ORIGINAL ARTICLE

Identification of Philadelphia Chromosome by Cytogenetic Analysis in Patients of Chronic Myeloid Leukemia - A Hospital-Based Study in Eastern Nepal

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ABSTRACT

Background: Philadelphia chromosome (Ph) is a consistent chromosomal abnormality in chronic myeloid leukemia. This abnormality is caused by reciprocal translocation involving breaks on chromosome 9 and 22 and translocation of genetic material t (9; 22) (q34; q11) giving rise to oncogene breakpoint cluster region - Abelson murine leukemia. The aim of the study is to identify the Ph in diagnosed CML cases and identify the percentage of Ph in positive cases.

Materials and Methods: A total of 30 cases of chronic myeloid leukemia which were diagnosed clinically were randomly selected for the study. Bone marrow samples were collected in heparinized vials. Cytogenetic analysis was done by karyotyping. Philadelphia-positive and -negative case was identified.

Results: Ph was detected in 29 cases of 30 cases. In this study, around 96.67% were detected Philadelphia positive.

Conclusion: Conventional cytogenetic can detect Ph in more than 95% of cases. In developing countries, where more sophisticated techniques are still not available, conventional cytogenetic is the gold standard for the detection of chronic myeloid leukemia.

Key words: Chronic myeloid leukemia, cytogenetics, Philadelphia chromosome

INTRODUCTION

Chronic myeloid leukemia is a clonal myeloproliferative disorder of the primitive hematopoietic stem cells.[1] Chronic myeloid leukemia is one of the most common leukemia in Asian population.[2] CML is the first cancer in which a consistent chromosomal abnormality the Philadelphia chromosome (Ph) was found, which was described by Nowell and Hungerford in 1962. This abnormality was identified due to reciprocal translocation involving t (9;22) (q34;q11.2) and involved the fusion of genes breakpoint cluster region (BCR) and tyrosine kinase human homolog of the Abelson murine leukemia virus (ABL).[3,4] The hybrid BCR-ABL oncogene produces abnormal RNA and causes increased tyrosine kinase activity changing normal hematopoietic cells into CML cells. The Ph is described as a shorter long arm of chromosome 22.[5]

Standard cytogenetic studies of the bone marrow identify the Ph in approximately 95% of the patients with the diagnosis of CML. The absence of the CML does not exclude the possibility of CML; it requires more sensitive genetic testing.[6]

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MATERIALS AND METHODS

This study was a hospital-based study done over a period of 2 years at B.P. Koirala Institute of Health Sciences.

Patient Selection

A total of 30 clinically diagnosed case of CML were randomly selected. Laboratory reports of hematological parameters were noted. Informed consent was taken from the participating patients'. Hematological malignancies other than chronic myeloid leukemia were excluded from the study.

Methods

The bone marrow aspirate was collected in a heparinized syringe from the posterior superior iliac spine. Bone marrow aspirates were examined for both direct and short-term culture. The collected sample was stored at 4°C before culture was initiated. Three sterile vials with 5 ml of RPMI-1640 were taken, 1 ml of fetal bovine serum was added to the culture vials, and heparinize bone marrow sample was added in each vial. They were gently mixed by shaking. The vials were kept in incubator at 37° for 1 h.

After 1 h, colchicine was added to arrest the dividing cells at metaphase stage and then again incubated for 1 h at 37°C. The culture was then transferred to sterile centrifuge

tube and then centrifuged at 1000 rpm. After discarding the supernatant, the cell pellet was suspended in pre-warmed hypotonic solution (0.56% KCl) at 37° for 30 min. The cells were then fixed with fixative 3:1 methanol:acetic acid for 20 min. The slides were prepared and stained with 5% Giemsa. Minimum 20 metaphase spread was analyzed using Zeiss light microscope. Homologous pairs were arranged according to International System for Human Cytogenetic Nomenclature 1995 on a predesigned format.

RESULTS

The clinically diagnosed and hematologically proven patients were randomly selected for this study. Chromosomal study of the selected cases revealed the presence of Philadelphia positive in 29 cases of 30, 96.67% of the total study case [Figure 1].

The Philadelphia percentage was calculated in the positive cases [Table 1].

Three patients of 30 had Philadelphia percentage on the range of 90–100%.

Higher number (n = 8) of patients had Philadelphia percentage in the range of 81–90%.

Only four cases of 30 showed below 50% of Ph.

DISCUSSION

The study included 30 clinically diagnosed case of CML for identification of Ph and the percentage of Ph if the case was Philadelphia positive.

Ph is identifiable by conventional cytogenetic as consistent chromosomal abnormality in more than 90% of cases of CML.[7] A similar study done in the Suzhou province in China by Xie *et al*. detected 95% of cases of Philadelphia positive (117). In a study by Zhu *et al*., in Nanjing, China 119 karyotype analysis detected 113 case i.e 91.1% case was Philadelphia positive out of which 83.9% showed standard translocation between chromosome 9 and 22 and rest were detected with del (9) by FISH technique.[8]

The Ph resulting from balanced translocation of t(9;22)(q34;q11) is a diagnostic hallmark of the chronic myeloid leukemia. CML is the first disease that was associated with a consistent cytogenetic abnormality - the Ph.

The present study showed 96.67% of Philadelphia-positive case. Philadelphia-positive CML was detected in 94.4% of cases in the Presov region in Slovakia, by the study conducted by Boronova *et al*.[9]

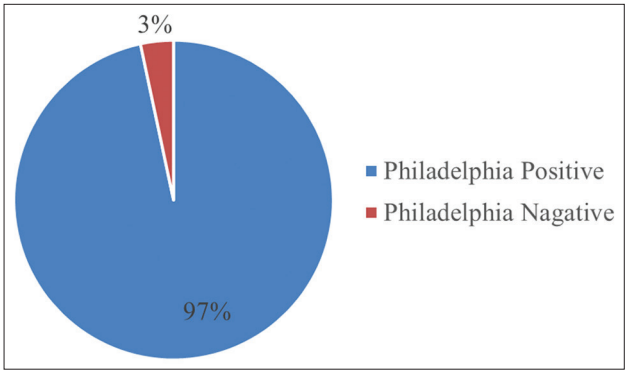

Figure 1: Pie chart showing Philadelphia-positive and -negative ratio

Table 1: Distribution of data according to age, sex, and Philadelphia percentage

Serial. no	Sex	Age	Philadelphia %
1	M	43	70
2	M	30	30
3	F	45	80
4	F	40	100
5	M	26	70
6	M	36	100
7	M	68	Negative
8	M	16	90
9	M	28	90
10	M	17	75
11	F	55	60
12	M	36	90
13	F	34	40
14	M	23	80
15	F	46	70
16	M	19	100
17	M	30	90
18	M	27	90
19	M	24	90
20	M	20	80
21	M	22	80
22	M	34	80
23	M	28	80
24	M	40	30
25	M	47	60
26	M	28	60
27	M	28	25
28	M	61	60
29	F	30	85
30	F	40	90

Within limited resources, conventional cytogenetic is still the first preference for many expertise. In a developing country, where trained professionals and highly sophisticated laboratory facilities are a distant dream,

conventional cytogenetic yields the best result to diagnose Philadelphia-positive case.

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