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**CYTOCHEMICAL CHARACTERISTICS OF ACUTE AND CHRONIC BLOOD  
CANCER**

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**ABSTRACT**

Lymphoblastic leukemia based on cell morphology dominant coloring Cytochemical into three main groups: 1L, 2L, 3L classify, Although the clinical value of the expected lifespan in adults is unknown, but in terms of prognosis and clinical course and response to treatment methods in children is important. 109 peripheral blood samples from patients suspected of Razi Hospital Hematology Center was developed leukemia, and follows Cytochemical staining was performed on each sample, 19 samples by alpha-naphthalenpropanoic acetate esterase staining positive 15/78 percent and 84/21 percent were negative. Of the 12 samples analyzed by acid phosphatase staining was found that 25% of the samples were positive and 75% negative, also the 25 samples that were analyzed by alkaline phosphatase staining, 100% of the negative and none of the samples were positive, and 34 samples were examined by staining Periodic acid shifts 97/55% of the negative samples and only 2/94 percent positive,

and from 12 samples by staining was evaluated Naphthile AS-D 16/66% positive and 83/33% of the samples were negative. The diagnosis of leukemia, the disease situation and select pin for the treatment of all cases with morphological characteristics of the cells was not possible, therefore, to reach the correct diagnosis Cytochemical tests and immune cell phenotype and cytogenetic studies are needed.

**Keywords: Acute and Chronic Lymphoid Leukemia, Cytochemical, Prognosis**

## INTRODUCTION

Hematologic malignancies involving bone marrow and lymph node diseases that originate, early disorders, including leukemia and bone marrow and immunoproliferative diseases (eg Myeloid Malignancy) and myeloproliferative syndromes (eg, myeloid metaplasia with myelofibrosis and Polycythemia) Yue et al. (2015). How to treat a patient with Polycythemia absolutely must be done through the logic to determine if patients with Polycythemia secondary or primary (Polycythemia Vera), (Shrestha S., 2013). The history and physical examination, the patient may not respond to this question, a man with lung and chest, wheezing, cyanosis and severe chronic obstructive pulmonary disease, barrel that is the speed of a patient HET (Paul Toric) with splenomegaly with Polycythemia Vera is most likely, will be diagnosed respectively (Yamamoto K et al., 2015), (Sharma P et al., 2014). Simple laboratory data in most patients with Polycythemia Vera are high, however, most patients with Polycythemia secondary

normal. Despite the low level of oxygen saturation, because that Polycythemia is a compensatory mechanism to hypoxemia, therefore, history, physical examination and laboratory investigation, will differentiate between primary and secondary Polycythemia, of course, sometimes the cause is not obvious Polycythemia secondary may need more tests to find the cause is rare. The rare causes include abnormal hemoglobin with increased affinity to oxygen, renal cysts secretion of erythropoietin, Hemangioblastoma brain, and hepatocellular carcinoma, Polycythemia Vera in the normal control of cell production in the bone marrow is removed, although the erythroid involved and this involvement will increase in red cell mass, the increased production of granulocytes and platelets in most cases it can be seen, Polycythemia Vera has some differences with other myeloproliferative clinical symptoms (ie, myelofibrosis with myeloid metaplasia, essential thrombocythemia, chronic myelogenous leukemia), Yue et al. (2015).

## MATERIALS AND METHODS

Sterile speculum, Sterile cotton swab, Glass slides, diamond stylus, ethanol, methanol and acetone, absolute homogeneity, toluidine blue staining kit. Alpha-naphthalenpropanoic acetate esterase kit, Naphthol AS-D chloroacetate esterase, acid phosphatase, PERIODIC-ACID SCHIFF (PAS), all of Manufacture, Sygma- Aldrich company, German product, Olympus company microscope imaging.

### Methods Sampling

Knowing documentation Razi Hospital in coordination with the previous sampling methods were referred to the hospital, and from those for blood disorders (leukemia) were referred for biopsy, biopsy was performed. Attending random cluster sampling, immediately after sampling, and drying the slides fixation solution, all the samples were fixed.

### Preparation of samples

Peripheral blood samples were taken every 11 samples were prepared slides, all slides were coded by a diamond pen, the dried slides temporary fixation with a solution that is 1 volume of ethanol, 1 volume of methanol and acetone was prepared 3 shares were fixed, and then slide the boxes we filing, and the Laboratory of Immunology we moved Lahijan Azad University Branch. to investigate the pattern of leukocyte 1 gram of series slides

stained with toluidine blue method, according to the morphology of the white blood cells, white blood cells, staining was noted in the population under study is preliminary, so if there is corruption in collecting samples so check white blood cells on is inefficient to investigate the differential diagnosis of adult cells based on pattern recognition Classic 5 cells, white blood cells, Naphthol AS-D chloroacetate esterase staining, alpha-naphthalenpropanoic acetate esterase, Acid phosphatase, periodic acid-Schiff with the books of commercial construction company Sigma-Aldrich was used.

### Check expand stained

It should all leukocyte cells and adult groups correctly and without error and the ability to separate high from each other be identified. The appearance of Morphological white blood cells in the development of stained, cytochemical staining and patterns observed in Cytochemical staining was compared and a broader pattern of white blood cells to be identified pentavalent. Open the painting that did not feature a suitable extension of the study subjects were excluded.

### Morphological changes of cells

All morphological changes of cells, including cell quality, inflammatory changes, stained with toluidine blue and examined.

### Check leukocyte cell

Cytochemical staining for evaluation of leukocyte cells are used, and the samples under a saw microscope. Stained with toluidine blue, acid phosphatase, neutrophils are observed. Eosinophils by Periodic acid-Schiff staining, and acid phosphatase, under an optical microscope with a 100× lens were observed. To view basophils with light microscopy of Periodic acid-Schiff staining and toluidine blue test. Mast cells in the toluidine blue and PAS staining visible, Monocytes by alpha-naphthalene propane acetate esterase and Naphthol AS-D chloroacetate esterase staining stained are staining. Lymphocytes by stain, alpha-naphthalene propane acetate esters, Naphthol AS-D chloroacetate, acid phosphatase, are stained. That's all positive and negative results for acute lymphoblastic leukemia as a percentage of results can be expressed.

### Photography

Open stained learn ways to take pictures of each of the white blood cells were used. All stained slides were examined and the typical image of each leukocyte cell of any Use Olympus optical microscope with conventional CCD a magnification of 100X with a calibrated optical 100 and microscope was harvested imaging software was harvested and Software database was coded.

### Toluidine blue staining

Slides with a solution of 1 volume of ethanol, 1 volume of methanol, and 3 volumes of acetone fixed, after transfer to the laboratory slides ready to put on a tray staining, and Toluidine blue on the grape fruit throw, after 15 to 20 minutes offer with water slides, after drying the slides can be viewed with the microscope.

### Naphthol AS-D chloroacetate esterase staining

In this method, proven solution by mixing 18 ml of citrate solution, and 27 ml of acetone and methanol (5 ml) was built, and the slides were placed in a minute, the solution to be prove, then washed with deionized water, and then a solution of 6/3 Trizymal by dissolving one part of concentrated buffer 6/3 Trizymal to 9 parts of deionized water was prepared, and added to the asalt capsule V fast Corrientes and then 2 ml naphthol AS-D chloroacetate esterase solution, added and mixed for 30-15 seconds. all slides were prepared solution for 5 min. were washed with deionized water for 3 minutes. Naphthol AS-D chloroacetate solution by dissolving a capsule Naphthol AS-D chloroacetate in 2 ml dimethyl formaldehyde obtained. painting background slides for 5 to 10 minutes in a solution of haematoxylin acid and then rinsed with running water and dried in the open air, target cells by light microscopy to

identify the specific and measurement were performed.

#### **Alpha-naphthalenpropanoic acetate esterase staining**

In this method, proven solution by mixing 18ml of citrate solution and 27ml of acetone and methanol (5ml) was built, and the slides were placed in one minute, the solution to be done was to prove, then rinsed with deionized water, then PH=7/6 Trizymal solution, by solving a 7/6 trizymal dense buffer to 9 parts of deionized water was prepared, and a capsule RR salt, and then add 2ml naphthalenpropanoic acetate was mixed for 20-15 seconds. All slides were prepared in the solution for 30 minutes and then washed with deionized water for 3 minutes. Naphthalenpropanoic acetate solution by dissolving a capsule naphthalene propanoic acetate in 2 ml of mono methyl ether glycol obtained, Painting background slides for 5 -10 minutes in a solution of haematoxylin and then washed in running water were dried in the open air. Target cells by light microscopy to identify species and micrometer measurements were examined and photographed.

#### **Acid phosphatase staining**

In this method, proven solution by mixing 25ml of citrate, 65ml of acetone, and 8ml of 37% formaldehyde was made, and the slides were immersed for 3 seconds in this

solution to be fixed, then washed with deionized water. Then the two tubes 5.0ml Fast Garnet GBC solution and 5.0 ml of sodium nitrite solution was poured and was mixed for 30 seconds. In a large glass jar, 45 ml of deionized water One ml Fast Garnet GBD solution that the Previously prepared 5.0 ml Naphthol AS-BI phosphate solution two ml of acetate was added, and the slides were placed for one hour in a Jar, after this time, washed with deionized water, and for coloring the background for two minutes in a solution of haematoxylin were placed. After washing with running water, dried in the open air, target cells by light microscopy to identify specific and micrometer measurements were examined and photographed.

#### **Periodic acid-Schiff staining**

In this method, fixation solution by mixing 5 ml of formaldehyde with 45 ml of ethanol 95° C was produced, the slides were exposed for one minute in a solution to the fixing operation to be performed, the slides were washed in running water for one minute and then placed 5 minutes in Periodic acid solution, the slides were washed well with distilled water. 15 minutes were Schiff solution, next 5min washing with running water, and in painting background were 90 seconds in a solution of haematoxylin, slides were washed in running water must be drained,

target cells by light microscopy to identify species and micrometer measurements were examined and photographed.

### **Alkaline phosphatase**

1-the first 45 ml of distilled water to bring the temperature 18 to 26 ° C.

2-then we prepared diazonium salt solution in which 1ml of sodium nitrite are added to 1ml of alkaline FRV.

3-and then the salt solution prepared in distilled water which had been prepared in the first step are added.

4-then 1ml Naphthol AS-BI to the diazonium prepared salt solution adding, and it into a glass jar poured, and well incorporate.

5-The sample is poured into jars and we wait 30 seconds and then the samples were washed with distilled water for 45 minutes, and then bring to the slides well dry, after all the samples in the baseline alkaline solution for 15 minutes, so stay from direct light, to get a good stain, because these stains are sensitive to light and the light loses its enzymatic activity and the disabled, after 15 min of incubation for 2 minutes were well washed with distilled water and bring to the dry slides, and then microscopic we evaluate the samples.

### **RESULTS**

Diagnosis of leukemia, to predict disease status and choice of treatment in all cases with the morphological characteristics of

the cells was not possible therefore, to reach the correct diagnosis Cytochemical tests and immune cell phenotype and cytogenetic studies are needed, knowing documentation Razi hospital in Rasht city in the context of sampling and in coordination with the previous was admitted to the hospital and from those for blood disorders (leukemia) were referred for biopsy, biopsy was performed Patients were randomized to and the cluster approach 11 slides from each patient (a total of 102 slides), blood samples were taken immediately after sampling and drying the slides fixation solution, all the samples were fixed. Peripheral blood was taken from each sample, 11 slides were prepared all slides were coded by a diamond pen are then the dried slides temporary with a fixation solution that is 1 volume of ethanol, 1 volume of methanol, and 3 volumes of acetone was prepared then fixed and then slide boxes Filing and we've moved to the Laboratory of Immunology Lahijan Azad University Branch to investigate the pattern of leukocyte 1 gram of series slides with toluidine blue stained method, according to the morphology of the white blood cells, staining was white blood cells, in the population noted under study is preliminary So if in collecting samples there is corruption so check white blood cells is inefficient on. To examine patterns and

differential diagnosis of adult cells based on pattern recognition Classic 5 cells, white blood cells, Cytochemical staining Naphthol AS-D chloroacetate esterase, Alpha-naphthalen propanoic acetate esterase, acid phosphatase, Periodic acid-Schiff staining Using the books of commercial construction company Sigma-Aldrich was used (fig 2).

Following results were obtained of the 19 samples analyzed by Cytochemical staining, Alpha-naphthalen propanoic acetate esterase 15/75 percent positive, and 84/21 percent were negative and 12 samples by acid phosphatase staining was found 25% of the samples over the stain positive and 75% were negative, and also the 25 samples that were analyzed by alkaline phosphatase staining, 100% of the samples were negative, and 34 samples were examined by staining Periodic acid shifts 97/05% of the negative and only 2/94 percent of the samples were positive, and from 12 samples by Naphthol AS-D staining was evaluated 16/66% positive and 83/33% of the samples were negative, in general it can be concluded staining was performed for acute lymphoblastic leukemia Single alpha-naphthalen propanoic acetate esterase, acid phosphatase, Periodic acid-shift, and Naphthol AS-D can show a positive reaction, that would be the staining

are specifically used for the diagnosis of acute lymphoblastic leukemia (fig 1, 2).

Cytochemical staining general results obtained indicate that it was:

Alpha-naphthalen propanoic chloro acetate or specific esterase (AChE) which acute lymphoblastic leukemia is negative, but in some cases of chronic lymphoid leukemia is positive.

PAS Stained, often with a rough granular cytoplasm has been PAS-positive acute lymphoblastic leukemia.

The positive reaction of the acid phosphatase is localized in 20% of cases of ALL can be seen, that the origin of leukemic T cells (T cell ALL) implies.

### **Discussion and Conclusion**

The FAB classification for acute leukemia has been the major system of classification for more than 20 years. This system provided structured criteria for the diagnosis of a variety of morphologic and cytochemical subtypes of acute leukemia. However, studies [1] indicate that the majority of categories in the FAB system do not delineate significant disease groups based on morphology and cytochemistry in terms of patient survival.

In our study FCA analysis in 50 cases of acute leukemia were analyzed and compared with their morphologic diagnosis.

cytochemistry did not aid in diagnosis and hence they opted for FCA to render a definitive

diagnosis. Hence, in their study, they stated that “Although cytochemical stains are essential to recognize the subtypes of AML, they are of limited use in differentiating the subtypes

of ALL and that the FCA has become a standard tool for the assessment and management of patients with leukemia.”

Acute leukemias being a heterogeneous group of malignancies varying in clinical, morphologic, immunologic and molecular characteristics and also in prognosis & specific therapy (fig 2).

Thus cytochemical analysis coupled with morphology can serve the purpose in the diagnosis of leukemias till immune-phenotyping and cytogenetics becomes available for everyone.

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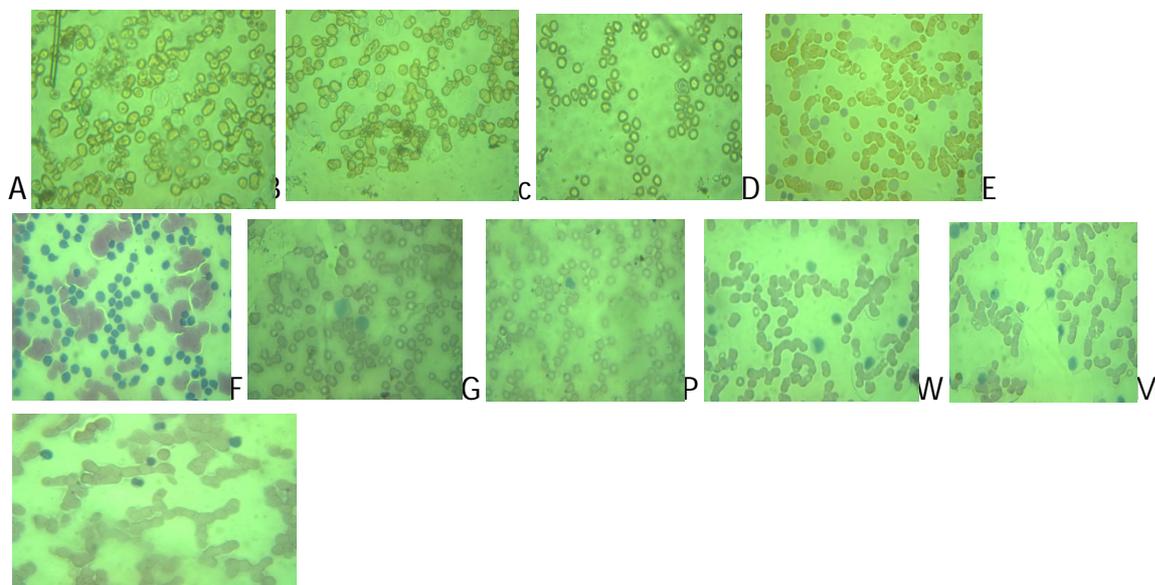
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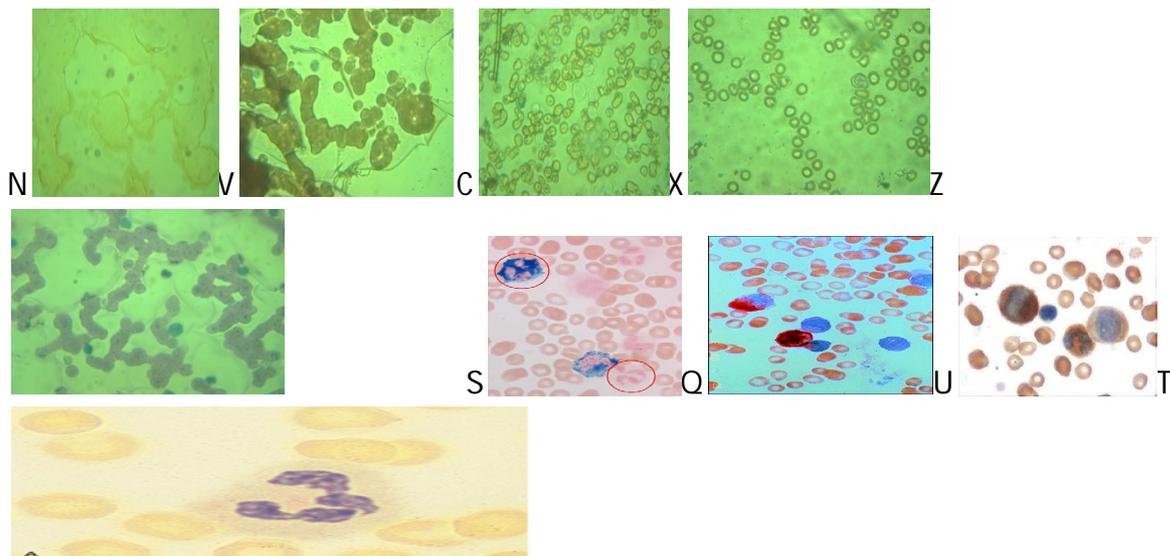


fig2; A: Alpha-naphthalenpropanoic acetate esterase staining (ANAE): Granulated positive T - lymphocytes and monocytes, B: Alpha-naphthalenpropanoic acetate esterase staining (ANAE): positive granules in B and T – lymphocytes, C: Alpha-naphthalenpropanoic acetate esterase staining (ANAE): positive reaction in monocytes, lymphocytes T cell positive, V: Alpha-naphthalenpropanoic acetate esterase staining (ANAE): positive granules in neutrophils and T - lymphocytes and T – prolymphocytes, X: Alpha-naphthalenpropanoic acetate esterase staining (ANAE): T and B lymphocytes positive reaction to platelets and cells positive for blasts- Alpha Color naphthalenpropanoic acetate esterase activity in monocytes show strong, D: Acid phosphatase (Aph): positive reaction in thrombocytes, lymphocytes, neutrophils and morphological characteristics Prolymphocytes and T - lymphocytes shows, Studies show that these colors in blast cells in the region show a positive reaction from the Golgi. T-CLL lymphocytes is also positive, Periodic acid shift (PAS):L: two blast cells with PAS have been extremely positive, one round and the other against the background of positive cytoplasmic granules, Z: for negative lymphocytes and neutrophils seem positively stained, E: For lymphocytes positive for positive thrombocytes, positive blast cells, F: Positive blast. As well as stained, G: Positive blast cells and Lymphocytes negative, P: Positive granules, W: Weak positive lymphocytes, M: Positive thrombocytes and Lymphocytes negative

Color Type	positive reaction	negative reaction
ANAE	15/78	84/21
APH	25	75
ALPH	0	100
PAS	2/94	97/55
NASD	16/66	83/33

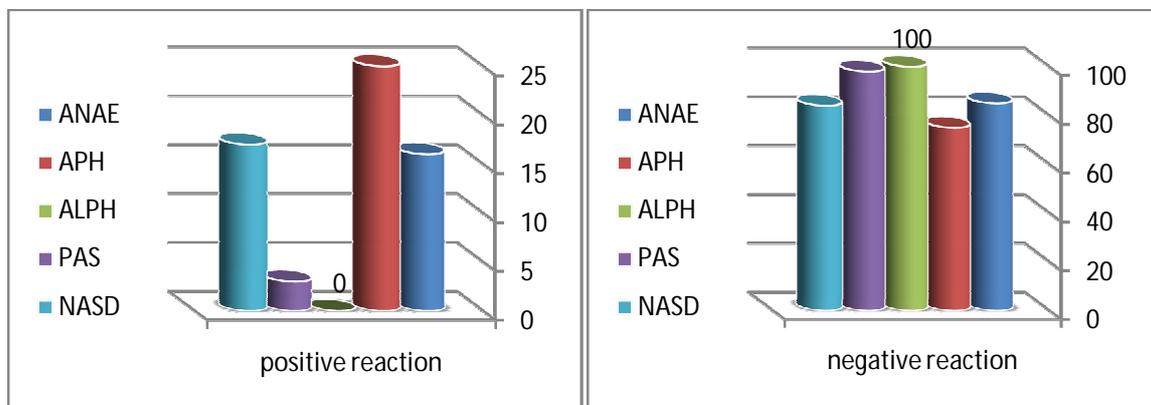


Figure 1: Cytochemical staining of positive and negative responses in lymphoid cells