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Research Article

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Cytogenetic Analysis of Chronic Myeloid Leukemia in an Eastern Indian Population

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Abstract

Chronic myeloid leukaemia (CML) is a common translocation known as BCR-ABL1, also referred to as the Philadelphia chromosome, that affects the primitive hematopoietic stem cell. The GLOBOCAN 2020 report estimates that there will be roughly 19.3 million new cancer diagnoses and 10 million cancer-related deaths in 2020. A fusion oncoprotein called BCR-ABL1, a constitutively active tyrosine kinase that is essential for the development of CML, can be inhibited to slow the course of the illness. The bone marrow and peripheral blood pile up different forms of immature and mature granulocytes or blast cells. In the previous study of CML in India, the annual incidence was reported to be 0.8 to 2.2 per 100,000 population. In leukemia, the most, common type is CML in India. Therefore, chromosomal analysis of CML plays an essential aspect in diagnosing leukemia patients. In the current study, 130 CML cases from the Eastern Indian population ranging in age from 7 to 79 years were cytogenetically analysed (mean of 36). In the study population, the male to female ratio was 1.24:1, with 72 males (55.38%) and 58 women (44.61%) participating. 122 (93.7%) of the 130 cases could be successfully karyotyped, whereas 8 (6.3%) failed culture tests. Out of the 130 cases that were noted, 25 (19.25%) had karyotypes that were normal, while 97 (74.61%) had the Philadelphia (Ph') chromosome, which has the distinctive translocation t(9;22); Ph'+ve. Furthermore, we conclude that while more advanced molecular techniques cannot entirely replace cytogenetics, which continues to be the focus of laboratory studies into the condition, they can be used in conjunction with it to help diagnose of chronic myeloid leukemia.

Introduction

A heterogeneous grouping of stem cell illnesses is encompassed by chronic myeloid leukaemia (CML), a hematopoietic somatic stem cell disorder. Body morphological anomalies, blood problem confirmation, and an accumulation of all types of mature and immature granulocytes or blast cells are its defining characteristics. In 2018, there were one new case of CML diagnosed globally for every 100,000 people, or 15% of all adult leukaemia cases [1]. According to a recent study, India's annual incidence of CML ranged from 0.8 to 2.2 per 100,000 people [2]. This is the most frequent type of leukemia in India, accounting for 30% to 60% of all leukemia [3]. The indication of CML in cytogenetics is the Philadelphia chromosome (Ph') initially observed in David Hungerford. The Ph' chromosome is found in approx 91-96% of CML cases. It is a balanced reciprocal translocation between the long arm of chromosome 9 and 22, t(9;22) (q34;q11) [4]. There are two genes involved in Ph' chromosome BCR (Break point Cluster Region) and ABL(Abelson) tyrosine kinase gene found in chromosome 9 and the breakpoint cluster (BCR) gene located in chromosome 22. This is translated into a fusion protein of different sizes major, minor and micro like p 230, p210, p-190. The molecular importance of this translocation is the generation of the BCR-ABL1 oncogene that cipher the chimeric BCR-ABL1 protein with built-in kinase activity [5]. Thus, the current study sought to detect chromosomal abnormalities in the Indian population using traditional cytogenetic analysis, as well as to determine their prevalence and frequency in the Indian context.

Material and Methods

Reagents

RPMI-1640 was Procured from Himedia, Fetal bovine serum (FBS), trisodium citrate, Giemsa stain were purchased from Sigma Aldrich (St. Louis, USA). In addition, trypsin, Pen Strep (Penicillin and Streptomycin), Colchicine were obtained from (Thermo Fisher Scientific, Inc.) phytohemagglutinin (PHA) from Invitrogen, KCl (Merck).

Sample Collection

Peripheral blood and bone marrow sample were collected in a sterile heparinized vial and transported within the laboratory as soon as possible from Sir Sunderlal Hospital, Banaras Hindu University, Varanasi, India. The ethics committee of the Institute

of Science, Banaras Hindu University (Ref. No: I.Sc./ECM-XII/2021-22) granted institutional approval for publishing, and signed informed consent was acquired from the patient/participant. The culture was set up by adding 400 microlitter of whole blood to 6.5 ml of RPMI culture media 700 microlitter bovine serum, 100 micro litre phyto hematoagglutinin (PHA). After 70 Hrs, we add cholchine for metaphase arrest and then harvest the cell following standard protocol.

Preparation of Metaphase and G-banding Staining

Mark and clean the slide, then resuspend the cells in a little amount of fixative and drop the suspension onto a cleaned microscope slide drop by drop in two places, demonstrating the heat flame for stabilising the cell's metaphase. Stain the slide in plain as well as G-banding. Giemsa stain is specific for the phosphate groups of DNAs. It binds to DNA areas with high levels of adenine-thymine bonding. Giemsa stain is used to stain chromosomes in Giemsa banding (G-banding), and it is frequently employed to construct a diagrammatic representation of chromosomes. Add 5 ml of Giemsa stain, 1 ml methanol and 44 ml of Giemsa water mix by pasture pipette. Inoculate the prepared slide in the Giemsa stain jar for 10-13 minutes, wash the slide in distilled water three times, clean the slide with tissue paper on the back side of the slide, dry and mount the slide by paramount with coverslip.

Microscopy

A total of 30 metaphases were taken with a microscope and karyotyping was performed with 450 G-banding resolution using Ikaros karyotyping system-Metasystem software (Carl Zeiss Microscopy Gmbh, Göttingen, Germany). The International System for Human Cytogenetic Nomenclature was used to evaluate the chromosomes (ISCN 2016).

Result

A total of 130 suspected CML patients residing in the Eastern Indian population like Bihar, Jharkhand, West Bengal, and Uttar Pradesh. This study was conducted from May 2019 to June 2022. The Ph' Chromosome of suspected male and female cases was found 55.38 % male and 44.61% female. Age and Sex distribution of our study shows in Table-1.

Table 1: Age and Sex Distribution of the 130 CML Patients in our study

CML Type		No. of Patients		Age in Years		Sex	
	(%)	5-25 (%)	26-50 (%)	51-75 (%)	>75 (%)	Male (%)	Female (%)
Ph' +ve	97 (74.61)	43 (33.07)	60 (46.15)	26 (20.00)	1 (0.76)	54	44
Ph'–ve	25 (19.23)	09 (6.92)	11 (8.46)	04 (3.07)	0	13	11
Culture Failed- 8		08					

Where we found 97 cases are Ph' positive and 25 cases are negative and 8 cases have failed in culture. Percentage of Philadelphia positive and negative in CML patients shows in Fig-1.

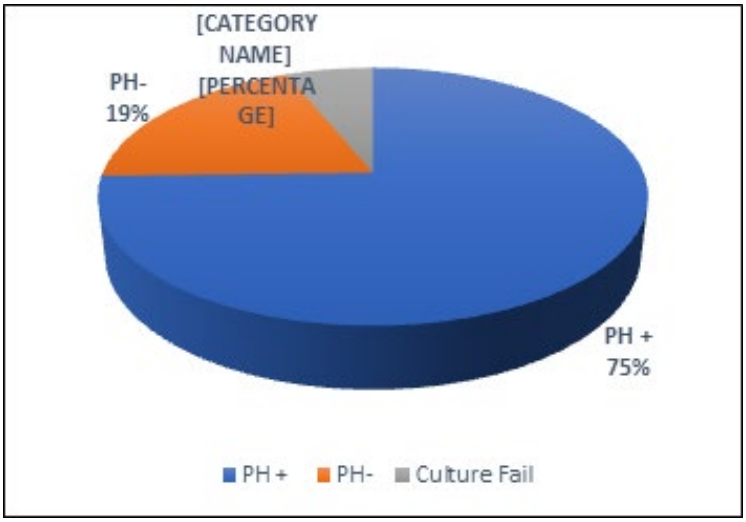

Figure 1: Percentage of Philadelphia Positive and Negative in CML Patients

While in most of the studied showed CML as most common type of leukemia as well as also reported higher percentage of frequency [6, 7]. Male to female ratio in patients with CML in our study was 1.24:1, shows in Fig-2.

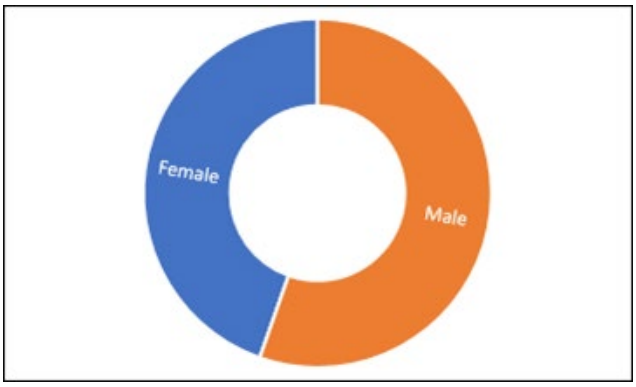

Figure 2: Analysis of Confirmed Philadelphia Cases Male and Female, Male Is More Affected Than Female

Which was similar to most of the studies from a different region of India like (2.3:1), (2:1), (2.4:1), (1.4:1). Other study carried out by also showed a higher ratio than our study (3:1). Studies carried out at the same hospital by (1.6:1) [8-15]. The exact cause of this variation is unknown. Still, it may be attributed to geographical, environmental or genetic factors. The range of age of our study is (7-79) Years and mean age of the diagnosis of CML cases in our study was 36.38 years. Most of the studies from different region of India range of age study like (31-45) and (31-75). Other study carried out by also showed range of age study (12-81). Studies carried out at the same hospital by (1.6:1). Distribution of CML cases in our study was seen between 7-79 years of age which was quite different from other studies. It might be due to childhood CML cases in our study. There were 8 cases reported in <20 years of age in our study out of 130 cases of CML. Most common age group in our study in CML cases were diagnosed between 25-35 years which was lower than study by (31-40 years) and by (31-45 years) and more similar to (16-30 years). Comparison of our data to various studies from the different geographical regions in India is shown in table-2.

Table 2: Comparison of our Data to Various Studies From Different Geographical Region In India

Region	Study	(Period of study)	No of case different leukemia (n)	CML
Calcutta	Chatterjee *et al.*[23] 1962	(1949–1961)	544	35.9
Mumbai	Advani *et al.*[24] 1979	(1960–1975)	1126	40
Pondicherry	Prakash *et al.*[25] 1981	(1970–1979)	278	30.8
Delhi	Rani *et al.*,[26] 1982	(1970–1979)	490	45.3
Kerala	Verghese *et al.*,[27] 1984	(1980–1983)	1016	16.4
Lucknow	Kushawaha *et al.*,[28] 1978	(1971–1984)	970	48
Chandigarh	Shome *et al.*,[29] 1985	(1975–1983)	820	36.7
Mumbai	Dicosta *et al.*,[30] 1989	(1975–1984)	242	38
Haryana	Rathee R *et al.*,[31] 2014	(2008-2012)	650	39
Loni, Maharashtra	Bhaviskar JB.[32] 2016	(2006- 2011)	156	33.97
Bhopal	Ahirwal R *et al.*, [33] 2018	(2013-2014)	73	47.97
Haryana	Singh G *et al.*[34] 2016	-(5 years)	356	28.3
Kamothe, Navi Mumbai	Jain A *et al.*[35] 2017	2014-2015	100	54
Nagpur	Salkar *et al.*[36] 2014	-(2 years)	n=110	30/110
Ranchi	Prasad C *et al.*,[37] 2017	(2009-2010)	46	34.7
Jammu	Kaur A *et al.*,[38] 2018	2017- 2018	60	26.66
Varanasi	Rajendra S *et al.*,[39] 2001	1985-2000	2057	28.29
Centre for Genetic Disorders, BHU	Our study, 2022	May 2019- June 2022	-	130

Karyogram of a Philadelphia chromosome human male and female karyotype shown in Fig.3. and Fig.4.

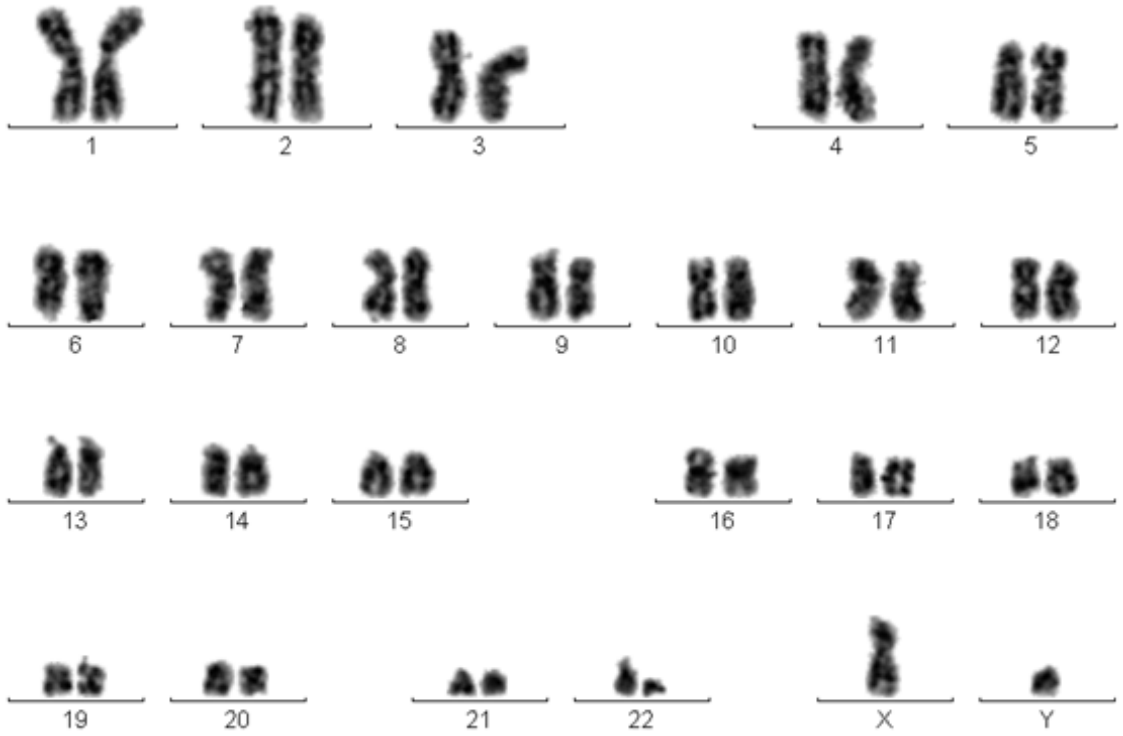

Figure 3: Karyogram of a Philadelphia Chromosome Human Male Karyotype

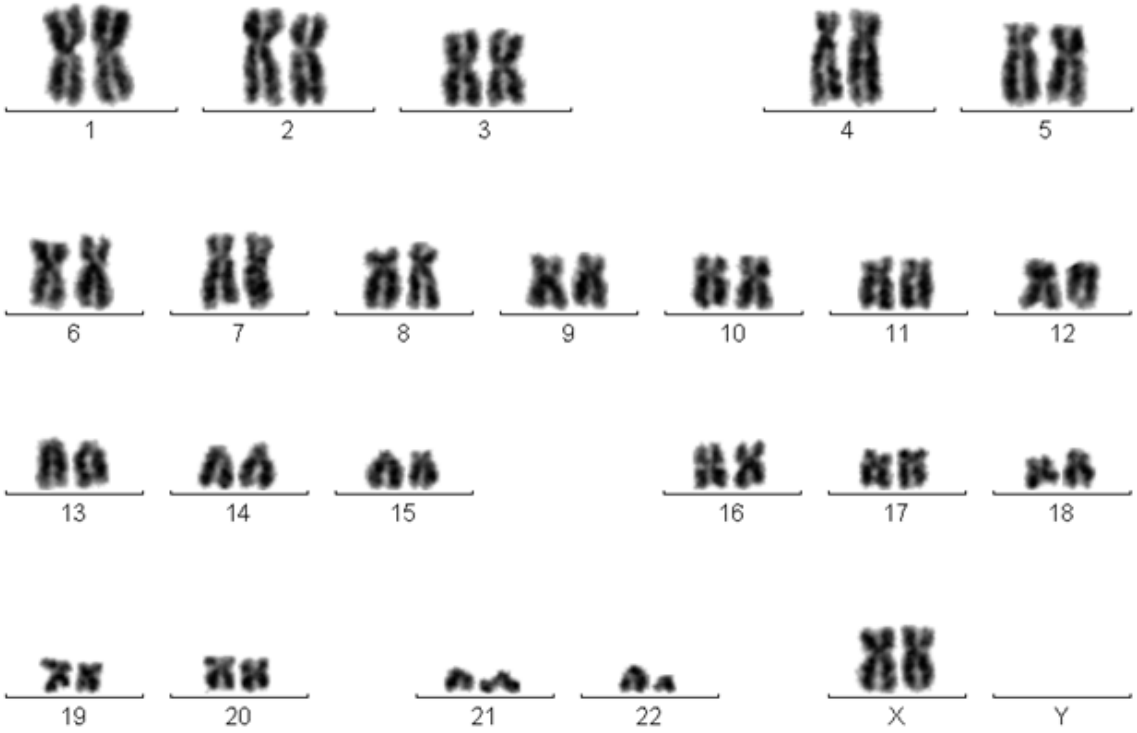

Figure 4: Karyogram of a Philadelphia Chromosome Human Female Karyotype

Discussion

Cytogenetic analysis is an important in CML diagnosis and serves as a prognostic indicator for continued care. The frequency has increased during the last few decades. As a result, CML is becoming more widely recognised throughout the world. The current research attempts to detect numerous Cytogenetic anomalies in CML and their prevalence in the Indian population. In this study, 130 cases were evaluated for cytogenetic analysis of suspected Ph' chromosomal abnormalities in CML patients referred to the Centre for Genetics Disorders, BHU, from Sir Sunder Lal hospital BHU, Varanasi, India. We observed Ph' chromosome was the most common chromosomal abnormality in hematological malignancies. Also reported, the frequency of Philadelphia chromosome was 10.20 % in 2019, which analyses 489 patients in different clinical stages of CML in India [16]. In our study most of the patients were presented in chronic phase (75.9%) which was comparatively lower than other studies like (90/98 cases) [17-39].

Different studied have been conducted in other regions of India, and after analyzing various studies finding. We found interesting finding that the incidence of CML was more common in Northern India.

Declaration of Patient Consent

The authors affirm that they got all necessary patient consent paperwork. The patient(s) has/have given written permission for their images and other clinical data to be published in the journal.

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Conflict of Interest

According to the authors, there are no claimed conflicts of interest.

Authors Contributions

AK: Conceptualization, Manuscript writing, Experiments performed and Data analysis. VT: Sample collection, Manuscript writing, primary inputs AA: Conceptualization the hypothesis, significant inputs, correspondence, corrections in the manuscript.

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