WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 51-58. Review Article ISSN 2277– 7105

CYTOCHEMICAL STAINS BENEFIT IN THE DIAGNOSIS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Article Received on
18 May 2015,

Revised on 12 June 2015,
Accepted on 06 July 2015

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ABSTARCT

Introduction: Acute leukemia is the most common type of childhood malignancy, acute lymphoblastic leukemia account for approximately 78%of all childhood leukemia. cytology and cytochemistry remain fundamental in diagnosis as it does not require high technology and can be applied in most laboratories throughout the world. **Aims of the study:** To evaluate the PAS positivity in bone marrow aspirate of newly diagnosed ALL children, to assess the positivity in each subtypes of ALL. and to evaluate the benefit of the special stain use in establishing diagnosis. **Patients and method:** bone marrow smears from newly diagnosed 122 patients all are children with mean age 5±3.06y and of range (6m-13y) with acute lymphoblastic leukaemia (ALL), the slides, were stained by the periodic acid-Schiff (PAS) and SBB, and the blast cell positivity was assessed quantitatively. the ALL cases were sub classified into L1,L2,L3 according to FAB classification and the special stains positivity for each subtype was mentioned. **Results:** the total number of patients was 122, out of 122 patients 89(73%)was positive for PAS stain ,33(27%) patient was negative .all 122 patients were negative for SBB. the patients further sub-classified into L1,L2,L3. the PAS positivity in ALL-L2 is 84% and this is good for hematologist as its more difficult in diagnosis than ALL-L1, while L3 is rarely positive. **Conclusion:** the cytochmical stains are still of much help to reach diagnosis of acute leukemia as the overall PAS positivity was 73%.

KEYWORDS: ALL, Cytochemical stain, PAS, SBB.

INTRODUCTION

Acute leukemia is the most common type of childhood malignancy ,its account for 30 % of all cancers diagnosed at children younger than 15 years. within this population acute lymphoblastic leukemia(ALL) account for approximately 78%of all childhood leukemia.[1,2,3,4] so it is the most common malignancy that is seen by the hematologists working at pediatric hospitals.

Despite advances in other areas ,careful microscopical examination of wright 's stained films remains fundamental in hematological diagnosis.[5] in addition to the combined techniques of cytochemistry and flow cytometry which currently used for definite diagnosis.[3,6,7,8]

The studies around the world still use the special stain as worth test beside the flowcytomrty.[8] The cytochemistry together with flow cytometry immunophenotype is worthwhile using for diagnosis and subclassification of acute leukemia.[3] Most cases can be appropriately designated as either AML or ALL by the addition of cytochemistry to the morphologic assessment.[7] and can establish lineage in 95% of the cases.[9] Sushma B. etal. show in their study that concordance rate as high as 86% between morphologic/cytochemical diagnosis and flowcytometric diagnosis. Of these, complete concordance was seen in 58% of the cases and partial concordance was seen in 22% of the cases. Non-concordance was seen in only 4% of their cases.[8] Mehawech P. etal show in their study that , cytochemical staining should be available for those cases in which flow cytometry results fail to allow a definitive diagnosis. In that study two patients with inconclusive flow cytometry results, cytochemical staining alone provided information sufficient for diagnosis.[11]

The Periodic Acid Schiff (PAS) reactivity is specific but less sensitive for ALL diagnosis. The sensitivity of a cytochemical-staining combination of PAS positivity and myeloperoxidase, Sudan black B(SBB) , and alpha-naphthyl butyrate esterase negativity in defining cases of lymphoblastic leukemia remained at 52%; however, the specificity of this combination for lymphoblastic leukemia was 100% (no false positives).[10]

Sudan black B(SBB)

Is the most commonly used and the most valuable in distinguishing AML from ALL.[6,7] This stain is specific for lipids, including neutral fat, phospholipids and sterols. Normal granulocyte precursors show increased sudanophilia that corresponds roughly to the number

of granules. Promyelocytes contain a few sudanophilic granules, and mature polymorphonuclear neutrophils contain large numbers of sudanophilic granules.[12]

Periodic acid–Schiff (PAS)

Is a staining method used to detect polysaccharides such as glycogen, and mucosubstances such as glycoproteins, glycolipids and mucins in tissues. PAS staining can be used to assist in the diagnosis of several medical conditions.[13]

Aims of the Study

This work was aimed to evaluate the benefit of the special stains in establishing diagnosis of ALL by assessing the PAS positivity in bone marrow aspirate of newly diagnosed ALL children and the positivity in each subtypes of ALL.

Patients and Methods

A Prospective study was done ,the patients described were seen at Central Child Teaching Hospital in Al-Iskan, Baghdad, Iraq from January 2014 to April 2015.

One hundred twenty two (122) patients with acute lymphoblastic leukemia were included in this study, all patients were diagnosed by clinical examination and laboratory investigations by leishmen's and cytochemical stains, PAS and SBB. and sub classified into L1, L2 and L3 according to FAB classification.

SBB

The SBB stain was manually prepared according to the procedure described by dacei and lewis:[13,14]

Fixative: Vapour from 40% formaldehyde solution.
Stain: SBB 0.3 g in 100 ml absolute ethanol.
Phenol buffer: Dissolve 16 g crystalline phenol in 30 ml absolute ethanol then add to 100 ml. distilled water in which 0.3 g Na2HPO4.12H2O has been dissolved.
Working stain solution: Add 40 ml buffer to 60 ml SBB solution.
Counterstain: Leishman stain.

PAS

A modified procedure used which was easier and give very good stain quality.[14]

Dissolve 1g of basic fuchsin in 200m l of boiling distilled water. (note: remove the flask from heat before adding the basic fuchsin).Allow the solution to cool to 50°C and add 2g potassium metabisulfite with mixing.

Allow to cool to room temperature then add 2ml concentrated HCL, mix and add 2g activated charcoal and leave overnight in the dark at room temperature .Next day filter the solution and it should be clear. Store in a dark container at 4°C.

Tow observers assessed the PAS reaction product in all cases by each counting tow hundred blast cells at least on each slide. Results were recorded as a percentage of such cells showing granular or block PAS-positivity.

The 5% is the cutoff point below which considered negative, above it considered positive.

Statistical Analysis

All statistical analysis were performed using Microsoft office excel,2007.the results were expressed as mean ± SD and percentage.

RESULTS

In this study 122 cases of ALL included with mean age of 5±3.06y and age range of (4m-13y) .Age distribution in ALL subtypes classification (FAB classification) is shown in table 1. We found that ALL-L3 presented at higher age group(6.6 ± 3.25y) than L1(4.9 ± 3.2y) and L2(4.85 ± 2.9y), while no cases of L3 seen below 1 year of age (the lowest age was1.17y), and the L2 seen in this study at a higher age range than L1. (Table.1)

Table (1): Age distribution and ALL subclass.

ALL subclass	Age range	Mean ± SD of age (year)
L1 (n= 59)	4 m – 11 y	4.9 ± 3.2
L2 (n=54)	11 m – 13 y	4.85 ± 2.9
L3 (n= 9)	1.17 – 11 y	6.6 ± 3.25
Total (n= 122)	4 m – 13 y	5 ± 3.06

The gender distribution seen in table (2), the male incidence were higher (56.6%) than female (43.4%) with male/female ratio 1.3/1 in this table about half of the patient was ALL-L1 (48.4%) while ALL-L2(44.2%) and ALL-L3(7.4%).

Table (2): Gender distribution according to ALL subclass.

Gender	L1	L2	L3	Total
Female	28 (47.5%)	22 (40.7%)	3 (33.3%)	53 (43.4%)
Male	31 (52.5%)	32 (59.3%)	6 (66.7%)	69 (56.6%)
Total	59 (48.4%)	54 (44.2%)	9 (7.4%)	122 (100%)

Out of the 122 cases included in this study, 89 cases (73%) were positive for PAS, 46 patients of L2 (85.2%) were positive for PAS ,42 case of L1 out of 59(71%) were positive for PAS. Cases of L3 show minimum positivity being 1 out of 9 cases(11.1%) (Table 3).

Table (3): show the PAS positivity in correlation with the ALL subclass

ALL subclass	PAS positive	PAS Negative
L1 (n=59)	42 (71.2%)	17 (28.8%)
L2 (n=54)	46 (85.2%)	8 (14.8%)
L3 (n=9)	1 (11.1%)	8 (88.9%)
Total (n=122)	89 (73 %)	33 (27 %)

DISCUSSION

In this study we collect 122 newly diagnosed ALL cases for one year and 5 months in the Central child Teaching Hospital in Baghdad. the availability of special stain make diagnosis much easier in many cases , those of granular ALL, those cases of ALL/L2 subtype which resemble AML/M1,and encourage our knowledge about.

In this study males comprised 56.6% of the total cases of pediatric ALL and females 43.4%, with a male/female ratio 1.3/1 agree with ameens study which was 1.6/1 [16] and balsam *etal* 1.7/1.[2] lisa L.H. *etal*. 1.14/1,[17]1.17/1 zuhair *etal* ,[18] all show male predominance.

By FAB classification ALL-L1 is the commonest (59%) .This is perfectly matched with khallid H.*etal* which was 59%.[19] in one study and also matched with another study by Khalid *etal* which was(61%).[20]

ALL-L2 coming next (54%) in this study ,for Khalid H. *etal* (34.3%) .[20] but zuhair *etal* show that L2 seen more in Iraqi patients being (89%).[18] (this was the same of our early practice) which is unlike the international reported cases, this may be due to the diagnosis of

ALL-L1 only for microblast morphology so that is why used to diagnose 90% of ALL as ALL-L2 but on revision of FAB classification for ALL-L1 :small in size, scant cytoplasm, inconspicuous nucleolus, variable cytoplasmic vacuoles.ALL-L2: show large heterogeneous in size, moderately abundant cytoplasm ,prominent nucleolus and variable cytoplasmic vacuoles in addition the cytological feature of the FAB L1 category of acute lymphoblastic leukaemia (ALL) are very likely to indicate that the diagnosis is ALL, this is not so of FAB L2 type ,which can be cytologically very similar to AML of FAB M0 or M1 subtype.[21]

We used to depend on the ALL score ignoring the previous important statement. The ALL-L3 was the lowest, being (9%), which agree with Khalid H. *etal* which was 3.3%.[20] and 5.4% in other study.[19]

Regarding the PAS positivity,in the current study the PAS positivity was 73% ,it is higher than J S Lilleyman *etal*. which show the positivity of 60% at their study.[15] but less than ekcol M.*etal*. who show PAS positivity in ALL of 82.3%.[3]

About 85.2% of ALL- L2 show PAS positivity which is higher than ALL-L1(71%) positivity, this observation is the reverse of J S Lilleyman *etal*. which stated that the (PAS negative) patients included a disproportionate excess of those with L2 morphology.[15]

The ALL-L3 cases show the minimum positivity, being 11% only. Keneth in his book.[22] show that ALL-L3 was negative for PAS, but Barbera J.Bain say it is usually negative.[23] ,rare reported cases being positive for PAS.[24] in agreement with this study in which a single positive case out of 9 ALL-L3 .

CONCLUSIONS

1- In our practice in Iraq we see acute lymphoblastic leukemia with male predominance for all subtype.
2- ALL-L1 is the commonest subtype seen, followed by ALL-L2 and ALL-L3 minimally seen.
3- We see ALL-L1 at lower age group (4M-11Y) than L2 (11M-13Y), and no L3 below 1 year in this study
4- The PAS positivity is making 73% of all ALL in children cases mostly in L2 (85%) than L1 (71%), and rare in L3 (11%).

5- Special stain are still helpful in the diagnosis of acute leukemia even with the availability of flowcytometry.
6- The cheap price and the easy use of these stains makes it the first choice for a hematologist to give the provisional diagnosis, and to decide if the diagnosis need further more sophisticated tests.

RECOMMENDATIONS

The stains should be available at any general hospital as it is simply prepared, interpreted and diagnostic.

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