FREQUENCY OF FAB SUBTYPES IN ACUTE MYELOID LEUKEMIA PATIENTS AT AGA KHAN UNIVERSITY HOSPITAL KARACHI

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease. Therefore, various parameters are needed to classify this disease into subtypes, so that specific treatment approaches can be utilized effectively. The commonly used method for diagnosis and classification is based on FAB criteria using morphology and cytochemical stains. For some of the categories, immunophenotyping is necessary. The aim of present study is to determine the frequency of various sub types in acute myeloid leukemia using FAB criteria in our population. This will aid in the correct diagnosis of acute leukemia and hence proper management of the patients. Materials and Methods: This is descriptive case control study conducted at Aga Khan University Hospital from January 1999 to December 2000. The total number of subjects was 116 that included both adults and children. The patients were diagnosed on the basis of bone marrow morphology using FAB classification. Cytochemistry was done in all cases, while immunophenotyping was considered only in those cases that were found to be problematic. Results: Among 116 patients, 70 were males and 46 were females with male to female ratio 1.5:1. The age ranged between 6 months to 85 years with a mean age of 32 years. AML-M4 was the predominant French-American-British (FAB) subtype (36.2%) followed by M2 (30.25%), M3 (10.4%), M1 (8.7%), M0 (7.7%), M5a (3.5%), M5b (2.5%) and M6 (0.8%). Conclusions: The most common FAB subtype observed in our study was Acute myelomonocytic leukemia (M4) which is in accordance with studies reported from Saudia Arabia and a previous study reported from our institution. However,other national and international studies have reported Myeloblastic Leukemia with maturation (M2) as the predominant subtype of AML.

Keywords: French-American-British (FAB) classification, Acute myeloid Leukemia (AML), subtypes.

INTRODUCTION

Acute myeloid Leukemia is a heterogeneous disease. Therefore parameters are needed to classify this disease into biologic entities to understand its pathogenesis and develop specific treatment approaches.[1, 2] As therapeutic advances were made, distinguishing the subtypes of acute leukemia became increasingly important.

Acute leukemias are classified on the basis of the presumed cell of origin.Concordance between experienced observers in the classification of acute leukemia increases from 70 to 99% when morphologic criteria are supplemented by cytochemical and immunphenotypic information.[3]

The modern era for classification of acute leukemias dates back to 1976, when international group of investigators from France, America and Britain developed a uniform classification system designated as French-American-British (FAB) classification[4], which was subsequently revised in 1985.[5]

The FAB classification of AML divides cases into eight major groups with subtypes for three of them (table 1). The classification criteria are based on morphologic and cytochemical features; however for some of the categories, immunophenotyping is necessary.[6, 7] It is lineage-based morphological classification that categorizes cases according to the degree of maturation of the leukemic cells and their lineage differentiation. The major

advantage of the FAB classification system is its ease of use. The cytological criteria are well defined; they do not require high technology and can be applied in most laboratories through out the world.[8] Keeping in view these advantages, the FAB proposal was adopted internationally. It provided long needed standard terminology and was quickly accepted by most of the multi-institutional study groups for management plans and comparison of treatment results between morphologic subtypes for their prognostic significance.

Present study was done to determine the frequency of AML types in our population. As the patients were from all over Pakistan belonging to various ethnic groups, our study thus well represent AML subtypes of the entire country.

Although, it is a single institution based study, the number of cases studied is largest that is ever reported at national level. Hence, our study tends to establish a trend of AML subtypes in Pakistan.

MATERIAL AND METHODS

This is descriptive case-control study done at hematology department of Aga Khan University Hospital. This is a tertiary care hospital receiving cases from all over the country. The study was conducted over a period of 2 years from January 1999 to December 2000.

Patients with history of previous hematological disorders like myelodysplasia, CML, aplastic anaemia and with history prior chemotherapy or radiotherapy were excluded from the study. The patients who were known cases of AML with or without treatment including relapsed cases were also not included.

Table-1: FAB Classification of AML

Myeloblastic leukemia minimally differentiated	M0
Myeloblastic leukemia without maturation	M1
Myeloblastic leukemia with maturation	M2
Hypergranular promyelocytic leukemia	M3
• Microgranular variant Myelomonocytic leukemia	M4
• With bone marrow eosinophilia	M4Eo
Monocytic Leukemia	M5
• Poorly differentiated	M5a
• Differentiated	M5b
Erythroleukemia	M6
Megakaryoblastic Leukemia	M7

Based on this, a total of 116 subjects with newly diagnosed untreated denove AML were included in the study. It included patients of all age groups and both sexes.

The diagnosis of AML was established according to the standard practice, and was based on peripheral blood and bone marrow morphology and cytochemistry. Immunophenotyping was done where considered essential. Hematological parameters were done on Coulter counter (model stak-s).

Bone marrow aspiration was done from posterior iliac crest. A written consent was taken from patients or parents as appropriate. In every case, 6-8 smears were made; two of them with peripheral smears were stained by Leishman's stain. In addition following cytochemical stains were carried out on peripheral blood and bone marrow smears in each case: Sudan blackB (SBB), periodic acid-Schiff (PAS), myeloperoxidase (MPO) and alpha naphthyl acetate esterase (ANAE) by commercially provided kits from Sigma Diagnostic according to manufacturer's instructions. Immunophenotyping was carried out in some patients with diagnostic difficulties and was performed by 2 color flow cytometric analysis of bone marrow aspirate or peripheral blood specimens with a Becton Dickinson (Mountain View, Calif) FAC Scan instrument. After mononucleaer cell enrichment by centrifugation over Histopaque-1077 (Sigma, St. Louis Mo), samples were studied for surface antigen expression using a panel of 15 monoclonal antibodies supplied by (Becton Dickinson, San Jose, Calif and Dako Denmark).

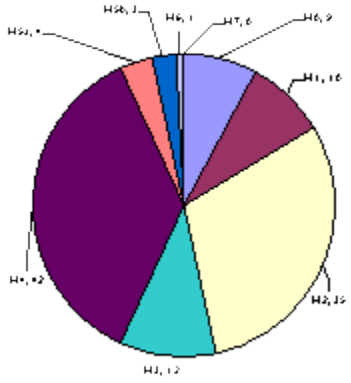
All the cases were reviewed by first and third author independently. On the basis of morphology, cytochemistry and immunophenotyping, AML was classified into its various subtypes. (M0-M7) using FAB criteria. (Table-1)[8.] The presence or absence of Auer rod in each bone marrow film was also noted.

RESULTS

Of 116 cases, 70 were males and 40 were females with male to female ratio1.5:1. The mean age was 32 years (range 6 months –85 years). 21 cases were up to the age of 15years comprising of 17 males and 4 females and their age ranges between 6 months- 15 years. Their haematological parameters are given in table 2. The CBC showed a wide range of variation in hemoglobin concentration and platelets ranging from subnormal to normal. Their Leukocyte count also showed variation from leucopenia to hyper leukocytosis.

In our study, we found AML (M4 FAB subtypes) to be the commonest comprising 42 out of 116 of total cases (36.25%). This also included 6 cases of M4Eo variant. The frequency of various AML subtypes according to FAB classification is given in Figure 1.

Figure 1: **French –American –British (FAB) Sub types in 116 cases of AML**



The cases were further divided in adult and children group. Both groups revealed M4 as a most common FAB type followed by M2. Relative frequencies of various subtypes for adults and children are illustrated in Table 3.

Auer rods were present in 36.2 %(42 cases). They were more common in M3 subtype (9/12, 75%) followed by M2 (18/35, 51.4%), M4 (12/42, 28.5%), M1 (2/10, 20%) and M5 (1/7, 14.2%).The number of Auer rods per cell was consistently greater in M3 subtype. No Auer rods were observed in the M0 or M6 subtype.

Table-2: Haematological parameters in AML patients (n = 116)

	Mean	Range
Hemoglobin (gm/dl)	8.4	2.2-14.4
White Cell Count (10^9/l	63.39	0.6-497.4
Platelet count (10^9/l)	48.9	1.0-270.0

Table 3: **FAB distribution of AML according to age group (n= 116)**

Fab Group	< 15 Years n %	> 15 Years n %
M0	05 (23.8)	04 (4.2)
M1	01 (4.7)	09 (9.4)
M2	06 (28.5)	29 (30.5)
M3	00 (0)	12 (12.6)
M4	07 (33.3)	35 (36.8)
M5 a	00 (0)	04 (4.2)
M5 b	01 (4.7)	02 (2.1)
M6	01 (4.7)	00 (0)
M7	00 (0)	00 (0)
Total	21 (100)	

DISCUSSION

With the introduction during the late 1960s and 1970s of increasingly effective therapy for acute leukemias, it became necessary to determine subgroups, which might require different treatment approaches. In 1976 FAB system [4] of classification was introduced, which was subsequently revised at various times to improve concordance.[5, 6, 7] This system provided structured criteria for the diagnosis of various sub types of AML and is based mainly on morphological and cytochemical features; for some of the categories, immunophenotyping is necessary.[8,9] Since than it has been widely adopted nationally and internationally for classification of acute leukemias, although ambiguities still give rise to confusion and it has been the subject of recent criticism.[10] So, in 1997 a revised WHO classification of AML was published which included cytogenetic studies as well.[11] However this classification is not practiced widely at national level because of financial constraints. The clinical and biological significance is also claimed for this system which accounts for distinct prognostic differences for various subtypes and their close association with chromosomal abnormalities. Although FAB proposals may be considered over simplification, but they do serve to provide the guidelines for both hematologists and non specialists and facilitate quick, consistent diagnosis and classification of these diseases. It is for these reasons that FAB is still favourite and popular among Pakistani hematologists.

The FAB distribution of AML has been extensively studied in the past decades at national and international levels.[9,12-19] In table 4 the adult results are compared with other series using FAB system. Most published data indicate the predominance of M2 as a most common subtype.[9.14-16,18] Occurrence of this subtype is also common after primary malignancy.[20] However, two studies from Saudi Kingdom reported predominance of M4 and

M5 subtypes.[13,17] Nakase et al showed AML-M4 as common subtype in Australian population compared to Japanese, where AML-M2 is common.[21] Present study also confirms M4 as the most common type followed by M2. This is in concordance with the previously published results from our institution by Kakepoto et al.[19]

Many of the differences in AML subtypes may be due to the subjectivity of morphologic diagnosis together with variable nature of acute myeloid leukemia subtypes, with no real demarcation. Some genetic factors may be responsible for a particular FAB subtype of AML in our population. Secondly most studies at national level have small number of patients and probably with underutilization of cytochemical stains. Moreover these studies were not subjected to immunophenotyping that may be because of error in diagnosis. The other reason for this discrepancy may be patients of different ethnic group and/or geographical variation.

Auer rods were seen in 36% of cases with highest frequency in M3. Spence et al [13] reported Auer rods in 40.6% of cases in their series. These results are consistent with present study.

Male to female ratio in present study is 1.5:1, which is in concordance with national and international studies.[14,16,17,22] The mean age (32 years) at presentation seems to be lower than the expected mean age reported in western countries where AML peaks in incidence after the 6th decade of life.[23] However, this is similar to mean age reported in studies from Saudi Arabia [17] and Pakistan.[16,19,24] The difference of mean age between present study and western studies is probably due to different geographical distribution.

Table 4: FAB Classification of AML in various centers in adults

Author/ Year/ No of Cases	M0%	M1%	M2%	M3%	M4%	M5%	M6%	M7%
Swirsky et al [12] 1986 (U.K) n= 619	00	30	25	5	23	13	2.4	00
Spence et al [13] 1988 (KSA) n= 121	1.7	1.7	14.9	8.3	57.8	13.2	1.7	00
Raina et al [14] 1990 (Libya) n= 54	00	7	57	15	13	4	4	00
Hassan et al [15] 1993 (Pak) n= 62	1.6	22.5	32.2	9.1	22.5	8.6	1.6	1.6
Chaudry et al [16] 1993 (Pak) n=54	00	13	44.4	11.1	24	3.7	3.7	00
Harakati et al [17] 1998 (KSA) n=52	00	2	4	17	40	33	00	04
Khalidi et al [18] 1998 (USA) n=78	8.9	19.2	27.0	9.0	20.5	11.5	2.6	1.3
Kakepoto et al [19] 2002 (Pak.) n=74	00	8.1	16	15	46	9.5	00	2.7
Arber et al [9] 2003 (USA) n=255	7.0	19.2	28.67	8.7	26.7	4.8	2.5	2.4
Present Study n= 95	1.2	9.4	30.5	12.6	36.8	6.3	00	00

CONCLUSION

In conclusion the most common type observed in our study was Acute Myelomonocytic Leukemia (M4) followed by Acute Myeloblastic Leukemia with maturation (M2). Although demographic features is not the aim of our study but this is in accordance with other national studies.

A multi-institutional study with large sample size from other areas of Pakistan is needed to confirm our findings. It is desirable that more than one experienced observer should examine the material before a final diagnosis is made. A full range of cytochemical stains is essential with immunophenotyping studies where appropriate for proper identification of the acute leukemias.

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REFERENCES

1. Tien HF, Wang CH, Lin MT, Lee FY, Liu MC, Chuang SM, et al. Correlation of cytogenetic results with immunophenotype, genotype, chemical features and ras mutation in acute myeloid leukemia: a study of 235 Chinese patients in Taiwan. Cancer Genet Cytogenet 1995;84:60-8.

2. Schoch C, Haferlach T. Cytogenetics in acute myeloid leukemia. Curr Oncol Rep 2002;4:390-7.

3. Browman GP, Naeme PB, Soamboonsrup P. The contribution of cytochemistry and immunophenotyping to the reproducibility of the FAB classification in acute leukemia. Blood 1986;68:900-5

4. Benett JM, Catovsky D, Daniel MT Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of acute leukemias. Br J Haematol 1976;33:451-8.

5. Bennett JM, Catovsky D, Dariel MT Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of FAB Cooperative Group. Ann Intern Med 1985:103;626-9.

6. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Criteria for the diagnosis of acute leukemia of megakaryocytic lineage (M7). A report of the French-American-British cooperative Group. Ann Intern Med 1985;103;460-2

7. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposal for the recognition of minimally differentiated acute myeloid leukemia (AML-MO). Br J Haematol 1991;78:325-9.

8. McKenna RW. Multifaceted approach to the diagnosis and classification of acute leukemias. Clin Chem 2000;46:1252-9

9. Arber DA, Stein AS, Carter NH, Ikle D, Formen SJ, Slovak ML. Prognostic impact of acute myeloid leukemia classification. Importance of detection of recurring cytogenetic abnormalities and multilineage dysplasia on survival. Am J Clin Pathol 2003;119:672-80.

10. Head DR. Revised classification of acute myeloid leukemia. Leukemia 1996;10:1826-31.

11. Harris NL, Jaffe ES, Diebold J,Flandrin G, Muller-Hermelink HK,Vardiman J, et al. World Health Organization classification of neoplastic diseases of hematopoietic and lymphoid tissue. Report of the clinical advisory committee meeting Airlie House, Virginia, November 1997.J Clin Oncol 1999;17:3835-49. .

12. Swirsky DM, de Bastos M, Parish SE, Rees JK, Hayhoe FG. Features affecting outcome during remission induction of acute myeloid leukaemia in 619 adult patients. Br J Haematol 1986;64:435-53.

13. Spence DG, Roberts GT, De Vol EB, Clink HM, Andrew Padmos M. Acute myeloid leukemia in Saudia Arabia: Morphologic classification using FAB subgroups. Ann Saudi Med 1988;8:179-84

14. Raina V, El-Habbash KI, Tenkovsky. Acute nonlymphoblastic leukemia in adults: experience in Tripoli Libya. Ann Saudi Med 1990;10:299-302

15. Hassan K, Qureshi M, Shafi S, Ikram N, Akhtar MJ. Acute myeloid leukaemia- FAB classification and its correlation with clinico- heamatological features. J Pak Med Assoc 1993; 43:200-3

16. Chaudhry MT, Tayyab M, Faooqi IA.Acute nonlymphoblastic leukemia in adults. J Pak Med Assoc 1993; 43:259-61

17. Harakati MSE, Al-Momen AM, Ajarim DS, Al-Moharib FI, Al- Theyab A, Fawzy EM, et al. Adult acute myeloblastic leukemia: Experience at King Khalid University Hospital. Ann Saudi Med 1998;18:221-5

18. Khalidi HS, Medeiros LJ, Chang KL, Brynes RK, Solvak ML, Arber DA. The immunophenotye of adult acute myeloid leukemia: high frequency of lymphoid antigen expression and comparison of immunophenotype, French-American-British classification, and karyotypic abnormalities. Am J Clin Pathol 1998;109:211-20.

19. Kakepoto GN, Burney IA, Zaki S, Adil SN, Khurshid M. Long-term outcomes of acute myeloid leukemia in adults in Pakistan. J Pak Med Assoc 2002; 52: 482-6

20. Pagano L, Pulsoni A, Tosti ME, Avvisati G, Mele L, Mele A, et al. Clinical and biological features of acute myeloid leukemia occurring as second malignancy: GIMEMA archive of adult acute leukemia. Br J Haematol 2001; 112:109-17.

21. Nakase K, Bradstock K, Sartor M, Gottlieb D, Byth K, Kita K, et al. Geographic heterogeneity of cellular characteristics of acute myeloid leukemia: a comparative study of Australian and Japanese adult cases. Leukemia 2000; 14: 163-8.

22. Sultan C, Deregnaucort J, Ko YW, Imbert M, D'Agay MF, Gouault-Heilman M, et al. Distribution of 250 cases of acute myeloid leukemia (AML) according to the FAB classification and response to therapy. Br J Haematol 1981; 47: 545-51

23. Mauritzen N, Johansson B, Albin M, Billström R, Ahfgren T, Mikoczy Z, et al. A single center population-based consecutive series of 1500 cytogenetically investigated adult hematological malignancies: Karyotype features in relation to morphology, age and gender. Eur J Haematol 1999; 62: 92-102.

24. Khalid A, Zahid M, Rehman A, Ahmad Z U, Qazi S, Aziz Z. Clinico- epidemiological features of adult leukemias in Pakistan. J Pak Med Assoc 1997; 47:119-22.

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