Role of Cytogenetic Evaluation in Diagnosis of Acute Myeloid Leukemia

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Abstract: Aim: Acute leukaemia represents clonal haematological disorders that arise from at least two or more genetics alteration in susceptible haematological cells. The cytogenetic study confirms a wide variety of common, rare and novel chromosomal anomalies in patients with haematological disorders providing valuable diagnostics and prognostic information. Method: Cytogenetic analyses were carried out in a total 4600 suspected patients. Of which, 68 patients were reported with Acute Myeloid Leukemia. Cytogenetic analyses from bone marrow cultures having age ranging from 5 years to 65 years were carried out. GTG banded metaphases were analysed and karyotypes by automatic karyotyping system and confirmation were made by using Florescent In Situ Hybridization technique (FISH). Results: Results revealed that out of 68 AML patients only 36 patients (52.9%) were found with translocation t(8; 21) (q22; q22) in AML-M2 subtype, 23 patients (33.8%) were found with a translocation t(15; 17) (q22; q12) in AML-M3 and only 09 patients(13.2%) were found with inversion in chromosome16 inv(16) (p13; q22) in AML-M4. Conclusion: It is concluded from the present study that a high prevalence rate of AML were found in t(8; 21) (q22; q22) followed by t(15; 17) (q22; q12) and inv(16) (p13; q22). The significance of results is discussed.

Keywords: G-banding, Karyotype, AML, FISH

1. Introduction

The nature of so-called complex karyotypic rearrangements associated with malignant hemopathies often undetected on conventional cytogenetics. The FISH technique is a new tool to discover cryptic translocations and to assign the chromosome origin of complex markers [1]. Genetics play an increasingly important role in the risk stratification and management of acute myeloid leukemia (AML) patients. Traditionally, AML classification and risk stratification relied on cytogenetic studies; however, molecular detection of gene mutations is playing an increasingly important role in classification, risk stratification, and management of AML [2]. Acute Myeloid Leukemia (AML) is a heterogeneous group of malignant haematopoietic disorder of rapidly proliferating neoplasm of immature haematopoietic stem cells. Recurrent chromosomal rearrangements such as t(8; 21) (q22; q22), t(15; 17) (q22; q12), and inv(16) (p13q22) are frequently identified as abnormality in AML. Several studies indicate that the AML patients with normal karyotypes represent the cytogenetically heterogeneous group which correlates with prognosis [3]. AML therapy is not targeted but the intensity of therapy is driven by the prognostic subgroup. Many prognostic scoring systems classify patients into favourable, poor, or intermediate prognostic subgroups based on clinical and genetic features. Current standard of care combines cytogenetic results with targeted testing for mutations in FLT3, NPM1, CEBPA, and KIT to determine the prognostic subgroup. Other gene mutations have also been demonstrated to predict prognosis and may play a role in future risk stratification, although some of these have not been confirmed in multiple studies or established as standard of care [4]. However, World Health Organization in 2008 revised its classification to recognize the impact of molecular markers on prognosis with normal cytogenetic findings, as the most of the patients achieve the complete remission with induction of chemotherapy. Thus, the complete diagnostic and prognostic testing of bone marrow is important to predict
the outcomes and post induction treatments [5]. Thus, the
cytogenetic study is important to confirm the wide variety of
common, rare and novel chromosomal anomalies in patients
with haematopoietic disorders may provide valuable
diagnostic and prognostic information [6].

Ahmad et al [7] showed that the t (8; 21) (q22; q22) is
most common recurrent chromosomal translocation seen in
nearly 10-15% of AML-M2 subtype. While t(15; 17) (q22; q12) found in only 5% of the specific type of AML such as
Acute Promyelocytic Leukaemia(APL) and inv(16) (p13q22)
found in approximately 8% of AML.

Velloso et al [6] have reported that the 50-60% of patients
shown intermediate risk group with chromosomal
constitution as t(9; 11), t8; 21 with normal karyotype, while
25-30% patients shown poor risk group with MLL, t(6; 9),
monosomy and deletion of chromosome 5 and 3, inv(3) and
25% of patients belong to good risk group with cytogenetic
chromosomal constitution as t(15; 17) (q22; q12), t(8; 21)
(q22; q22) and inv (16) (p13; q22).

In the present study, we describe the role of cytogenetic
evaluation in the diagnosis of AML, as it is an important
prognostic marker in the management of AML along with
recognition of specific subtypes. We also report, the
prevalence rate of cytogenetic abnormality found in adults
and its incidence increases with age.

2. Materials and Methods

A total of 4600 Patients were screened from January 2015
to December 2015 who were suspected to have AML.
Conventional Cytogenetic was performed on 24 hours
unstimulated short term culture of bone marrow cells. The
cells were grown in culture medium Marrow Max (GIBCO)
supplemented with 20% of Fetal Bovine Serum (FBS). The
colcemid was added for 30 minutes followed by KCL (75
mM) at room temperature for 27 min and Carnoy’s fixative
for four times. Slides were stained with Giemsa Trypsin
Giemsa (GTG) banding technique. GTG banded metaphases
from each culture were analysed and karyotyped by using
automatic IKAROS karyotyping software. The karyotypes
were described according to International system for human
cytogenetic nomenclature [8]. The cytogenetic findings
were confirmed by using FISH.

3. Results

In the present investigation total of 4600 AML suspected
patients were studied. Of which, 68 were reported with
AML. Among 68 patients, 36(52.9%) were reported with t(8; 21)
(q22; q22) in AML-M2 subtype, 23(33.8%) were with a
t(15; 17) (q22; q12) in AML-M3 and only 09
patients(13.2%) were found with inversion in chromosome16
inv(16) (p13; q22) in AML-M4. The abnormal karyotypes
with complex translocations are shown in [Figures 1, 2, and
3]). The results suggest that a high prevalence rate of AML
were found in t(8; 21) (q22; q22) followed by t(15; 17) (q22;
q12) and inv(16) (p13; q22) respectively [Figure 4].

The results of dual colour FISH analysis using AML1 and
ETO probes are shown in Figures 5 and 6” In Figure 5 there are
two separate red and green signals showing normal
chromosomes, while Figure 6 clearly shows two fusions, one
red and one green signals of AML1/ETO confirming t(8; 21).
While using PML and RARA probes, Figure 7 showed two
separate signals red for PML and green for RARA. While
Figure 8, showed two fusions with red, green and yellow
indicating t(15; 17). Interstitial, FISH analysis using inv(16)
shown in Figure 9 with two fusions of red and green signals
indicating normal chromosome #16. While Figure 10 shows
fusion of red and green signals in one which is normal
chromosome #16 and separate green and red signals
indicating inv(16) chromosome.

4. Discussion

Acute myeloid leukemia (AML) represents a group of
clonal hematopoietic stem cells disorders that is
characterized by both failure to differentiate and over
proliferation of the stem cell compartment by non-functional
cells called myeloblasts at the expense of normal cells [9].”
Cytogenetic analysis plays a critical role in the diagnosis,
classification, prognosis, and management of acute myeloid
leukemia (AML). It has become an essential technique that
helps doctors identify leukemia and provide treatment
guidance.

Chromosomal abnormalities in AML classified as
numerical and structural, chromosomal abnormality can be
deemed as disturbances in normal composition of
chromosomes. Numerical abnormalities take the form of an
aberrant copy number of particular chromosomes. This
phenomenon occurs due to the fact that chromosome
misreggregation takes place when the cell divides resulting in
the loss or gain of specific chromosomes [10].”

If AML is untreated, most patients will die over a period
of days or weeks based largely on the level of blasts in the
blood and bone-marrow. Cytogenetics is recognized as one of
the most important valuable prognostic determinators in
AML. An abnormal karyotype has been found in
approximately 60% of AML patients have favourable
cytogenetics that involve t(8; 21), t(15; 17) and inv(16); these
patients have complete remission (CR) rate over 90% and
five year survival of 65% [11]”

The aim of the study was to evaluate a role of cytogenetic
in the diagnosis of AML. A large numbers of AML suspected
patients were screened. Overall AML prevalence rates of
15% were observed in AML M2, M3 and M4e0 subtypes
which are similar to previously reported study. But, in the
present study t(8; 21) (52.9%), t(15; 17) (33.82%) and
inv(16) (13.23%) were higher as compared to those reported
by the 10%, 10% and 5 % in t(8; 21), t(15; 17) and inv(16)
respectively [12].”
Figure 1. G-banded karyotype of a female AML patient t(8; 21) (q22; q22).

Figure 2. G-Banded karyotype of a male patient showing t(15; 17) (q22; q12.).

Figure 3. G-Banded Karyotype of Male AML Patient Showing inv(16) (p13; q22).

Figure 4. Frequency of prevalence rate found in AML.

Figure 5. FISH showing AML(red) and ETO (green).

Figure 6. FISH analysis showing AML1/ETO (red and green) fusion signals.
Identification of t(8; 21) (q22; q22), t(15; 17) (q22; q12), and inv(16) (p13; q22) or its molecular equivalent rearrangement of genes is considered to be most valuable tool for cytogenetic and FISH studies [13]. Thus, for diagnosis, a FISH was more sensitive and accurate than conventional cytogenetic in detecting rearrangements besides confirming abnormality. Thus, all AMLs type namely AML M2, M3 and M4e0 are considered to be a good risk factor for prognosis.

5. Conclusion

Cytogenetics is considered one of the most valuable prognostic determinants in AML. In the present study, a total of 4600 patients were analysed with different age-groups. AML associated with t(8; 21), t(15; 17) and inv(16) predicted as a good-risk group in comparison to the complex karyotypes. For good-risk group, an autologous or allogeneic SCT should be reserved for patients who relapsed after chemotherapy. The study highlights the importance of diagnostic cytogenetics as an independent prognostic factor in AML.

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