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Karyotypic Profile of Chronic Myeloid Leukemia in Patients Diagnosed at Tertiary Level in Afghanistan

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Abstract

Background: Current guidelines for chronic myeloid leukemia (CML) management include utilization of both conventional cytogenetics as well as advance molecular analysis at diagnosis and while monitoring the therapy. The reason being the fact that karyotype analysis is one of the indispensable tools for identification of additional chromosomal abnormalities of prognostic significance. **Methodology:** We conducted a descriptive case-series study of 33 patients to demonstrate the profile of karyotype abnormalities in CML. **Results:** 75.7% of patients had a single Philadelphia chromosome (Ph), while in addition to Ph, one patient had t (11;17), one patient had t (7;14), one patient had 5q deletion, one patient had double Ph and one patient had a complex karyotype with 6q deletion, monosomy 11, monosomy 12 and marker chromosome. Two patients with pathognomonic clinical feature of CML had normal karyotype and thus were advised to proceed with further molecular studies, since they showed significant clinical improvement with tyrosine kinase inhibitor therapy. **Conclusion:** Detection of additional chromosomal abnormalities in CML patients is a key to identification of patients requiring advance therapeutic modalities other than the conventional tyrosine kinase targeting agents. This is true both at diagnosis as well as during the tyrosine kinase inhibitor therapy.

Keywords: Karyotypic, Chronic myeloid leukaemia, Tertiary level, Afghanistan

Abbreviations: ABL_1: Abelson Gene; BCR: Breakpoint Cluster Region; CML: Chronic Myeloid Leukaemia; PCR: Polymerase Chain Reaction; qRTPCR: Quantitative Real Time PCR; DMR: Deep Molecular Response; FISH: Fluorescence in-situ Hybridization; Ph: Philadelphia Chromosome; TKI: Tyrosine Kinase Inhibitor; FMIC: French Medical Institute for Mothers and Children; CP: Chronic Phase; AP: Accelerated Phase; BC: Blast Crisis.

Introduction

Balanced translocation resulting in fusion of the Abelson gene (*ABL_1*) from chromosome 9q34 with the breakpoint cluster region (*BCR*) gene on chromosome 22q11.2 is the pathognomonic molecular driver of CML [1]. The resulting *BCR-ABL_1* fusion gene is both the diagnostic as well as therapeutic target of CML [2]. The first agent with tyrosine kinase inhibitor activity that was licenced in 2000 for treatment of CML patients, was Imatinib, gradually followed by multiple agents with higher efficacy [3,4].

Although, the current management protocols have adopted molecular analysis including quantitative real time polymerase chain reaction (qRT-PCR) for *BCR-ABL1*, now aiming to assess patient for deep molecular response (DMR), conventional karyotyping and fluorescence in-situ hybridization (FISH) still have a strong hold not only during the diagnosis but

also during monitoring [5-7]. Where on one side, if patients who have achieved DMR and are able to maintain it for a specific time period, are entitled to be allowed for complete discontinuation of TKI therapy, others, especially those who have developed new clones of Ph positive neoplastic cells with complex karyotypic abnormalities may need to opt for therapeutic modalities beyond TKI, including allogenic bone marrow stem cell transplantation [8,9].

Thus, in-spite of molecular progress in diagnosis and monitoring of CML, conventional cytogenetics is still very significant [10]. With an aim to help establish quality health services in a country with no history of availability of cytogenetic diagnostic modalities in the past, French Medical Institute for Mothers and Children (FMIC) with the help of Agha Khan University, Pakistan, established Afghanistan's first cytogenetic laboratory. Accordingly, we conducted a case series study to demonstrate the karyotypic profile in CML diagnosed at FMIC during the first year of establishment of our cytogenetic laboratory.

Materials and Methods

Study Design

A descriptive "case-series" study was conducted from 1st January, 2020 to 31st January. 2021, including 33 patients who were diagnosed with CML, after acquisition of informed consent from patient or next of kin. For inclusion in the study, patients either had to have demonstrable "pathognomonic" clinic-morphological features of splenomegaly, bone marrow and/ or peripheral blood hyperleukocytosis, and bi-modal peak of mature neutrophils with myelocytes, or presence of leucoerythroblastic picture with myeloproliferative features, where conventional karyotyping revealed presence of Ph, into chronic phase (CP), accelerated phase (AP) and blast phase/ crisis (BC) considering the recommendations by World health Organization (WHO) classifications [11]. The statistical analysis was performed using statistical package for social sciences (SPSS), version 25.

Results

As shown in Table 1, out of 33 patients included in our study, 18 (54.5%) were female and 15 (45.5%) were male. The age at diagnosis ranged between 12 to 75 years with median age at diagnosis being 42 years. 20 patients were permanent residents of Kabul, 3 patients were from Wardak province, two patients were from Ghazni province, while from Badakhshan, Balkh, Bamyan, Jouzjan, Khost, Nangarhar, Paktia and Parwan, there were only one patient each. Considering the leukocyte count at diagnosis, 29 (87.9%) of patients presented with hyperleukocytosis defined as white cell count equal to or more than 100,000/ cubic millimetres of blood, while only

Table 1: Demonstrating the demographic, clinical and karyotypic features.

Medical Record Number	Age (years) /gender	Clinical Features	Karyotype
PW-76-20	42, Female	Hyperleukocytosis, Bimodal peak Response to TKI	46, XX, t (9;22) (q34 q11)
PW-77-20	12, Male	Leukoerythroblastic- anaemia	46. XY, t (9;22) (q34 q11)
PW-78-20	45, Female	Hyperleukocytosis Bimodal peak Response to TKI	46, XX, t (9;22) (q34 q11)
PW-81-20	67, Female	Hyperleukocytosis Bimodal Peak, Blast 12% Response to TKI	46, XX, Del (13q) (q21.2 q34)
PW-75-20	45, Female	Hyperleukocytosis Lost Contact	46, XX, t (9;22) (q34 q11)
PW-69-20	55, Female	Leukocytosis Lost Contact	46, XX, t (9;22) (q34 q11)
PW-66-20	28, Male	Hyperleukocytosis Bimodal Peak Response to TKI	46, XY
PW-64-20	16, Female	Hyperleukocytos, Bimodal peak and Response to TKI	46, XX, t (9;22) (q34 q11)

PW-63-20	60, Female	Blast 33%, Leukoerythroblastic anaemia Response to TKI	46, XX, t (9;22) (q34 q11)
PW-62-20	75, Male	Hyperleukocytosis Bimodal peak Lost Contact	46, XY, t (9;22) (q34 q11)
PW-59-20	28, Male	Hyperleukocytosis Blast 15% Response to TKI	46, XY, t (9;22) (q34 q11)
PW-58-20	33, Male	Hyperleukocytosis Bimodal Peak Lost contact	46, XY, t (9;22) (q34 q11)
PW-53-20	41, Male	Hyperleukocytosis, Blast 23%, Bimodal Peak Response to TKI	46, XY, t (9;22) (q34 q11), Del (6) (q23.3q27), t (9;22) (q34; q11.2),-11,-12,add(12)(p13.3),mar[20]
PW-51-20	26, Female	Hyperleukocytosis Bimodal Peak Lost contact	46, XX, t (9;22) (q34 q11)
PW-45-20	42, Male	Hyperleukocytosis Bimodal Peak Response to TKI	46, XY, t (9;22) (q34 q11)
PW-35-20	46, Female	Hyperleukocytosis Bimodal Peak Lost Contact	46, XX, t (9;22) (q34 q11)
PW-30-20	35, Male	Hyperleukocytosis Bimodal Peak Lost Contact	46, XY, t (9;22) (q34 q11)
PW-28-20	25, Male	Hyperleukocytosis Bimodal Peak Lost Contact	46, XY, t (9;22) (q34 q11)
PW-27-20	50, Male	Hyperleukocytosis Bimodal Peak Lost Contact	46, XY, t (9;22) (q34 q11)
PW-24-20	45, Female	Hyperleukocytosis Bimodal Peak Response to TKI	46, XX, t (9;22) (q34 q11), t (11;17) (q21 q25)
PW-23-20	31, Female	Hyperleukocytosis Bimodal Peak Lost Contact	46, XX, t (9;22) (q34 q11), t (7;14)
PW-20-20	32, Male	Hyperleukocytosis Bimodal Peak Lost Contact	46, XY, t (9;22) (q34 q11)

PW-13-20	48, Male	Hyperleukocytosis Bimodal peak Response to TKI	46, XY
PW-10-20	45, Female	Hyperleukocytosis Bimodal peak Lost Contact	46, XX, t (9;22) (q34 q11)
PW-09-20	22, Female	Hyperleukocytosis Bimodal Peak Response to TKI	46, XX, t (9;22) (q34 q11)
PW-02-20	38, Female	Hyperleukocytosis Bimodal Peak Response to TKI	46, XX, t (9;22) (q34 q11)
PW-06-20	35, Female	Hyperleukocytosis Bimodal Peak Lost Contact	46, XX, t (9;22) (q34 q11)
PW-90-20	25, Female	Hyperleukocytosis Bimodal Peak Lost Contact	46, XX, t (9;22) (q34 q11)
PW-89-20	45, Female	Hyperleukocytosis Bimodal Peak Lost Contact	46, XX, t (9;22) (q34 q11), del(5q) (5q13.3 to q35.3)
PW-82-20	50, Female	Hyperleukocytosis Bimodal Peak Lost Contact	46, XX, t (9;22) (q34 q11)
PW-102-20	45, Female	Hyperleukocytosis Bimodal Peak Response to TKI	46, XX, t (9;22) (q34 q11)
PW-101-20	74, Male	Hyperleukocytosis Bimodal Peak Lost Contact	46, XY, t (9;22) (q34 q11)
PW-87-20	57, Male	Hyperleukocytosis Bimodal Peak Response to TKI	46, XX, t (9;22) (q34 q11), Der (22) t (9;22) (q34 q11)

4 (12.1%) of patients had white cell count more than 10,000 but less than 100,000/ cubic millimetres [12]. Similarly, considering the disease phase at diagnosis, 30 (90.9%) of patients were in chronic phase (CP), one patient (3%) in accelerated phase (AP) and two patients (6.1%) in blast crisis/ phase (BC). Considering the cytogenetic profile at diagnosis, as shown in Figure 1 and Table 1, 25 (75.7%) patients had only a single Ph chromosome, while in addition to Ph, one patient (3%) had t (11;17), one patient (3%) had t (7;14), one patient (3%) had derivative of chromosome 22 (t (9;22) (q34 q11)) resulting in double Ph, one patient had *de-novo* 5q deletion (5q13.3 to q35.3) that we have reported elsewhere in the literature [13] and two patients who presented with pathognomonic clinic-morphological feature of CML had normal karyotype, thus were advised to proceed either with fluorescent in situ hybridization or Multiplex Qualitative PCR for BCR-ABL_1, for disease confirmation. Unfortunately, due to financial constrains the two patients opted to rather consider tyrosine kinase inhibitory (TKI) therapy and achieved complete hematological remission (CHR) within a span of one month.

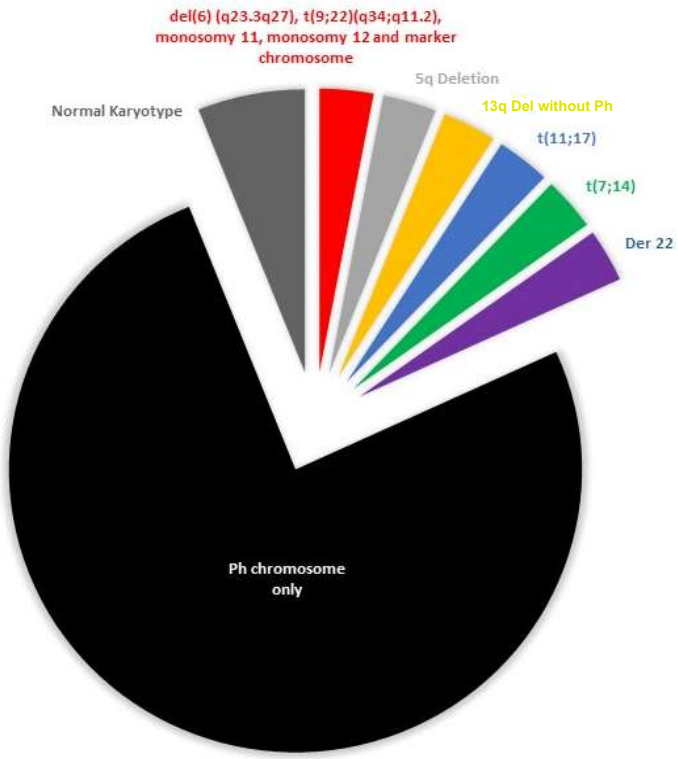

Figure 1: Conventional karyotype profile of patients at diagnosis of CML.

One patient (3%) presented with typical clinic-morphological features of CML in CP but karyotyping revealed only presence of 13q deletion. Fortunately, after starting TKI therapy, this patient too had CHR within a span of one month. Of all the patients, one patient (3%) was diagnosed as CML in CP based on clinic-morphological features and he too, due to financial constraints, opted to proceed with TKI therapy. Unfortunately, the patient did not respond to first line TKI and subsequently consented for peripheral blood cytogenetic analysis, that revealed presence of del (6) (q23.3q27), monosomy 11, monosomy 12 and marker chromosome in addition to Ph chromosome, t (9;22) (q34; q11.2), which we already have reported elsewhere in the literature [8]. None of our patients presented either with monocytosis or basophilia.

Discussion

CML diagnosis and treatment has tremendously evolved with the progressive advent in the field of genetic studies [1]. Although, molecular analytical techniques, including qualitative and quantitative real time polymerase chain reaction (qRT-PCR) studies for BCR-ABL$_1$ fusion gene are the mainstay diagnostic as well as therapeutic modalities, conventional cytogenetic analysis has persistently retained its role as a significant tool, not only at diagnosis but also for patient monitoring during therapy [8]. Complex chromosomal abnormalities which would be missed by molecular studies are identified by conventional karyotyping and fluorescent in-situ hybridization (FISH) studies, making them important pillars in therapeutic decision making [8].

Usman et al. conducted a study including 461 CML patients diagnosed at Shaukat Khanum Memorial Cancer Hospital and Research Centre, demonstrated that 64% of their patients were male while 36% of the patients were female. Median age at diagnosis for male patients was found to be 31 years while median age at diagnosis for female patients was 36 years [14].

Mu et al. performed a large-scale study on the cytogenetic profile of 1,863 Philadelphia chromosome positive CML patients in china and demonstrated that median age at diagnosis was 41 years, which they concluded to be younger than the age at diagnosis, in western population [15]. They also demonstrated that additional chromosomal abnormalities (ACA) were diagnosed in 3.1% of newly diagnosed CML patients, in CP, 35.4% of patients diagnosed in AP and 52.9% of patients diagnosed in BC [15]. In our study, the median age at diagnosis was 42 years, similar to the Chinese population and thus younger than the age at diagnosis in western population [15]. In our study while most of the patients were diagnosed in CP, one patient who was diagnosed in accelerated phase demonstrated presence 13q deletion without Ph chromosome.

Since the patient had remarkable response with achievement of CHR within one month of starting imatinib, it was presumed that patient had cryptic BCR-ABL$_1$ translocation. Due to financial reasons, she did not proceed with Multiplex PCR analysis or FISH studies.

In another study by Thielen et al. including 3585 patients, the incidence of CML was highest in male patients [16], while in our study the incidence was more in female. Similarly, in a study by Safaei et al. including 134 patients, conducted in Shiraz province of Iran, the mean age was 44 years, with an age range of 4 to 90 years, where 81.3% of patients were diagnosed in chronic phase, 8.2% in accelerated phase and 10.4% in blast crisis [17]. These figures were close to our results with median age at diagnosis being 42 years, while 90% of patients being diagnosed in chronic phase, 3% in accelerated phase and 6.1% in blast crisis. In our study, the youngest age recorded at diagnosis was 12 years.

In an observational-retrospective study conducted at Fundeni Clinical Institute, Bucharest by Crisan et al., median age at diagnosis of CML was reported to be 50.5 years. It was demonstrated that the most frequent minor route additional cytogenetic abnormalities in Ph positive cells were monosomies and structure abnormalities like inversions and translocations [18]. On the other hand, the most frequently encountered major route cytogenetic abnormalities were trisomy 8, trisomy 19 and a second Ph chromosome [18].

Wang et al. in a study conducted at MD Anderson Cancer Institute, demonstrated that additional chromosomal abnormalities were associated with bad prognosis, of note when patients harbored more than 2 additional chromosomal abnormalities, the impact on survival was the worst [19]. Similarly, one of our patients, who presumably had del (6) (q23.3q27), t (9;22) (q34; q11.2), monosomy 11, monosomy 12 and marker chromosome at diagnosis, had no response to TKI and thus was counselled to undergo Allogenic bone marrow stem cell transplant [8]. Clonal evolution occurs in excess of 10% of CML patients while being treated with TKI agents, therefore cytogenetic analysis plays an important role not only at diagnosis but also during management [1].

Conclusion

Where modern molecular modalities have now enabled deep scrutiny into the disease process, conventional cytogenetic analysis maintains its strong hold as an irreplaceable modality to be implemented at diagnosis as well as during TKI therapy. This is because it identifies the additional cytogenetic abnormalities which are markers of clonal evolution of prognostic significance in CML patients, that harbors significant impact upon the management plan. Since most of our patients deferred follow-up, it would be ideal to counsel the patients regarding significance of regular follow-up, to ensure better outcomes.

Limitations

The current research was based on the data collected in a single institution over a period of one year. Similarly, due to serious logistic reasons during the COVID19 pandemic, further specialized tests such as FISH and PCR were not possible to be requested at Agha Khan University Hospital, the parent institution for FMIC.

Acknowledgement

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Authors' Contributions

AMH and AHS conceived the idea. AMH, SR, ZAA, JAG and ASZ were the major contributor to the writing of the manuscript. AHS, ASI, FI, ZWS and MA collected the laboratory data via integrated laboratory management system (ILMS). JAG, ZAA, AMH and SRN contributed towards the final version of the manuscript. SRN, SHN, AJR and ASZ provided the clinical information of the patients. SOR, MA, MH FE, ZWS and SS performed cytogenetic studies. JAG, AMH, HAM, EE and RS were the major contributors for critically revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethical Approval

Ethical approval was acquired from Ethical Review Committee (ERC), of French Medical Institute for Mothers and Children (FMIC), Kabul Afghanistan. ERC Code: 100-FMIC-ER-21

Consent for Publication

Written informed consent was acquired from all the patients at the time of blood/ bone marrow sampling and they were verbally informed about the research and its possible impact towards the betterment of medical practice.

Availability of Data and Materials

All the generated data are included in this article.

Competing Interests

The Authors declare that they have no competing interests.

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