DIAGNOSTIC ACCURACY OF CYTOCHEMISTRY TAKING FLOW CYTOMETERY AS GOLD STANDARD IN DIAGNOSING ACUTE LEUKEMIA.

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Abstract

Background & Objective: Acute leukemia are malignant neoplastic diseases that arise when the hematopoietic stemcells have malignant transformation into undifferentiated cells permanently. Flow cytometry is the gold standard to diagnose acute leukemia but it is available at few laboratories among private and public setups in Punjab and that too is very costly. Cytochemistry is an alternative and relatively cheap option which is available at most of the setups. So this study was planned with an aim to evaluate the diagnostic accuracy of cytochemistry using Sudan black B and Periodic Acid Schiff. stains, for acute leukemia diagnosis in a resource-constrained laboratories.

Methods: After meeting the inclusion criteria and taking appropriate ethical review board approval ,80 patient were enrolled in this cross sectional analytical study. Detailed history and physical examination with complete blood cells count and numerous hematological indices were obtained by using semi- automated analyzer; SYSMEX KX-21. Slides were made by using peripheral blood / bone marrow smears, fixed by methanol and stained for different cytochemical stains. Leukemia cell analysis was done by using the conventional immunofluorescence method by using the monoclonal antibodies directed against cMPO, CD2, CD5 , CD7 , CD 10 , CD11 c , CD 14 , CD 19 ,CD 20, CD33 , CD 34 , CD 45 , CD 64 , CD 79a, HLA-DR , KAPPA, LAMBDA and TdT. The results of flow cytometry and cytochemistry were noted in a pre-designed proforma and results were entered in SPSS for analysis.

Results: The mean age of patients included in the study was calculated to be 39.40±14.76 years, and male to female ratio was 1.3:1. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of cytochemistry using SBB stain for diagnosis of acute leukemia was 91.94%, 100%, 100%, 78.26% & 93.75% respectively taking Flow cytometry as gold standard. Similarly, the sensitivity, specificity, PPV, NPV and diagnostic accuracy of cytochemistry using PAS stain for diagnosis of acute leukemia was 100%, 91.94%, 78.26%, 100% & 93.75% respectively taking flow cytometry as gold standard

Conclusion: According to this study, the cytochemistry using both SBB and PAF stains showed good accuracy for acute leukemia. Therefore, it may substitute Flow ctometry in many cases, in restricted resource settings.

Keywords: Cytochemistry, Sudan black B , Periodic Acid Schiff. stains

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Acute leukemia is a kind of cancer that develops when a hematopoietic stem cell transforms into a primitive, undifferentiated cell with an abnormally extended lifespan. Acute leukemia is divided into two types: acute lymphoblastic and acute myeloblastic leukemia. Because acute leukemia is a diverse collection of cancers with different clinical, morphologic, immunologic, and molecular features, it requires different treatment and prognosis.[1]

AML is a kind of aggressive haematological cancer that can affect people of any age group but mostly adults. AML is the most common type of leukemia in adults in the Western world, accounting for around 25% of all leukemias in adults. Male to female ratio was 1.5:1 in a research done by Naeem et al. in Lahore in 2017. Pancytopenia was observed in 12 individuals (15.5%).[2] Acute lymphoblastic leukemia (ALL) is a clonal illness that causes malignant growth and lymphoblast accumulation in the medullary cavity. Due to differences in disease biology and related genetic anomalies, adult acute lymphoblastic leukemia is a heterogeneous illness.[3,4]

There are currently just a few cancer registries in Pakistan that keep track of the prevalence and incidence of various cancers, including leukaemia. Advances in flow cytometry technology, as well as the availability of a wider spectrum of antibodies and fluorochromes, have increased our capacity to detect various cell populations, even when they are present in only a tiny percentage within complex of the cells examined.[5,6] With broad acceptance of the 2016 World Health Organization categorization, the criteria used to define different disease entities have been refined during the last decade. Immunophenotyping by flow cytometry in acute leukemia is routinely advised for lineage assignment of cells populations, for example the analysis of immune cells in a liquid medium.[7,8]

This study aims to evaluate the diagnostic accuracy of cytochemistry using Sudan black B and Periodic Acid Schiff stains, for acute leukemia diagnosis in a resource constrained laboratory. Keeping in view the resource constraints and avid demand in our country, such a study may also help to develop guidelines more suitable for our healthcare facilities.

METHODS

It was a cross-sectional analytical study carried out at department of Pathology, Allama Iqbal Medical College from January to December 2021 after approval from ERB (49th/ERB JHL). About 80 cases of suspected acute leukemia were selected using nonprobability consecutive sampling. Patients referred from JHL Oncology Department and those presenting directly to Hematology department, AIMC, with a provisional diagnosis of acute leukemia constituted the study population. All patients with provisional diagnosis of acute leukemia for the first time and Age group of 5-65 years belonging to both genders were included in the study. Patients on treatment of leukemia and relapsed or follow up cases of leukemia were excluded.

Under aseptic measures, 2-3 cc of venous sample was collected from the subjects in EDTA vacutainers. Samples were processed the same day. SYSMEX KX21 was used for CBC. Using aseptic techniques, the bone marrow samples were obtained from posterior superior iliac spine of children aged more than two years. The site was cleaned with pyodine and then anesthetized. A local anesthetic, lidocaine injection (5cc), was given in the subcutaneous tissue, intramuscular area and the periostium. After an interval of five minutes, 11 gauge bore bone marrow needle was introduced by boring movements. After fixing the needle in the marrow, stylet was removed and 60 cc syringe was attached to the top of the needle. Negative pressure was applied and bone marrow aspirate was collected. Part of the aspirate was immediately spread on slides and the other part was collected in EDTA vacutainer and was sent for flow cytometry. The prepared slides of smear were fixed by using methanol and were stained by using different cyto-chemicals stains (PAS & SBB). For both these stains, commercially available kits (MEDLINE for SBB and MERCK for PAS) were used. BD Fluorescence Activated Cell Sorter CALIBUR cytometer, having four colored -dual lasers was used within 12 hours of sample collection, to process BMAs and peripheral blood samples for flow cytometric analysis.

Results from the flow cytometry were also recorded in terms of CD markers positivity or negativity, along with the deduction. Data was then analyzed statistically to gauge the diagnostic accuracy of the cytochemistry in diagnosing acute leukemia.

IBM SPSS Statistics 24 was used for data analysis. Mean ± Standard Deviation were given for numeric variables i.e. age. Frequency and percentages were given for type of leukemia, diagnosed by flow cytometric

immunophenotyping and Cytochemistry. PPV and NPV were calculated to assess the diagnostic accuracy of cytochemistry. Data was stratified for type of leukemia, age and gender for diagnosis by Cytochemistry and chi-square test was used to evaluate the significance. P-value ≤ 0.05, was considered to be statistically significant.

RESULTS

The mean age of the patients was 37.52 years and their ages ranged from 5 to 65 years.

Total 80 newly diagnosed Acute Leukemia cases including 43 (53.75%) males and 37 (46.25%) females, were included in the study. The male to female ratio was 1.3:1. The summary statistics of Complete blood count (CBC) of these patients is given in table 1. In terms of peripheral smear findings, the mean number of peripheral smear blast of the patients was 60.47 ± 23.96%, the mean neutrophils of the patients was 22.90 ± 10.21%, the mean lymphocytes of the patients were 30.67±20.76%, the mean eosinophils of the patients was 2.30±1.19%, the mean red blood cell of the patients was 4.24±1.17 and the mean number of blasts in bone marrow was 70.31±20.38%. Among these 80 cases, 56(70% in total & 90.3% among AML cases) were positive for SBB while 6 AML cases (7.5%) were negative by cytochemistry for SBB but were diagnosed as of Myeloid origin on flow cytometry. 5 of these cases were later diagnosed as M0and 1 case as M1. 2 cases (3.2%) reported as M2 on cytochemistry were later categorized as M4 on flow cytometry. No discrepancies were seen in diagnosis of AML-M3 through cytochemistry using SBB. All acute lymphocytic leukemia cases were positive for PAS. Although 5 M0 cases also stained positive/partial positive with PAS giving aberrant results.

Table 1: *Summary statistics of CBC parameters*

	Mean	Standard Deviation	Minimum	Maximum
HB	7.36	1.51	3.30	11.20
Red blood cell count	3.89	1.04	1.86	5.90
MCV	90.35	8.14	75.40	105.00
MCH	27.52	1.35	24.80	35.00
PTLRDW	5.60	7.31	0.16	31.89

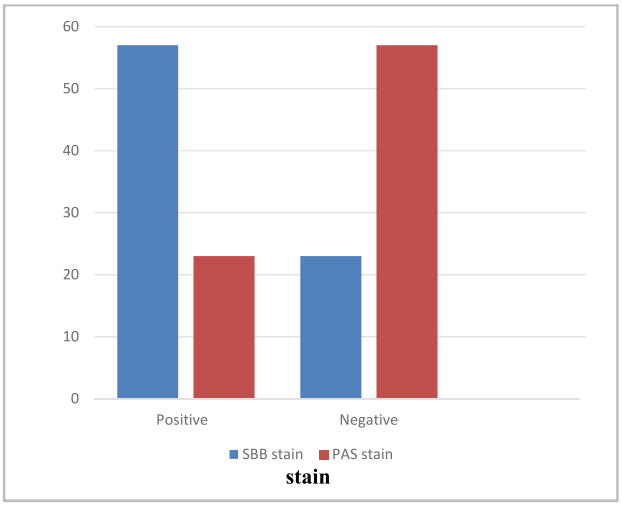

Figure 1: *Frequency distribution of detection of acute leukemia by using SBB and PAS stain.*

This study showed that the cytochemistry using SBB stain diagnosed positive acute leukemia in 57 (71.25%) patients while the cytochemistry using PAS stain diagnosed positive acute leukemia in 23(28.75%) patients (Fig 1). Along with the ALL cases, it was positive in cases of M0.

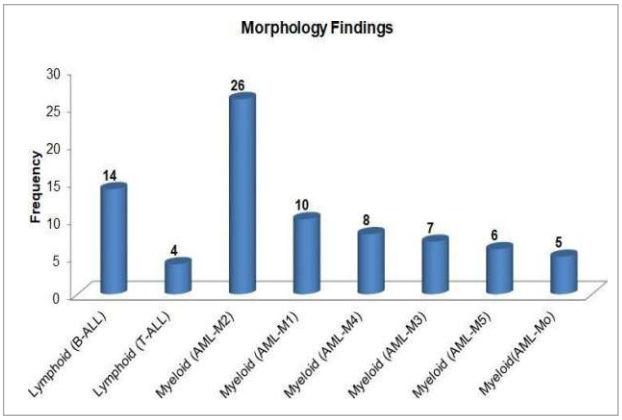

Figure 2: *Distribution of different subcategories of acute leukemia*

B- ALL was reported in 14 patients and T-ALL in 4 patients. Among myelod leukemia, AML-M2 cases predominated with 26 cases (32.5%) followed by AML-M1in 10 (12.5%), AML-M4 in 8 (10%), AML-M3 in 7 (8.75%), AML-M5 in 06 (7.5%) , and AML-M0 in 5 (6.3%) as shown in figure 2. No cases of AML-M7, AML-M6 were reported during this study. Presence of Auer rods helped in diagnosis of 17

Table 2: *Validity of Cytochemistry using SBB stain and PAS for detection of acute leukemia taking Flowcytometry as gold standard*

Acute leukemia		Flowcytometry for Myeloid CD markers		Total
		Positive	Negative	
Cytochemistry using SBB stain	Positive	57 100.0%	0 0.0%	57 100.0%
	Negative	5 21.7%	18 78.3%	23 100.0%
Total.		62 77.5%	18 22.5%	80 100.0%
Cytochemistry using PAS stain	Positive	18 78.3%	5 21.7%	23 100.0%
	Negative	0 0.0%	57 100.0%	57 100.0%
Total		18 22.5%	62 77.5%	80 100.0%

patients.

According to this study's results, the sensitivity, specificity, Positive Predictive Value, Negative Predictive Value and diagnostic accuracy of cytochemistry using SBB stain for diagnosis of acute leukemiawas 91.94%, 100%, 100%, 78.26% & 93.75% respectively taking Flowcytometry as gold standard. The study results showed that the sensitivity, specificity, PPV, NPV and diagnostic accuracy of cytochemistry using PAS stain for diagnosis of acute leukemia was 100%, 91.94%, 78.26%, 100% & 93.75% respectively taking flowcytometry as gold standard. (table 3)

Table 3: *Diagnostic validity of cytochemistry taking flowcytometry as gold standard*

Diagnostic Validity	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
SBB	91.94%	100%	100%	78.26%	93.75%
PAS	100%	91.94%	78.26%	100%	93.75%

DISCUSSION

Chloroacetate Esterase, α-naphthyl Acetate Esterase, double esterase, MPO, SBB, PAS, are among some of the cytochemical stains. In the vast majority of instances, these methods can distinguish between lyphoid and myeloid lineages. While in decline, the cytochemical staining technique is a straightforward and cost-effective method for diagnosing acute leukemia.[9]

Cytochemical staining was detected in 89.5 percent cases, according to a study[8]. In 30 of the 36 AML patients, MPO was positive (83.33 percent). In 29 of 35 instances, SBB was positive (82.9 percent). For the 31 patients (100%) with acute lymphoblastic leukemia, both responses (MPO and SBB) were negative (ALL). 31 cases of ALL (100%) were reported to be positive for PAS along with one case of AML (3.2%). This PAS positive AML case was positive for MPO and SBB as well and on morphological examination, had monoblasts. The staining of Acetate Esterase non-specific esterase processed in 10 instances of probable AML cases indicated positive in 7 out of 10 cases, according to immunophenotyping (70 percent).[8]

One study by Deghedy et al. found that cytochemicals stains (specifically MPO & SBB) are helpful in diagnosing acute leukemia, particularly the Myeloid type. The significance of these cyto-chemicals is specifically important in unindustrialized countries, because they are simple and no special equipment or exceedingly proficient healthcare professionals are required.[9]

In their investigation, Samir et al., discovered that PAS stain was negative in 20% of the lymphoid cells.10 Although some studies have shown negative in some instances of T-ALL, this response has granular positivity for lymphoblasts.[11]

PAS in conjunction with negative MPO and SBB, continues to play an essential role in distinguishing lymphoblastic from myeloblastic leukemia, and immunophenotyping backs this analysis.[12]

In their study, author showed that positive findings of PAS-stain, when in combination with negative findings of myeloperoxidase, SBB, and α- naphthyl butyrate esterase stains, can still distinguish ALL from AML. The PAS stain alone has a sensitivity and specificity of 52 percent and 81 percent for ALL, respectively. The sensitivity of cyto-chemical staining in combination with PAS-stainpositive findings and negative findings of myeloperoxidase, SBB and α-naphthyl butyrate esterase in detecting the occurrences of ALL

was still 52 percent[1].

In developing countries, where Flow cytometry is yet not available or scarcely accessible, Cytochemistry for SBB/ MPO, and PAS play a significant role in diag-nosing acute leukemia patients.

CONCLUSION

Incidence of acute leukemia is rising day by day. Flow cytometry is the gold standard for its diagnosis but financial constraint is a huge factor for common man, especially in our set up, in going further with the expensive diagnosis even before start of treatment. In underdeveloped countries, where the whole burden of disease and financial burden lies with the patient and his family, sustainable, affordable and accessible techniques and tests need to be researched, with a perspective for 'Health for All'. The results of this study shows that cytochemistry can be used for diagnosis of acute leukemia being a low cost and workable technique that can be easily made available even at primary & secondary healthcare level.

Ethical Approval:
The ethical Approval was obtained vide letter no. 49th /ERB JHL

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