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PHILADELPHIA CHROMOSOME IN PRETHERAPEUTIC CASES OF CHRONIC MYELOGENOUS LEUKEMIA

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ABSTRACT: Chronic myeloid leukemia (CML) is a clonal myel0 proliferative disorder due to neoplastic transformation of myeloid stem cells. The characteristic Philadelphia Chromosome translocation t(9:22) (q^{34}: q^{11}) juxta poses the c-abl oncogene from chromosome 9 with break point cluster region (bcr) on chromosome 22 resulting in the generation of aberrant bcr/abl transcripts. The abnormal bcr/abl tyrosine kinase gene product has enhanced activity compared to the wild type c-abl tyrosine kinase and is believed central to the pathogenesis of CML. Most of the patients in chronic phase who are treated with cytotoxic agents eventually develop a fatal blast phase that is the end stage in which the patient usually fails to respond combination chemotherapy and only treatment remain after that is allogenic bone marrow transplantation. Since the philadelphia chromosome is the most consistant abnormality in CML patients and as it possess both diagnostic as well as prognostic importance, it's detection is mandatory to establish the diagnosis of CML and for planning of chemotherapy. Our study is a preliminary work to re-establishing the finding of previous workers and to detect out any other associated or new reccuring chromosomal anomalies in CML patients. The bone marrow samples of 89 CML patients (54 males & 35 females) diagnosed clinically and pathologically were collected in heparinised RPMI-1640 culture media aseptically with informed consent from patients and their guardian in Hematology section of pathology Dept. during the time period from Nov-2006 to May 2008. But the most common additional structural chromosomal aberration found here was t(15:17), which is relatively rare in available literature. To establish this as a newly recurring additional chromosomal aberration in CML patients, large database analysis is required.

INTRODUCTION: **C**hronic Myelogenous Leukemia (CML) is a clonal expansion of haematopoeitic progenitor cells characterised clinically by myeloid hyperplasia, leukocytosis with basophilia and splenomegaly.

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The cytogenetic hall mark of CML is the philadelphia (Ph) translocation t(9:22) (q^{34}:q^{11}), resulting in P^{210} BCR-ABL which is essential for the development of the disease. In 1960, Nowell and Hungerford described the characteristic philadelphia chromosome associated with CML [1]. The first recurrent chromosomal abnormality associated with a specific human malignancy. They reported that myeloid cells from patients with CML showed a deletion of long arm of chromosome 22. Subsequently, the translocation t(9:22) was defined. Present work is a cytogenetic study on CML patients to detect philadelphia chromosome

and other associated structural and numerical chromosomal aberrations, before any therapy introduced to them. For this bone marrow samples were collected and analysed by direct harvesting and giemsa banding. Karyotyping was done and the findings were compared and correlated with that of previous workers.

MATERIAL & METHODS:

The study was carried out at S.C.B Medical College, Cuttack in the Dept. of Anatomy in collaboration with Hematology section of Pathology, Dept. for sample collection and Telemedicine Dept. for microphotography. In the present study, bone marrow from CML patients were sampled for analysis. The samples were collected from both male and female patients coming to Hematology Section of Pathology Department of S.C.B Medical College, Cuttack. Patients within the age group of 2 - 70 yrs have been analysed for this study.

Criteria for selection of Patients:

The patients were selected on the basis of clinical finding such as hepatosplenomegaly, unexplained anemia, and pyrexia of unknown origin along with pathological feature of leukocytosis, basophilia and presence of blast cells in peripheral Smear. Detailed inquiry about family history was done and informed consent was taken from the patients for cytogenetic analysis.

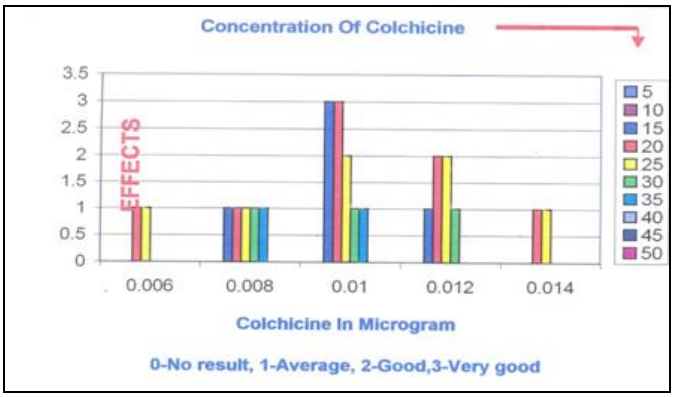
Cytogenetic Study:

The following reagents were used for the present study.

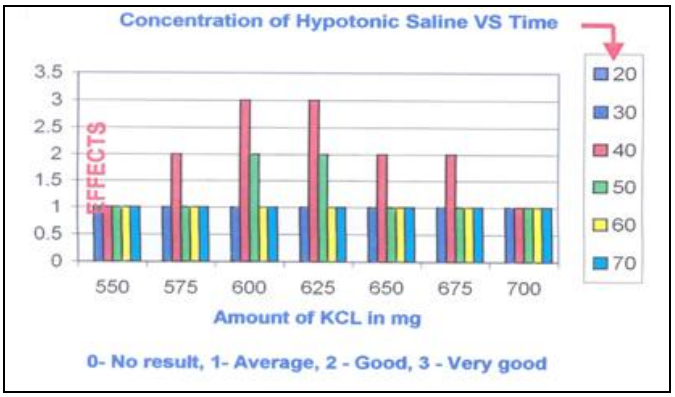
Anticoagulant: Heparin solution was used as anticoagulant in the dose of 100 IU per ml of bone marrow. Heparin was added to the culture medium and mixed thoroughly before sample collection.

Culture Media: Heparinized RPMI 1640 culture media with L. glutamine was used for sample collection. The necessary micronutrients and essential amino acids required for the cell survival are present in this culture medium.

Spindle Inhibitor: Colchicine was used as spindle inhibitor which arrest the mitosis at metaphase stage. This result in accumulation of chromosomes in rappidly dividing cells. For better results 0.005 mg of colchicine was added in 10ml of distilled water from which 0.2ml was added to culture and was kept for 20 minuites (**Graph 1**). Hypotonic Solution: Hypotonic solution in compare to that of cytoplasm of normal cell is used as a pre treatment agent. Due to endosmosis, water from the hypotonic solution entered to the cytoplasm of cell through semipermeable plasma membrance, resulting in swelling of the cells and subsequent dispersion of the chromosomes. Potassium chloride was used in a concentration of 0.600mg for the above purpose (**Graph 2**).



GRAPH 1: THE CONCENTRATION OF 0.01 MICROGRAM WITH 15-20 MINUTES EXPOSURE GIVES VERY GOOD RESULT



GRAPH 2: THE BEST EFFECTS ARE OBSERVED IN THE CONCENTRATION OF 600-625 MG% OF KCL FOR 40 MINUTES IN OUR STUDY.

Fixatives: For the preservation of the cell and their contents in original form and to make them stable during further processing, fixatives were used. In the present study the reagents used as fixatives were glacial acetic acid and absolute methyl alcohol in the ratio of 1:3.

Staining: In above study conventional Giemsa stain was used for the staining of slides. This Giemsa stain was prepared from stock solution by

addition of 2ml of stock concentrate to 38 ml of Phosphate buffer (pH -7.2). The prepared stain was filtered using Whatman filter paper. This filtered stain solution was added over air dried slide for five minutes after which slides were rinsed with distilled water twice and again kept air dried. The stained slides were screened for wide spread metaphase.

G. Banding: After dropping of cell suspension over hot and moist slides, they were air dried and kept in incubator at (37ºC) for 7days for maturation. This matured slides after 7days incubation were treated with 0.05 mg% of trypsin (1:250) solution. Chemical denaturation of A=T and C G bonds in between polynucleotide chains occured at places as a reasult of treatment with proteolytic enzyme (trypsin). On Giemsa staining to this trypsin treated slides, alternate light and dark bands of chromosomes were visible under microscope and now the slide are called as banded slide and the procedure is G. Banding.

Cytogenetic analysis:

The Bone Marrow was aspirated aseptically from Posterior Superior illiac Spine of CML patient by sterilized bone marrow niddle and a 10ml disposable Syringe and 0.5 - 1 ml of the sample was transferred immidiately to a culture bottle containing 5 ml of preheparinized RPMI 1640 culture media. Then the bone marrow was thoroughly mixed with the culture media by shaking. This bone marrow in culture media was then subjected for direct harvesting. To the culture bottle containing bone marrow and heparinized RPMI 1640 Culture media, colchicine was added in a dose of 0.002 mg/ ml of bone marrow and mixed thoroughly. It was then kept in incubator at 37ºC for 20 minutes.

After 20 minutes of incubation the culture bottles were removed and samples were transferred to clean and sterilized centrifuse test tube. The test tubes were marked by marker pencil and then subjected for centrifugation in 900 rpm for 9 minutes. The supernantant was discarded with a micropipette and the remaining cell pellet was broken properly by fingers to make a homogenous cell suspension. The hypotonic salt solution of 0.06 % KCL was added to the above homogenous cell suspension and mixed thoroughly with the help of micropipette. This hypotonic treated cell suspension was kept in incubator at 37ºC for 40 minuites to swell up the cells. The swelled cells after incubation was centrifused for 9 minutes at 900 rpm and supernatant fluid was removed. The cell pellet thus obtained was broken slowly and carefully to avoid rupture of fragile cell membrance of swollen cells and resuspended in 0.5ml of same fluid. To the above solution, chilled fixative (3 Parts of methyl alcohol, 1 part of glacial acetic acid) was added slowly drop wise along the wall of the inclined centrifuse test tube to the cell pellet with a micropipette and kept for 10 min at room temperature.

The chilled fixative treated cell suspension was again mixed with 5 ml of fresh fixative and centrifused for 9 minutes at 900 rpm. After removing Supernatant fluid the remaining cell pellet was broken properly to make a homogenous cell suspension to which 0.5ml of fresh fixative was added for resuspension and kept for 2hrs in refrigerator. After 2hrs the resuspended cell suspension was mixed with 5 ml of fresh fixative and centrifused for 9 minutes in 900 rpm. The Supernatant was discarded and the remaining cell pellets were broken properly to get homogenous cell suspension. 5 ml of fresh fixative was added to the suspension and recentrifused after thorough mixing. This above procedure was repeated for 3-4 times till a clear white cell pellet was obtained. 0.5 ml of fresh fixatives was added and mixed to obtain a homogenous cell suspension. This clear cell suspension was now ready for dropping.

Slide Preparation:

Air drying method for slide preparation was followed for this study. New clean and grease free slides were used for dropping. 2-3 drops of fixative treated cell suspension were dropped on hot and moist slide from the height of 20-24 inches. The slides were kept in vertical position with slight inclination during dropping and also kept in same position after dropping. The dried slides were coded properly by marker pencil.

Banding Protocol:

The coded slides were kept inside the incubator at 37ºC for 7days for maturation. The mature slides

were treated with trypsin solution (50mg trypsin in 100 ml distilled water) in a cuplin jar for 20 seconds. The trypsin digested slides were rinsed with 0.9 % NaCl solution twice which were kept in two cuplinjars. The slides were then washed with distilled water in a cuplin jar and stained with 2 ml of 4% Giemsa stain. The stain was poured over the slide and kept for one minute after which 2 ml of distilled water was added and a mixed thoroughly and allowed for air drying. The stained air dried slides were examined under light microscope for Screening and selected for microphotography.

During taking microphotograph each slides were screened for 15-20 well spreaded metaphases out of which five of them were photographed. One out of 5 photographs having minimal over lapping was karyotyped.

Karyotyping:

For this study, conventional ISCN-91 (International System For Human Cytogenetic Nomenclature) and Human chromosome (2^{nd}edition) was reffered. Karyotyping was done manually basing on the following criteria. Lenth of the chromosome, Position of the centromere, Presence of satelliate bodies, banding pattern. The results were confirmed by microscopic examination and photographic observation. More importance was given to direct microscopic observation, as the photographs are less reliable than direct observation.

RESULT: Chronic myeloid leukemia CML is a clonal myeloproliferative disorder of the pluripotent haematopoietic progenitor cells characterized by excessive proliferation of marrow granulocytes and presence of philadelphia chromosome. Since the presence of philadelphia chromosome is an important criteria for the diagnosis of CML and also gives prognostic information about its chemotherapy, CML patients are usually subjected for cytogenetic analysis for the detection of philadelphia chromosome. In the present study 89 CML patients diagnosed clinically and pathologically were analysed cytogenetically. Out of 89 cases 54 were males and 35 were females. The male to female ratio was 1.54:1. In this stydy bone marrow samples were collected from the patients with informed consent. In case of minor or seriously ill patient, consent was taken from the parents or from their guardian. Cytogenetic analysis of all the patients were done according to the standard protocol (ISCN-1991) [2]. The chromosomes were banded, karyotyped and studied in detailed for structural and numerical aberration.

The detection of philadelphia chromosome along with presence of other chromosomal aberrations were taken into account in the present study. In the expression of the results, the patients having similar findings were grouped and arranged in tables, interpreted in ba1r charts and represented by pie diagrams for comparision.

TABLE 1: PHILADELPHIA CHROMOSOME POSITIVITYIN CML PATIENTS

Chromosomal Aberration	No of Cases	Percentage
Philadelphia Positive	72	81%
Philadelphia Negative	17	19%
Total	89	100%

This **Table 1** shows the incidence of philadelphia chromosome in clinically and pathologically diagnosed CML patients. In the present study 89 cases of clinically and pathologically diagnosed patients were analysed cytogenetically and karyotyped. Out of 89 patients 72(81%) cases have translocation t(9:22), i.e. philadelphia positive. Among these 72 Ph positive cases, some are only Ph Positive without any other chromosomal aberration, while some others have additional chromosomal aberrations along with the presence of philadelphia chromosome. They may be either structural or numerical aberrations. The remaining 17 (19%) patients are cytogenetically philadelphia negative. Again among this group some patients showed other structural and numerical aberrations, while rest arekaryotypically normal.

TABLE 2: AGE SPECIFIC INCIDENCE AND MALE FEMALE DISTRIBUTIONIN CML PATIENTS DIAGNOSED PATHOLOGICALLY.

Age	Male	Female	Male To Female Ratio	Total
0-20 yrs	2	2	1:1	4
21-40yrs	9	6	1.5:1	15
41-60yrs	25	16	1.56:1	41
>60yrs	18	11	1.63:1	29
Total	54	35	1.54:1	89

This **Table 2** shows the Age and Sex distribution of the pathologically diagnosed CML patients. In the

present study 54 males and 35 females are analysed. The male to female ratio is 1.54:1. The patients are within 2-70yrs of age. Median age is 47.5yrs. In the age group of 0-20yrs, there are 4(4.5%) patients. Out of them 2 are males and 2 are females having the male to female ratio 1:1.15(17%) patients are within the age group of 21-40yrs. 9 males and 6 females with male to female ratio of 1.5:1 are present in this group. Maximum patients (46%) are within the age group of 41-60yrs. With 25 males and 16 females this group comprises 41 patients. Male to female ratio is 1.56:1. The no. of patients having age more than 60 yrs are 29 (32.5%). There are 18 males and 11 females patients in this age group. The male to female ratio is 1.63:1.

TABLE 3: CYTOGENETIC OBSERVATION VS AGE DISTRIBUTION

Chromosomal Aberration (Ph +ve as only)	0-20yrs	21-40yrs	40-60yrs	>60yrs	Total
Karyotypic anomaly	2	7	18	3	30
Ph -ve with normal Karyotype	0	2	5	2	9
Ph +ve with other aberration	2	5	15	20	42
Ph -ve with other aberrations.	0	1	3	4	8

This **Table 3** shows the distribution of different chromosomal aberrations in different age groups. There are 30(34%) patients having philadelphia chromosome positive as the only karyotypic anomaly. Among them 2 patients are in the age group of 0-20 yrs, 7 patients are within 21-40yrs, 18 patients are within 40-60yrs and 3 patients have age more than 60yrs.In the present study 9(10%) patients are Karyotypically normal. They have neither the philadelphia chromosome nor any other chromosomal anomaly. In this group 2 patients are within 21-40yrs, 5 patients are within 40-60yrs and 2 patients have age more than 60yrs.

Maximum number of patients have the philadelphia chromosome positive along with other chromosomal aberrations. Out of 42(47%) patients of this group, 2 patients are below 20yrs, 5 patients are within 21-40yrs, 15 patients are within 41-60yrs and 20 patients have age more than 60yrs. Though 8 (9%) patients have ph -ve, they have some other chromosomal aberrations. One patient of this group has age within 21-40yrs, 3 patients have age within 40-60yrs and 4 patients have age more than 60yrs.

TABLE 4: CYTOGENETIC OBSERVATION VS MALE FEMALE DISTRIBUTION

Chromosomal Aberration	Male	Female	Total
Ph +ve as only Karyotypic anomaly	18	12	30
Ph -ve with normal karyotype	5	4	9
Ph +ve with other chromosomal anomaly	25	17	42
Ph -ve with other aberration	6	2	8
Total	54	35	89

This **Table 4** shows the male female distribution in different types of chromosomal aberrations of CML patients. Out of 30(34%) patients showing philadelphia chromosome as only Karyotypic anomaly, 18 are males and 12 are females. Male to female ratio is 1.5:1.

5 males and 4 females with male to female ratio of 1.25:1 are present in the group of CML patients having normal Karyotype. Among the 42(47%) patients having additional chromosomal aberrations along with philadelphia chromosome, 25 are males and 17 are females. The male to female ratio is 1.47:1.

8 patients are there in the group of CML patients where philadelphia chromosome is absent but other chromosomal aberration present. In this group there are 6 males and 2 females with male to female ratio 3:1.

TABLE 5: CYTOGENETIC OBSERVATION VS MEAN & MEDIAN AGE DISTRIBUTION

Chromosomal Aberration	Age Range	Mean Age	Median Age
Ph +ve as only Karyotypic anomaly	2-68	42.5	46
Ph -ve with normal karyotype	36-65	50.5	53
Ph +ve with other chromosomal anomaly	8-70	52.7	58.5
Ph -ve with other aberration	38-69	55	58
Total	2-70	50	54

The above **Table 5** describes the mean & median age distribution among the different types of chromososmalaberration. In the present study 89 patients are undertaken within 2-70yrs of age.

Mean age is 50yrs and median age is 54yrs. The patients having philadelphia chromosome as only karyotypic anomaly are within 2-68yrs of age having mean age 42.5yrs and median age 46yrs.

The CML patients having normal karyotype are within 36-65yrs having mean and median age 50.5yrs and 53yrs respectively. Other chromosoamal aberrations present along with philadelphia chromosome in a group of patients within 8-70yrs having mean age 52.7yrs and median age 58.5yrs.The Ph -ve CML patients having other chromosomal aberrations were within 38-69yrs with mean and median age 55yrs and 58yrs respectively.

TABLE 6: CML PATIENTS HAVING PHILADELPHIA CHROMOSOME AS ONLY KARYOTYPIC ANOMALY.

Sl. No	Case No.	Age	Sex	BM No.	Karyotype	No of abnormal Karyotype per 20 metaphases	Remark
1	1	29	M	176/2006	t(9:22)46XY	15/20	75%
2	3	47	M	188/2006	t(9:22)46XY	16/20	80%
3	8	57	F	219/2006	t(9:22)46XX	17/20	85%
4	9	2	M	224/2006	t(9:22)46XY	13/20	65%
5	13	23	M	249/2007	t(9:22)46XY	14/20	70%
6	14	66	F	256/2007	t(9:22)46XX	18/20	90%
7	16	45	M	269/2007	t(9:22)46XY	16/20	80%
8	18	48	F	284/2007	t(9:22)46XX	16/20	80%
9	19	22	F	290/2007	t(9:22)46XX	14/20	70%
10	22	44	M	312/2007	t(9:22)46XY	15/20	75%
11	29	68	F	41/2007	t(9:22)46XX	17/20	85%
12	35	28	F	86/2007	t(9:22)46XX	15/20	75%
13	38	49	M	108/2007	t(9:22)46XY	15/20	75%
14	43	47	F	146/2007	t(9:22)46XX	14/20	70%
15	49	41	M	185/2007	t(9:22)46XY	13/20	65%
16	51	49	F	199/2007	t(9:22)46XX	14/20	70%
17	52	24	M	208/2007	t(9:22)46XY	13/20	65%
18	55	43	M	238/2007	t(9:22)46XY	15/20	75%
19	57	63	M	256/2007	t(9:22)46XY	17/20	85%
20	61	53	M	281/2007	t(9:22)46XY	16/20	80%
21	62	45	M	288/2008	t(9:22)46XY	15/20	75%
22	63	55	F	295/2008	t(9:22)46XX	14/20	70%
23	68	39	F	324/2008	t(9:22)46XX	14/20	70%
24	71	23	M	341/2008	t(9:22)46XY	13/20	65%
25	73	52	M	356/2008	t(9:22)46XY	15/20	75%
26	77	52	F	20/2008	t(9:22)46XX	16/20	80%
27	81	42	M	49/2008	t(9:22)46XY	16/20	80%
28	84	18	F	68/2008	t(9:22)46XX	13/20	65%
29	87	43	F	90/2008	t(9:22)46XX	14/20	70%
30	89	60	M	106/2008	t(9:22)46XY	16/20	80%

In the present study 30(34%) patients have philadelphia chromosome as only Karyotypic anomaly (**Fig. 1 & 2**). These patients are within 2-68yrs of age and median age is 44.5yrs. 60% patients are within 40-60yrs. Male to female ratio is 1.5:1. It is observed from the above **Table 6** that the number of abnormal karyotype per 20 metaphases is more in advance age.

TABLE 7: CML PATIENTS WITH NORMAL KARYOTYPE

Sl. No	Case No.	Age	Sex	BM No.	Karyotype	No of normal Karyotype per 20 metaphases.	Remark
1	7	54	M	212/2006	46 X Y	17/20	85%
2	17	65	M	275/2007	46 X Y	15/20	75%
3	41	40	M	132/2007	46 X Y	18/20	90%
4	48	56	M	179/2007	46 X Y	16/20	80%
5	54	48	F	226/2007	46 X X	17/20	85%

6	66	41	M	319/2008	46 X Y	17/20	85%
7	72	62	F	346/2008	46 X X	14/20	70%
8	78	36	F	28/2008	46 X X	18/20	90%
9	83	53	F	62/2008	46 X X	17/20	85%

Though philadelphia chromosome is chief criteria for diagnosis of CML, it is not always found. In the present study 9 CML patients are karyotypically normal (**Fig. 3 & 4**).

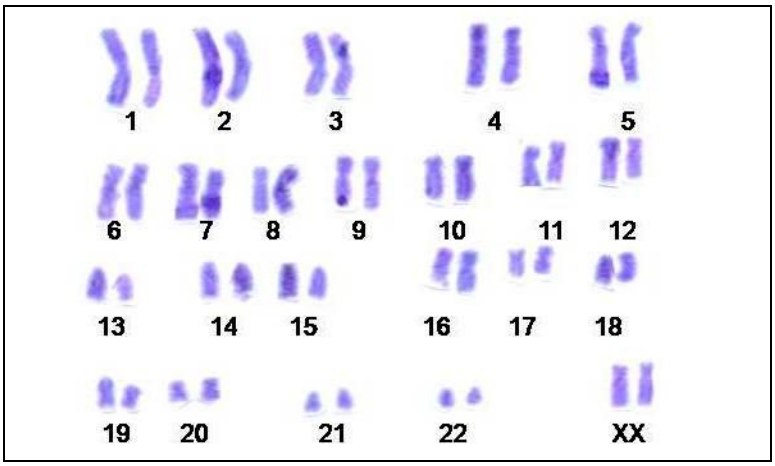


FIG.3: G. BANDED KARYOTYPE OF BONE MARROW CELLS OF FEMALE CML PATIENT SHOWING NORMAL KARYOTYPE 46XX.

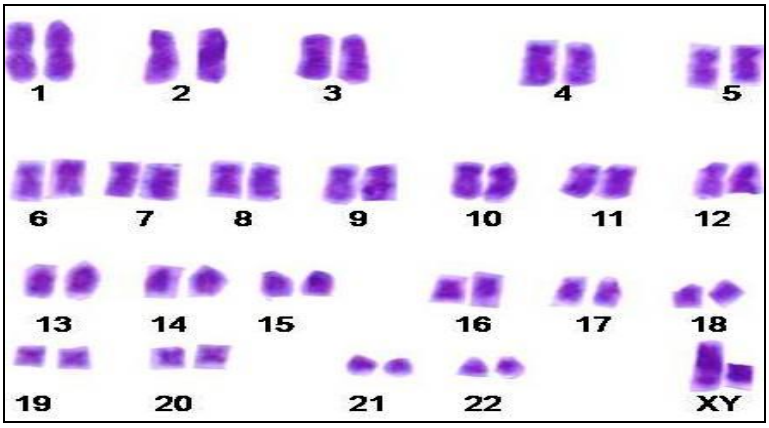


FIG.4: KARYOTYPE OF BONE MARROW CELLS OF MALE CML PATIENT SHOWING NORMAL KARYOTYPE 46XY.

These patients are within 36-65 yrs of age. Median age is 53yrs. Male to female ratio is 1.25:1. In the above **Table 7** it is observed that number of normal Karyotype per 20 metaphases is more in relatively younger age group.

TABLE 8: PHILADELPHIA POSITIVE PATIENTS WITH OTHER NUMERICAL ABERRATIONS.

Sl. No.	Case No.	Age	Sex	BM No	Karyotype	No of abnormal Karyotype per20 metaphases	Remark
1	2	42	F	182/2006	t(9:22)47XX+8	16/20	80%
2	5	21	M	201/2006	t(9:22)45XY-7	13/20	65%
3	12	61	F	241/2007	t(9:22)47XX+8	17/20	85%
4	15	56	F	262/2007	t(9:22)45XX-4	16/20	80%
5	20	68	M	296/2007	t(9:22)47XY+8	18/20	90%
6	23	58	F	322/2007	t(9:22)45XX-7	16/20	80%
7	26	67	F	19/2007	t(9:22)46XX+8	17/20	85%
8	28	24	F	34/2007	t(9:22)44XX-3	13/20	65%
9	30	58	M	49/2007	t(9:22)47XY+8	16/20	80%
10	32	64	M	66/2007	t(9:22)45XY-7	17/20	85%
11	33	50	M	72/2007	t(9:22)47XY+8	15/20	75%
12	36	51	F	94/2007	t(9:22)44XX-7	15/20	75%
13	39	64	M	114/2007	t(9:22)47XY+8	17/20	85%

14	44	30	F	151/2007	t(9:22)47XX+8	15/20	75%
15	46	12	M	167/2007	t(9:22)47XY+8	13/20	65%
16	50	67	M	192/2007	t(9:22)45XY-4	18/20	90%
17	53	63	F	216/2007	t(9:22)47XX+8	17/20	85%
18	56	64	F	244/2007	t(9:22)44XX-7	17/20	85%
19	60	59	M	273/2007	t(9:22)47XY+8	16/20	80%
20	65	66	F	307/2007	t(9:22)46XX-3	17/20	85%
21	69	46	F	329/2008	t(9:22)47XX+8	15/20	75%
22	74	65	F	362/2008	t(9:22)45XX-3	18/20	90%
23	75	46	M	3/2008	t(9:22)47XY+8	16/20	75%
24	79	44	F	36/2008	t(9:22)44XX-4	14/20	70%
25	82	66	M	356/2008	t(9:22)46XY+8	15/20	75%
26	85	69	M	74/2008	t(9:22)45XY-7	18/20	90%
27	88	30	M	96/2008	t(9:22)47XY+8	14/20	70%

Along with the philadelphia chromosome other chromosomal aberrations are also found in CML patients. Such aberration may be structural or numerical. In the present study 27(30%) cases show other numerical aberrations in addition to the presence of philadelphia chromosome (**Fig 5** and **6**).

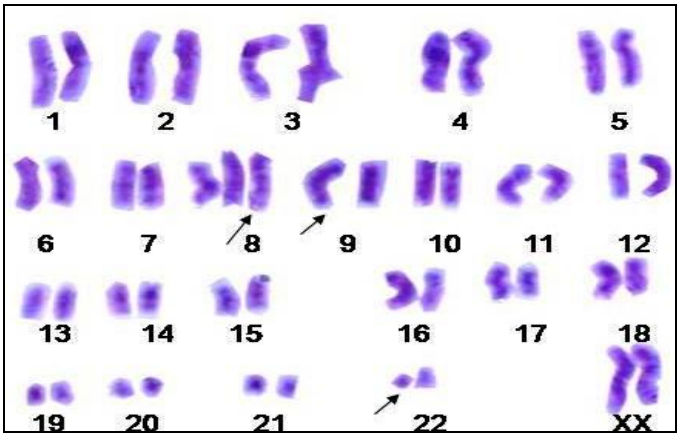


FIG.5: G. BANDED KARYOTYPE OF BONE MARROW CELLS OF FEMALE CML PATIENT SHOWING TRANSLOCATION t(9:22) & TRISOMY 8. [t(9:22) 47XX + 8].

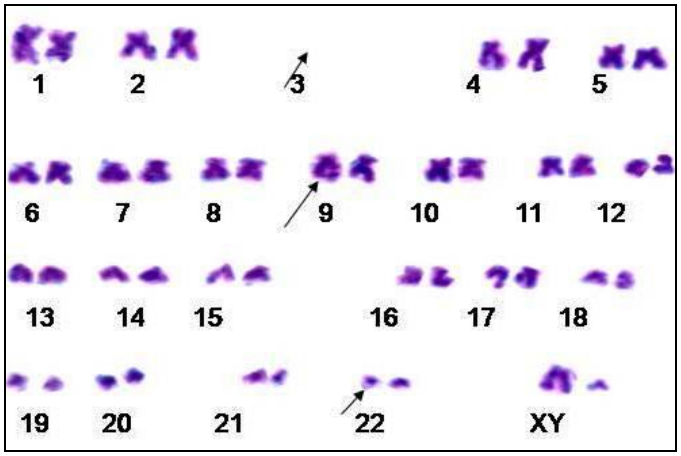


FIG.6: G. BANDED KARYOTYPE OF BONE MARROW CELLS OF MALE CML PATIENT SHOWING TRANSLOCATION t(9:22) & DEL-3 [t(9:22) 44XY -3].

These patients are within 12-69 yrs of age. Median age is 57yrs and mean age is 52.25yrs. Maximum (81%) patients of this group are within 40-70yrs of age. Male to female ratio is 0.93:1. The most common numerical aberrations found in this group is trisomy 8. Out of 15 cases having trisomy 8 in Ph +ve clone, 9 are males and 6 are females. Male to female ratio among them is 1.5:1.The next common

numerical aberration found in the above table is del-7. Out of 6 cases of del-7 in Ph+ve group, 3 are male and 3 are female having the male to female ratio of 1:1.Besides the above numerical aberrations in philadelphia positive patients, 3 cases have del-4 and 3 cases have del-3.

Out of 3 patients having del-4, only one patient is male and 2 are females, while all cases of del-13 are females. So the male to female ratio has reversed here with female predominance. The number of abnormal Karyotype per 20 metaphages is more towards late age.

TABLE 9: PHILADELPHIA POSITIVE CML PATIENTS WITH STRUCTURAL ABERRATIONS

Sl. No.	Case No.	Age	Sex	BM No	Karyotype	No of abnormal Karyotype per20 metaphases	Remark
1.	4	65	M	194/2006	t(9:22)46XY, t(15:17)	17/20	85%
2.	6	61	M	208/2006	t(9:22)46XY, t(2:6)	16/20	80%
3.	11	55	M	238/2007	t(9:22)46XY, t(15:17)	16/20	80%
4.	21	65	F	304/2007	t(9:22)46XX, t(15:17)	17/20	85%
5.	25	8	F	11/2007	t(9:22)46XX, t(15:17)	13/20	65%
6.	27	66	M	27/2007	t(9:22)46XY, t(6:7)	18/20	90%
7.	31	48	M	56/2007	t(9:22)46XY, t(15:17)	14/20	70%
8.	37	57	M	102/2007	t(9:22)46XY, t(1:7)	16/20	80%
9.	40	43	M	124/2007	t(9:22)46XY, t(15:17)	15/20	75%
10.	45	70	M	158/2007	t(9:22)46XY, t(2:6)	18/20	90%
11.	58	28	M	262/2007	t(9:22)46XY, t(6:7)	14/20	70%
12.	67	64	F	320/2008	t(9:22)46XX, t(15:17)	17/20	85%
13.	70	62	M	336/2008	t(9:22)46XY, t(1:7)	16/20	80%
14.	80	61	M	42/2008	t(9:22)46XY, t(15:17)	16/20	80%
15.	86	51	M	81/2008	t(9:22)46XY, t(6:7)	15/20	75%

The previous table shows the distribution of structural chromosomal aberrations found in philadelphia positive CML patients. Like the numerical chromosomal aberration, there are also different varieties of structural aberrations, found in philadelphia positive CML patients (**Fig.7** and **8**).

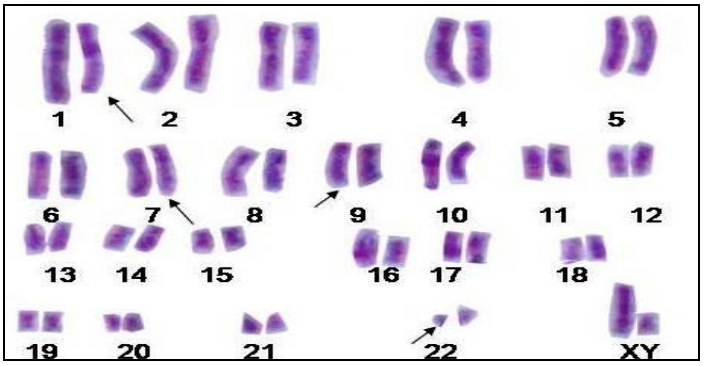


FIG.7: G. BANDED KARYOTYPE OF BONE MARROW CELLS OF MALE CML PATIENT SHOWING TRANSLOCATION T(9:22) & TRANSLOCATION t(1:7).[t(9:22)46XY t(1:7)].

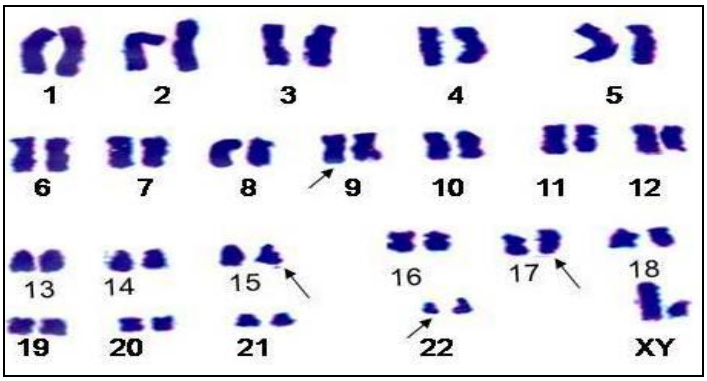


FIG.8: KARYOTYPE OF BONE MARROW CELLS OF MALE CML PATIENT SHOWING TRANSLOCATION t(9:22) & TRANSLOCATION t(15:17).[t(9:22)46XY t(15:17)].

In the present study 15(17%) cases show structural chromosomal aberrations in addition to the philadelphiachromosome. The most common structural chromosomal aberrations found in Ph+ve patients is t(15:17). Out of 8 patient having t(15:17), there are 5 males and 3 females having male to female ratio 1.6:1.These patients are within 8 - 70 yrs of age. The mean age is 53.6yrs and median age is 59 yrs. Male to female ratio is 1.6:1.The rest of the patients showing additional structural aberration are males. Among them 3 cases show t(6:7), 2 cases show t(2:6) and 2 cases have t(1:7)along with philadelphiachromosome. In the above table it is observed that structural aberration are more seen in males and more number of abnormal Karyotypes per 20 metaphases are seen in older age in comparision to younger age.

TABLE 10: PHILADELPHIA NEGATIVE CML PATIENTS WITH NUMERICAL& STRUCTURAL ABERRATION.

Sl. No.	Case No.	Age	Sex	BM No	Karyotype	No of abnormal Karyotype per20 metaphases	Remark
1	10	42	M	231/2007	t(2:6)46XY	15/20	75%
2	24	62	M	4/2007	t(15:17)46XY	17/20	85%
3	34	54	F	80/2007	47XX + 8	16/20	80%
4	42	67	M	139/2007	t(2:6)46XY	18/20	90%
5	47	63	M	172/2007	t(15:17)46XY	17/20	85%
6	59	69	F	268/2007	45XX – 7	18/20	90%
7	64	38	M	301/2008	t(2:6)46XY	14/20	70%
8	76	44	M	9/2008	47XY + 8	15/20	75%

There may be both structural and neumerical aberration found in a single patient in addition to philadelphia chromosome but in present study we have not detected any such case. The structural and numerical chromosomal aberrations are also found in philadelphia negative CML patients **(Fig.9** and **10**).

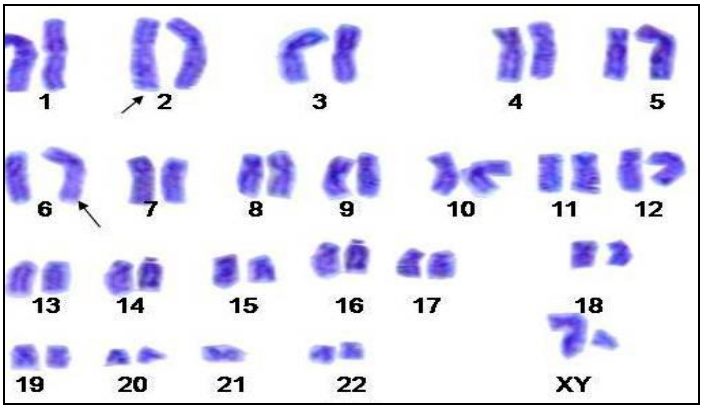


FIG.9: KARYOTYPE OF BONE MARROW CELLS OF MALE CML PATIENT SHOWING TRANSLOCATION t(2:6). [t(2:6)46XY)]

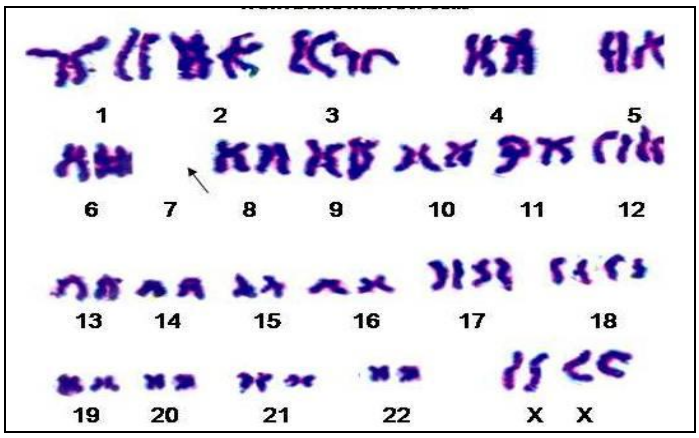


FIG.10: G. BANDED KARYOTYPE OF BONE MARROW CELLS OF FEMALE CML PATIENT SHOWING DEL-7.(44XX-7)

In the present study 8(9%) cases show such abnormality having 6 males and 2 females. Male to female ratio is 3:1. The patients are within 38-69 yrs of age. Mean age is 55yrs and median age is 62yrs. 5 cases show structural aberrations and 3 cases show neumericalaberrations.Thestructural chromosomal aberrations seen in philadelphia -ve clone are t(2:6) in 3 cases and t (15:17) in 2 cases. The numerical aberrations found in philadelphia -ve patients are trisomy 8(2 cases) and del-7 (1Case).From the above table it is observed that the additional chromosomal aberration in Ph -ve clone are found more in males.

DISCUSSION: Chronic myeloid leukemia is a disease of clonal proliferation of multipotenthaematopoietic stem cells characterized by marrow hyperplasia of granulocytes, erythroid precursors, megakaryocytes and connective tissue forming cells. Association of philadelphia chromosome with CML is well established. This is the 1st consistent abnormality observed in human cancer and is present in the bone marrow of 90-95% cases of CML patients. After it's 1st identification in 1960, many researchers worked in this field and found the same specific abnormality[1]. Cytogenetic study play a dominant role in the identification of philadelhia chromosome which is diagnostic as well as prognostic indicator of CML.

In the present study 89 samples of bone marrow from clinically diagnosed CML cases were analysed. The conventional method (ISCN-1991) was followed in this study. Out of 89 patients, 72 (81%) cases has 9:22 translocation and 17(19%) cases were Ph -ve (**Table - 1**).

D. Costa GG et al. carried out their cytogenetic study on 102 cases of CML [3]. The samples were analysed cytogenetically which showed 94% patients having Ph chromosome positive.

In the study of ANGLO M et al.10 CML patients were investigated [4]. Cytogenetic study was carried out in bone marrow samples using short term culture. It was observed that 9 out of 10(90%) patients have positive Philadelphiachromosome. The cytogenetic analysis of 23 pathologically diagnosed cases of CML were done by Jorge E Corte et al [5]. They observed that 17(73%) patients were Ph +ve. Bone Marrow samples were collected and analysed cytogenetically. 28(90%) cases shows philadelphia positive. Similar to our observation, 16(84.2%) cases were Ph+ve in an analysis of 19 CML patient diagnosed pathologically [6]. Nisha Singh (2006) studied 16 CML patients diagnosed pathologically [7].

In his conventional cytogenetic analysis Ph chromosome was found in 13 (81%) cases and the same (81%) Ph positivity was obtained in our study. Our observation almost corroborates with the data obtained by the above workers but there are little variations, which may be due to a small group subjected for analysis and geographical variations.

In the present study age specific incidence and male-female ratio of CML were observed (**Table-2).**The maximum cases were observed in 41 to 60 yrs of age group. The male-female ratio was almost equal in 0-20yrs of age group where it is maximum i.e. 1.63 in the patients having age more than 60yrs.Similar age distribution in CML patients were also seen in the study where 137 cases between 15-68 yrs median (33yrs) were analysed [7]. Maximum patients i.e. 67(48%) were within 40-60yrs of age but incontrast to our finding in age distribution had undertaken a cytogenetic study on 34 pathologically diagnosed CML children having age group between 5-11.5 yrs (median 8.2yrs) [9].

Male to female ratio was 1.2:1. So the male female ratio of CML patients in 0-20yrs of age group is almost similar to our finding even though the age distribution in both studies are different. However both age distribution and male female ratio in that age group was similar to our observation with another study where 19 CML patients within 60-65yrs of age with male to female ratio 2:1 were analysed [10]. Late age distribution in CML patients were also noticed in the study [11].

They analysed 30 diagnosed CML patients in the age group of 26 - 57yrs out of which 14 (46%) patients belonged to 5th and 6th decades. So our observation tallies with some observers in age distribution where with other observer in age specific male female ratio. In few observers both age distribution as well as male female ratio

showed similarity with the present study. The small variation may be due to different geographical distribution. One of the most important observation of the present study is that, maximum 42(47%) cases had Ph +ve with other chromosomal aberrations and such patients had age more than 60yrs. (**Table 3**) So the patients having Ph chromosomes with other aberrations have nearly same or slightly higher percentage than the patients having Ph chromosome as only anomaly. The same thing was reported by S.I. Sonata et al [12].

They studied 67 cases of chronic myeloid leukemia diagnosed pathologically. The patients were in the age group of 20-68yrs. Out of 67 patients 28 had Ph +ve as only karyotypic anomaly and 29 patients had other chromosomal aberrations along with philadelphia chromosome. However exactly same percentage of distribution between only Ph positive group and Ph chromosome with other anomaly group occurred in the study [13].

In their study they analysed 32 CML patients cytogenetically. 15 patients had Ph +ve as only karyotypic anomaly where as 15 cases had Ph +ve with other chromosomal aberrations. Again the result appeared in the same manner as our study in the work of Ahmed Aribi et al where 19 CML cases within age group of 60-65yrs were studied [10]. Out of 13 Ph +ve cases 6 (32%) had Ph +ve as only karyotypic anomaly and 7(37%) had Ph +ve with other chromosomal aberrations. So there is striking similarity in this regard between above workers and our present studies. In the present work there are 54 males and 35 females suffering from CML, having male predominance, the male to female ratio being 1.54:1. **(Table 4).**

36 patients with myeloid metaplasia of age group between 13-65yrs were studied by J PM Geraedtscytogenetically [14]. The male to female ratio was 1.47:1.The similar male predominancy in CML was also noticed by the following workers where male to female ratio ranges from 1.4:1 to 1.8:1. Anwar N. et al analysed 137 cases of CML diagnosed pathologically. They were between 15-68yrs (Median 53yrs) [8]. The male-female ratio was 1.7:1. SettinA et al conducted a study on 63 cases of CML having 40 male & 23 female patients [15]. The male to female ratio being 1.74:1. In an another study of 21 patients between the age group of 29-82 yrs(Median age 62yrs) were analysed by Jorge Cortes et al andthe male to female ratio was 1.8:1[16].Michael W. et al analysed 32 patients of CML. The patients were in the age group of 18-80yrs, male to female ratio being 1.4:1 [13]. However very high male predominancy in CML was also reported by many authors where male to female ratio was 4:1, 2.8:1 and 3:1 respectively [4, 6, 11].

They have analysed 10 patients, 19 patients, and 30 patients respectively. These variation may be due to small group of patients taken into consideration in their study. Another finding in the present study was the median age of the patients being 54yrs. (graph I & J, **Table 5**) which is corroborating with following workers. The patients were in the age group of 18-68yrs, median age being 54yrs. In an another study 137 cases of CML diagnosed pathologically [8]. The cases were between 15-68yrs (median 53yrs) which is very close to our observation. Similar finding was obtained in an another work where 69 diagnosed cases of CML having BCR-ABL positive were studied by Claudia Haferlach et al [17].

The patients were between 28-70yrs (median age was 58yrs).From the report of another study, 32 patients of chronic phases of CML were investigated [13]. The patients were within 18-80yrs. Median age 57yrs.With a close observation and analysis of the findings of the above workers it is concluded that CML patients present nearly similar median ages with minimal variations as this is a disease of 5th and 6th decade. CML patients having philadelphia chromosome as only karyotypicanomaly.

In the present study 30(34%) patients have philadelphia chromosome as only karyotypicanomaly (**Table 6, Fig.1** and **2**).

S.I. Sonata et al studied 67 cases of CML. Out of them 28(42%) have philadelphia chromosome as only karyotypic anomaly which is little higher than that in our study [12]. Again M.S. Anand et al studied 32 CML patients. Out of them 26 were in chronic phase and 6 in blast crisis [19]. 16(50%) had philadelphia chromosome as only karyotypic anomaly which is of still higher percentage. Radio

Oudat et al. studied 31 cases of CML. 20(69%) cases had Ph +ve as only karyotypic anomaly [18]. Thus it shows extermely higher percentage and had marked differenece from that in our study. Supporting to the above workers R.K. Marwaha et al had undertaken 34 pathologically diagnosed CML children in which 18(52%) cases had Ph +ve as only karyotypic anomaly9. Anwar N et al analysed 137 CML patients diagnosed pathologically where 73 (54%) cases had Ph +ve as only karyotypic anomaly [8].

Here we found a marked difference in the percentage of absolutely Ph+ve CML patients from the above workers. The relatively low incidence was observed in the only Ph +ve clone. This may be due to new recurring chromosomal aberrations.

CML patients with normal karyotype:

In the present study 9(10%) patients had normal karyotype. They had neither philadelphia chromosome nor any other chromosomal aberration (**Table 7, Fig.3** and **4)**.Supporting to our finding, 3 (13%) patients were karyotypically normal in the cytogenetic analysis where 23 pathologically diagnosed cases of CML were analysed [16]. Similarly in the study of MS Anand et al, 26 patients of CML in chronic phase and 6 in blast crisis were recruited. 2(8%) patients had normal karyotype in their cytogenetic analysis which is very close to our finding [19]. In an another study 137 cases of CML diagnosed pathologically. 16 (12%) patients were karyotypically normal [8]. The percentage of CML patients having normal karyotype in our study almost tallies with the above mentioned workers.

Ph +ve CML patients with other numerical aberrations:

In the present study 27 (30%) cases showed other numerical aberrations in addition to the presence of philadelphia chromosome **(Table 8, Fig.5** and **6**). Corroborating completely to this finding of our study, S.I. Sonata et al studied 67 cases of chronic myeloid leukemia diagnosed pathologically [12]. After karyotyping they found that 20(30%) cases had other numerical changes in addition to philadelphia chromosome in the form of hyperploidy with frequent involvement of chromosome +8, +17, +19, +21. The same percentage of CML patients was also noticed in the study where 26 patients of CML in chronic phase and 6 in blast crisis (BC) were recruited [19]. In their study 8 (30%) patients demonstrated with trisomy 8 and del-13.R.K. Marwaha et al had undertaken a cytogenetic study on 34 pathologically diagnosed CML children [9].

They observed that 6 cases were with trisomy 8 and 4 cases had del-13 in addition to philadelphia chromosome. So additional numerical aberration was seen in 30% cases which is matching cent percent to our study. Close corrobation was also seen [20]**.** They had conducted a study on 174 cases of CML patients on different phases. 54(31%) patients showed additional chromosomal anomaly like del-3, trisomy 8 and monosomy-7.Similar similarity was again observed in a study of 69 diagnosed cases of CML having BCR-ABL positive[17]. Cytogenetic data of 30 CML patients had additional chromosomal changes with trisomy 8 (10 cases), del-13 (6 cases), del-9 (4 cases) and remaining 10 cases showed structural chromosomal changes like t(1:7) 4 cases, t(13:15) 6 cases. So 20(29%) cases had additional numerical aberrations.

Our finding corroborates 100% in this particular aspect with some of the above observer and tallies very closely to other mentioned workers.

Ph +ve CML patients with other structural aberrations:

In the present study 15 (17%) patients have other structural aberration in addition to Philadelphia chromosome (**Table 9, Fig.7** and **8**). Similar result obtained as the outcome of a study [12]. They examined 67 cases of CML diagnosed pathologically. In their study 9 (14%) cases had structural chromosomal aberrations like t(2:8) and t(2:7) in addition to the philadelphia chromosome. Like this, 69 diagnosed cases of CML, having BCR-ABL positive, were studied byClaudia Haferlach et al [17]**.**

In their study 10(15%) cases showed structural chromosomal aberrations like t(1:7) in 4 cases and t(13:17) in 6 cases along with philadelphia chromosome. In the same manner Susan Branford et al studied 25 patients of CML. Karyotypic

analysis showed 23(92%) patients with philadelphia chromosome, Out of them 8 had additional numerical aberrations and 4 (16%) had additional structural chromosomal aberrations which is more closer to our observation [21]. However nearly similar percentage obtained in this group of CML patients in the study [4]. They analysed 21 CML patients. In their study 18(80%) cases showed Ph +ve. Out of them, 6 cases had additional numerical chromosomal aberrations and 4(19%) had additional structural chromosomal aberrations.

But the report given by Richabourg S et al tallies perfectly to our finding. They carried out their cytogenetic study on 41 CML patients with variant philadelphia chromosome[22]. 38 (93%) cases showed philadelphia positive and in 3 patients other aberration like trisomy 8 (2 cases), del-13 (1 Case) were detected where as other 4 patients had t(13:17) and 3 patients had t(1:7) as additional structural chromosomal aberrations in Ph +ve patients. So in 17% cases additional structural aberration was present. From the comparison of the reports and opinion of the above workers and that of our study, it could be concluded that the rate of occurrence of additional chromosomal aberation in CML patients runs parallel to almost all workers mentioned above, with little variations, which is minimal and usual.

Philadelphia negative CML patients with other chromosomal aberrations:

Another finding in the present study is that, 8 (9%) cases are Philadelphia negative but with other chromosomal aberrations.(**Table 10, Fig.9** and **10**). Such statement also found in the report of S.I. Sonata et al [12]. They studied 67 cases of CML diagnosed pathologically.

In their analysis 6(9%) patients in Ph negative clone had other chromosomal aberrations as 4 numerical and 2 structural aberrations. Like the carbon copy to this report, B. Anger et al also claimed the same thing in their analysis where they had examined 69 clinically and pathologically diagnosed CML patients [23]. Out of 69 cases 57(83%) showed philadelphia negative. Among them 6 (9%) had other chromosomal aberrations. So both the above workers had cent percent similarity with our findings. However though not cent percent, very closely related result was observed in the study of Nisha Singh who studied 16 CML patients diagnosed pathologically [7]. He found that 13 (81%) cases were Ph +ve, the remaining 3 were Ph -ve. In Ph -ve group, 1 had structural aberration, 1 had numerical aberration and 1 had normal karyotype. So the percentage of Ph -ve CML patients with other chromosomal aberation was 12.5%. They observed that in 7 patients, philadelphia chromosome was absent. Among them 2 (3%) patients had trisomy 8 and 3 (4%) patients had t(13;15) and 2 were karyotypically normal.

Ahmed Aribi et al also reported the similar thing from their analysis which tallies with above workers [10]. They had studied 19 CML patients and found that 13(69%) cases had philadelphia chromosome positive. In philadelphia negative group 2(11%) patients had t(13:15). Even though philadelphia chromosome is very much popular and essential for the diagnosis of CML, a small group of stamped CML patients are devoid of it. Instead they may possess other structural and numerical anomaly which were proved from the reports of the above workers and corroborating to them, similar finding were also obtained in our present study.

Finally it is observed that many of our findings are corroborating to a number of previous workers, at places they are matching exactly or at places with little variation. However in findings like absolutely Ph +ve CML cases we found marked variation which may be due to emergence of newly occurring chromosomal aberation in addition to philadelphia chromosome.

CONCLUSION: The marked variation was observed in the percentage of only Ph +ve group and this may be due to emergence of additional chromosomal aberration in Ph+ve CML patients. We found trisomy 8 as most common additional numerical chromosomal aberration which is also reported by many of the previous observer. But the most common additional structural chromosomal aberration found here was t(15:17), which is relatively rare in available literature. To establish this as a newly recurring additional chromosomal

aberration in CML patients, large database analysis is required.

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