syringe. 1ml of aspirate was collected in a sterile tube containing 5 ml of RPMI 1640 (Gibco, BRL) culture media supplemented with 10-units/ ml of heparin. Samples were transported at room temperature.

BONE MARROW CULTURE

The bone marrow aspirate was centrifuged (1000 r.p.m; 10 minutes) and washed with RPMI 1640 media in the same tube in which it was transported. After discarding the supernatant, the pallet was supplemented in 5ml of RPMI 1640 reconstituted with 20% fetal bovine serum (Gibco, BRL). Two vials were set up for direct harvesting and for short-term culture for 24 hours in Co₂ incubator at 37° centigrade.

CHROMOSOME PREPARATION

Harvesting for bone marrow was done using colcemid (Gibco, BRL) treatment for 1 hour at a concentration of 0.2/ml. The culture was centrifuged and the cell pallet was suspended in 10ml of hypotonic solution (0.075m potassium chloride (KCL) for 30 minutes at 37° c. The suspension was again centrifuged and the cells were then fixed in methanol acetic acid fixative 3:1 for over night at -20°c. Precleaned slides were used for chromosome preparations. The cell suspension was dropped from an approximate distance of 40-50 cm on the slide placed at 30°. Conventional staining was done in 4% Giemsa (Gibco, BRL) solution.

GTG- BANDING

On slide stored for 2-4 days at room temperature, GTG- banding was done using modified method of seabright5. Slide was then washed in normal saline and stained with 2% Giemsa (Gibco, BRL) stain.

METAPHASE SCREENING

The preparation was screened under X 10 objective using zeiss light microscope. Well spread metaphases were further analysed under x100 oil immersion objective. Minimum of 30 well spread metaphases were analysed. In samples showing absence or mosaicism for the Philadelphia chromosome 40-50 metaphases were screened. This was done to detect presence of few malignant cells and to ascertain the percentage of ph positive cells respectively. Two to five spread metaphases were photographed and karyotyped.

MICROPHOTOGRAPHY AND KARYOTYPING

Microphotography was done under x100 oil immersion using automatic exposure system of earl zeiss photo-micrographic equipment. The exposed

film was developed with kodak's developer at 20°C using standard method. Prints were developed in

standard print developer followed by fixation and washing. Karyotype analysis was done by cutting individual chromosomes from photographs of metaphase spreads (Plate land 2). The homologous pairs were arranged according to international system for cytogenetic nomenclature⁶ on a pre-designed format.

Fig. 1

Fig.1. G - banded metaphase spread showing t (9;22) (q34, q11). Arrows indicate 9q+and 22q.

RESULTS

At the time of diagnosis, the cytogenetic analysis was done in all the cases and treatment started with hydroxyurea(HU) or HU and interferon(IFN) combination chemotherapy (from 15 days- 2yrs duration). The following results were obtained.

CYTOGENETIC ANALYSIS

All 35 patients were available for cytogenetic analysis at time of diagnosis, out of 35 patients, 17

Fig. 2

Fig.2. G - banded karyotype showing t (9;22) (q34, q11). Arrows indicate der (9) and der (22).

were 100% ph positive (49.9%), 10 were 50-90% ph positive mosaicism (28.5%) and 3 were 100% ph negative (8.5%). In 14.25% of patients the cytogenetic analysis was failure at the time of diagnosis. 49.9% patients showed standard ph translocation9; 22 (q^{34} ; q^{11}).

Out of 35 patients, only 13 were available for follow-up cytogenetic analysis after 6 months. At the time of diagnosis among these 13 patients, 8 were 100% ph positive, 3 were 50-90% ph positive mosaic and 2 were 100% ph negative. Two of the mosaic patients remained unchanged and one became 100% ph positive. Of the eight 100% ph positive patients, 5 became ph mosaic with ph positivity (50-70%) and 2 patients remained unchanged, while one patients become 100% ph negative in combination chemotherapy (IFN and HU).

Cytogenetic results showed better response with combination chemotherapy (IFN+HU). With cytogenetics results, total Leucocyte count (TLC) reduced and hemoglobin increased significantly after 3 months of therapy. There was significant changes at 3rd and 6th months in haematological parameters (Fig.3). The platelet count was not

Fig. 3 : Comparison of pre and On therapy analysis of peripheral blood samples

changed significantly after commencement of therapy, where as the liver and spleen were found reduced in size (Fig.4).

Fig. 4 : Comparision of Liver and Spleen Size-pre and on Therapy

DISCUSSION

In the present study, out of 35 patients, only 13 patients were available for follow up cytogenetic analysis after 6 months. The cytogenetic hallmark of CML, the truncated chromosome No. 22 (ph chromosome) which is found due to translocation $t(9;22)(q^{34}; q^{11})$ in 90-95% cases of CML. There have been various reports showing complete disappearance of ph chromosome following therapy with Busulfan or Hydroxyurea accompanied with prolonged haematologic remission. In this study, out of 13 patients 2 were ph negative, 8 were 100% ph positive and 3 were ph positive mosaic before therapy, of the 3 mosaic patients, 2 remained unchanged after therapy and one patients became 100% ph positive. Out of eight 100% ph positive patients, 5 became mosaic with ph positivity (50-70%) and 2 patients remained unchanged, while one patients became 100% ph negative. Mosaicism in these patients may be transient and induced by treatment, in studying the ph status it has been demonstrated the uncomplicated ph in patients lymphnodes stimulated with epstein - Ban- virus. However, ph status was not studied in lymph nodes. Further, a ph reduction was considered good risk

factor.⁷ Thus, a study has shown a 30-50% ph reduction. A minority of patients with CML achieves a complete cytogenetic remission defined as disappearance of ph chromosome.⁸ In the present study one patients has shown complete disappearance of ph chromosome.

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