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RESEARCH ARTICLE

A retrospective study of clinico-hematological and cytogenetic profile of erythroleukemia from South India

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ABSTRACT

Objective of the study is the retrospective evaluation of clinico-hematological and cytogenetic profile of patients with erythroleukemia (EL) in a south Indian population. Case records of all patients with acute myeloid leukemia seen in the Department of Medical Oncology at Kidwai Memorial Institute of Oncology, Bangalore, between January 1997 and December 2004 were reviewed. Clinical details were noted and slides were reviewed. A total of 326 AML patient were diagnosed of whom 14 patients had AML M6. Contribution of EL to all forms of AML was 4.3%. The mean age was 37.1+13.9 yrs (range: 16-65); most patients were in their 4th decade, with a male:female ratio of 3.67:1. Mean duration of symptoms in the present series was 10.9+6.9 weeks. Cytogenetics were normal in 71% of cases, and minor abnormalities were observed in 21% of cases. As a conclusion relative low incidence of secondary EL, more frequent normal karyotype, and relatively younger age observed in our series makes the picture of EL in our subcontinent different from that in other series reported thus far.

Key Words: AML M6, South India, cytogenetics, clinical profile.

INTRODUCTION

Giovanni Di Guglielmo first described erythroleukemia (EL) in 1928, and it is still referred to as Di Guglielmo syndrome [1]. With the better understanding of the biology of this entity and observation of variability in the clinical courses and survival outcomes, it has been sub-classified into three distinct subgroups[2]. To date, there have been only a few case reports and small series of EL reported from the Indian subcontinent [3,4,5,6,7]. There are no published series of this rare entity from the southern subcontinent (MEDLAR). We are thus reporting a retrospective analysis, in which we evaluated the clinico-hematological and cytogenetic profile of the patients with EL. However, comparison between the present series and older ones is not practical because of the changes in classification and definition of the disease.

MATERIALS And METHODS

This study is a retrospective data analysis. Case records of all the patients with acute myeloid leukemia (AML) seen in the Department of Medical Oncology at Kidwai Memorial Institute of Oncology, Bangalore, between January 1997 and December 2004 were reviewed. A total of 326 AML patients were diagnosed in this period, of whom 14 patients had AML M6; thus, the contribution of EL to all AMLs was 4.3%.

Clinical details: The clinical details as available in the hospital records were noted. Age, sex, symptoms with duration, examination findings and any other relevant data were reviewed.

Slide review: Peripheral smear slides and bone marrow slides along with cytochemistry comprising MPO, PAS and other staining were reviewed.

Definition of AML M6: World Health Organization (WHO) sub- classification was followed. M6a is 50% or more erythroblasts and 30% or more non-erythroid elements (i.e., myeloblasts I, myeloblasts II, monoblasts). In M6b, the erythroid component is singularly involved. It is characterized by the presence of 50% or more erythroblasts and pronormoblasts, and 30% or more basophilic erythroblasts. M6c (mixed type) is 50% or more erythroblasts, 30% or more non-erythroid components, and 30% or more pronormoblasts.

Cytogenetic analysis: Cytogenetic analyses of bone marrow samples were performed using conventional metaphase analysis. At least 20 metaphases were analyzed and 10 of them kary-

Table 1. Clinical characteristics of the AML M6 patients

Pt. no	*Age*	*Sex*	*Fever*	*Jaundice*	*Asthenia*	*Bleeding*	*Lymphadenopathy*	*Organomegaly*	*Pallor*
1	52	F	-	-	+	-	-	-	+
2	30	M	-	+	+	-	-	-	+
3	40	F	+	-	+	+	-	-	+
4	53	F	+	-	+	-	-	+	+
5	20	M	-	-	+	-	+	+	+
6	46	M	+	-	+	-	-	-	+
7	16	M	+	+	+	+	-	+	+
8	28	M	-	-	+	-	-	-	+
9	65	M	+	-	+	-	-	-	+
10	30	M	-	-	+	+	-	+	+
11	40	M	+	-	+	-	-	+	+
12	23	M	-	-	-	+	+	+	+
13	35	M	+	+	+	-	-	-	+
14	42	M	+	+	-	-	-	-	+

+ Present. – Absent.

otyped. Chromosome identification and karyotyping followed the International System for Chromosome Nomenclature (ISCN, 1985; 1995).

The karyotypic aberrations are divided into three categories as normal, minor karyotypic aberrations ([MIKA], i.e., less than 3 chromosomal abnormalities), and major karyotypic aberrations ([MAKA], i.e., 3 or more chromosomal abnormalities).

RESULTS

A total of 326 AML patients were diagnosed in this period, of whom 14 patients had AML M6. The clinical profile of the patients is shown in Table 1.

The hematological parameters are presented in Table 2.

DISCUSSION

AML M6 is a distinctive bone marrow disorder characterized by the neoplastic proliferation of the dysplastic erythroid elements mixed with blasts of myeloid origin. There are very few reports of this entity from India. A single case in 10 years of follow up of the TMH data [7], 20 cases in the series from AIIMS Delhi[3], and 10 cases from Chandigarh have been reported [5]. It is relatively uncommon and accounts for 3-5% of all de novo AMLs and 20-30% of secondary leukemias. However, in the present series there was not even one case of secondary EL, and the contribution of EL to all forms of AML was 4.3%, which is in agreement with the existing literature [8-14].

It usually presents in the 5th decade, but Mazzella *et al.*[12] described two peaks, one in the seventh decade of life and a second, smaller peak in the fourth decade of life. Although rare in children, reports exist from the newborn period through the age of seven years. In the present series, the mean age was 37.1 +13.9 yrs (range: 16-65), and most of the patients were in their fourth decade. The reason for this could be that all the cases in the present series were de novo. In most of the series, due to unknown reasons, males have been predominant, as in the present series, with a male:female ratio of 3.67:1.

Clinical profile: The signs and symptoms of EL are nonspecific and are due to the replacement of bone marrow by leukemic cells. Patients rarely present with symptoms lasting longer than six months, and they are usually diagnosed within 1-3 months after the onset of symptoms. The mean duration of symptoms in the present series was 10.9+6.9 weeks, which is consistent with the existing data [5,8,14,16]. The symptoms in the present series were asthenia (100%), fever (61.5%), jaundice (23.1%) and bleeding (15.3%). Surprisingly, none of our patients had significant bone pains, which have been reported in 33% of all cases [5,8,14,16,17].

Table 2. Hematological parameters of patients with AML M6 at presentation

Pt no	*Hb (g/dl)*	*WBC ($x10^6$/dl)*	*Plt ($x10^6$/dl)*	*BM Mega**	*No. of series with Dysmyelopoiesis*	*WHO classification*	*Cytogenetics*
1	6.2	9900	73,000	Adequate	2(E+Me)	M6a	Not done
2	4.5	2300	29,000	Reduced	3(E+My+Me)	M6a	46,XY
3	4	3800	95,000	Adequate	3(E+My+Me)	M6a	46,XX
4	8	3100	318,000	Adequate	1(E)	M6b	46,XX
5	4	8000	150,000	Adequate	2(E+My)	M6a	46,XY/ t (3:5)
6	10.5	6000	100,000	Adequate	3(E+My+Me)	M6a	46,XY
7	8	2000	10,000	Reduced	1(E)	M6a	46,XY
8	8.5	10000	120,000	Adequate	2(E+My)	M6b	45,XY /-7
9	7	4500	160,000	Adequate	1(E)	M6a	ND
10	10	8000	19,000	Reduced	2(E+Me)	M6a	46,XY
11	4.4	18200	39,000	Reduced	3(E+My+Me)	M6b	46,XY /-5
12	3.8	16000	42,000	Reduced	2(E+Me)	M6a	46,XY
13	5.6	5,600	38,000	Reduced	2(E+My)	M6b	46,XY
14	6.8	6,900	150,000	Adequate	1(E)	M6a	46,XY

BM Mega*: Bone marrow megakaryocytes.

Table 3. Clinical characteristics of the patients with AML M6 in the present series compared to other series

Series	*No. of patients*	*Organomegaly*	*Lymphadenopathy*	*Anemia*
Tsuji *et al.* 1995[15]	20	2	ND	18
Mazzella *et al.* 1998[13]	21	15	ND	18
Kowal-Vern *et al.* 2000[10]	19	13	ND	ND
Olopade O 1992[14]	26	10	ND	26
Present	14	6	2	14

Table 4. Morphological characteristics of the bone marrow smears in AML M6

	Present series	*Domingo-Claros et al.*[18]
Megaloblastic	86%	85%
Inter-nuclear bridging	36	17
Multi-nuclearity	72%	65%
Dysplasia	100%	100%
Hypocellularity	14%	3%

Examination findings included splenohepatomegaly, lymphadenopathy and pallor. The incidence of these findings in the present study was compared with other studies and the data are shown in Table 3 [10,13-15].

Hematological parameters: Morphological diagnosis was based on bone marrow and peripheral smear findings. The presence of large numbers of erythroid cells showing varying degrees of dysplastic features (100%), megaloblastic change (86%), multinuclearity (72%), internuclear bridges (36%) and nuclear budding (64%) were observed. The erythroid cells showed a difference in granular PAS positivity. The megakaryocytes showed dysplastic feature as mononuclear form and 50% cells with disparate nuclear dysmyeloplastics was seen in granulocytic series showed hypogranularity and hyporegimentation. Blasts with or without Auer rods showed MPO positivity. Diagnosis was made based on WHO criteria. However, the morphological characteristics were not widely studied in the reported series. We compared our findings with one of the largest studies and the results are shown in Table 4. [18]

The patterns described to date by FAB identify EL as AML M6 only. But a lot of research has since been done by Mazzella *et al.*[12,13]. The proposed pattern was based partly on the old FAB criteria and also upon morphological, cytochemical, and immunophenotypical criteria. But the most consistent and important is the morphological pattern based on the percent of the erythroblasts and myeloblasts. In 1997, WHO proposed a new subclassification that recognizes two subtypes, M6a and M6b. A third subtype, M6c, was added in 1998.

In M6a, there are 50% or more erythroblasts and 30% or more non-erythroid elements (i.e., myeloblasts I, myeloblasts II, monoblasts). In M6b, the erythroid component seems to be singularly involved. This subtype is characterized by the presence of 50% or more erythroblasts and pronormoblasts, and 30% or more basophilic erythroblasts. Together, these erythroid components may involve 90% of bone marrow. The myeloblast count is usually less than 30%, and distinguishing the myeloblasts from primitive erythroblasts is difficult. For this reason, Auer rods are never observed in this subtype. M6c (mixed type) is 50% or more erythroblasts, 30% or more non-erythroid components, and 30% or more pronormoblasts.

When treated with the standard myeloid protocol, the M6a and M6c subtypes demonstrate a very high remission rate, whereas most patients with the M6b subtype remain refractory to treatment. Notably, patients with the M6c subtype remain in remission for a significantly shorter time than the M6a group. Mean survival for these subtypes is: M6a, 31.4 (SD, 32) months; M6b, 3.15 (SD, 4.2) months; M6c, 10.5 (SD, 12.7) months. In the present series, we found three cases of M6b and 11 cases of M6a, and not even a single case of M6c. Myelodysplastic

Table 5. Prognostic implication of cytogenetic profile in AML M6

Prognosis	*Good*	*Intermediate*	*Poor*
Karyotypes	t(8;21), +14.	Normal karyotype Not fitting into either of the two	5/5q,-7/7q-, inv3, 11q,17p, del20q, +13, t(9;22), or >2 abnormalities.

Table 6. Cytogenetic profiles of the patients with AML M6

	Present	Olopade *et al.*[14]	Colita A *et al*.[20]	Sakurai M *et al*.[19]	Davey FR *et al.*[22]	Wells *et al.*[8]	Cuneo *et al.*[11]	Cigudosa JC *et al.*[21]
No of patients	14	26	54	17	52	33	20	23
Normal	10	6	11	ND	13	17	2	0
MIKA	3	3	9	ND	5	4	4	0
MAKA	0	17	15	8	9	6	14	23
No data	1	0	19	ND	25	6	0	0

MIKA: Minor karyotypic aberrations. **MAKA:** Major karyotypic aberrations.

changes were observed in at least one cell lineage in all cases, in a minimum of two cell lineages in 10 cases, and in all three lineages in 4 cases. The frequency is not different from that described in the literature.

Cytogenetic analysis: The cytogenetic analysis in patients with EL is critical for determining the diagnosis and prognosis of disease as with other leukemias. Based on the current knowledge, their prognostic implication was tabulated in Table 5.

Comparison of the cytogenetic abnormalities of the present study revealed that the relative frequency of MAKA was significantly low in our study. The comparison is shown in Table 6 [8,11,14,19-2].

The cytogenetic abnormality pattern is quite varied in the above-mentioned series, with a majority of the studies showing a dominance of MAKA. The incidence of the normal karyotype in various series ranged from 0-65%, which was not different from the present series (71%), as was the case with MIKA (0-25%, present series 21%). However, MAKA, which was observed at rates of 22-100% in all the series, was not observed in our series. In a population based study in the United Kingdom, the frequency of the normal karyotype was similar to that observed in our population[8]. We are of the opinion that in the rest of the studies, which were not population–based, secondary (to either MDS or therapy-related) AML-M6 was higher and thus more karyotype abnormalities could be expected. A number of chromosomal abnormalities were described by various authors, of which 5q and 7 were more common in some subsets of the population[14], involvement of chromosomes 11 and 19 in de novo patients[20], and various other non-specific translocations. In the present series, we had a single case each of chromosome 5 and 7 deletions (probably secondary AML-M6) and a case of t (3:5) which was not described previously. The rest of the cases had normal karyotype, the reason for which (very high incidence of normal karyotype) needs to be explored.

Following the WHO classification, Kowal-Vern *et al.*[10] reported that the M6b subtype is associated with the highest number of MAKA. In the remaining subtypes (M6a&c), equal distribution among normal, MIKA, and MAKA was observed. M6a has an intermediate prognosis, M6b has an unfavorable prognosis and the shortest survival time, and M6c has an intermediate-to-unfavorable prognosis. Due to poor follow-up and non-uniformity in treatment received, we did not study the survival in our study.

Relative low incidence of secondary EL, more frequent normal karyotype, and relatively younger age observed in our series makes the picture of EL in our subcontinent different from that reported in other studies thus far.

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