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Original article

Application of Cytochemical staining in the diagnosis and classification of acute and chronic leukemia

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ABSTRACT

Background: 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. **Methods:** In the 109 peripheral blood samples from patients suspected of Razi Hospital Hematology Center was developed leukemia, and follows Cytochemical staining was performed on each sample. **Results:** In 19 samples by alpha-naphthalen propanoic 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. **Conclusion:** 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.

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1. Introduction

The recognition of subgroups of leukemias by morphologic and cytochemical criteria has greatly contributed over the past decades in the differential diagnosis, classification, and prognosis of the leukemic proliferations. In 1964 Hayhoe and his associates in a combined morphologic and cytochemical study of acute leukemias were able to determine four different types of leukemias: acute myeloblastic, acute myelomonocytic, acute lymphoblastic and erythremic myelosis. A few years later Schmalzl and Braunsteiner and Daniel et al. separated the acute monocytic leukemias from the other acute leukemias using the sodium fluoride sensitive naphthol AS-D acetate esterase reaction (NASDA + NaF). In 1976 Bennet et al. proposed the French-American-British (FAB) classification of acute leukemias based on morphologic and certain cytochemical criteria (myeloperoxidase and NASDA-NaF when necessary). In the FAB classification six classes of myeloid leukemias, were recognized on the basis of cell maturation and differentiation (table 1). This classification of

myeloid leukemias appears to have prognostic significance. In the same classification (FAB) three groups of lymphoblastic leukemias (L 1-L3) have been proposed. These subgroups of lymphoblastic leukemias did not correlate with immunologic and cytochemical markers, however. In recent years the simultaneous study of lymphocytic cell with morphologic, immunologic, and cytochemical methods resulted in the demonstration of significant qualitative and semiquantitative enzyme differences in normal, and leukemic B- and T-lymphocytes. As a result of these studies, T-cell lymphocytic proliferations, acute or chronic, could be recognized on the basis of the pattern and degree of positivity of the acid phosphatase, a-naphthyl acetate esterase and f3- glucuronidase reactions. In this chapter the practical aspects of certain cytochemical reactions are analyzed, on the basis of their contribution in the following: the differential diagnosis and classification of acute leukemias (myeloperoxidase, Sudan black B, naphthol AS-D chloroacetate esterase, NASDA + NaF, and periodic acid-Schiff); the recognition of leukemic lymphocytic cell subpopulations according to their immunologic phenotype (acid phosphatase, a-naphthyl acetate esterase and f3- glucuronidase), and the diagnosis and differential diagnosis of certain chronic hematopoietic disorders (neutrophil alkaline phosphatase and acid phosphatase resistant to tartrate).

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Cytochemical Reactions for the Differential Diagnosis and Classification of Acute Leukemias

Within the group of acute leukemias, the most common problem is the differential diagnosis of acute lymphoblastic leukemia (ALL), especially the L2 class of the FAB classification, from the acute myeloblastic leukemia (AML) without apparent morphologic evidence of granulocytic differentiation (M1 class of the FAB classification). It is also of interest to distinguish the myelomonocytic leukemia (M4 class), from the hypergranular promyelocytic (M3) class of acute myelocytic leukemia and the monocytic variant (Ms class) of the FAB classification. Finally it is important to confirm the monoblastic nature of the M5 acute monoblastic leukemia variant of the FAB classification, which is likely to be confused with acute myeloblastic or acute immunoblastic leukemia. For the differential diagnosis and classification of the leukemias just named, the application of the following cytochemical reactions may be necessary, myeloperoxidase, Sudan black B (SBB), periodic acid-Schiff (PAS), esterase with naphthol AS-D chloroacetate as substrate, esterase with naphthol AS-D acetate as substrate (NASDA), and esterase with naphthol ASD acetate as substrate and inhibition with sodium fluoride (NASDA + NaF).

Myeloperoxidase Reaction

Myeloperoxidase appears during the early stages of differentiation of the granulocytic series (from myeloblast to promyelocyte) and is localized in the lysosomes or primary granules. Neutrophils and eosinophils are myeloperoxidase strongly positive, whereas basophils are myeloperoxidase negative. This enzyme is also present in the cells of the monocytic series. The cytochemical demonstration of myeloperoxidase activity in the blast cells of acute leukemias without apparent morphologic evidence of myelocytic differentiation, such as the M1 class of the FAB classification, determine the myeloblastic nature of this proliferation (figures 1 and 2). Thus demonstration of myeloperoxidase activity in this type of leukemia is very important, since the M1 class may be confused with the acute lymphocytic leukemia (L1 and especially L2 of the FAB classification), which is by definition myeloperoxidase negative. Auer rods, which are considered to be abnormal lysosomes, when present are myeloperoxidase positive. The myeloperoxidase reaction is highly specific for the granulocytic and monocytic series. According to certain investigators more than 5% myeloperoxidase positive blast cells are required in order to classify a case of acute leukemia as myeloblastic. The percentage of positive blast cells may range from 5 to 100%, with faint to very strong positivity. This varying percentage of myeloperoxidase positive blast cells is probably due to maturation arrest of the abnormal leukemic clone at different stages of differentiation. In certain cases the enzyme may be localized in the paranuclear space, the endoplasmic reticulum, and in the Golgi apparatus, as has been shown by electron microscopy cytochemistry. Such observations explain the finding of myeloperoxidase positivity in blast cells without other morphologic evidence of granulocytic differentiation. Myeloperoxidase positivity may also be found in the acute monocytic leukemias (M5 class of the FAB classification). Deficiency of this enzyme has been demonstrated in the granulocytic series of patients with preleukemic states.

Sudan Black B Reaction

Although the exact nature of the SBB stain is not known, its cellular distribution is similar to that of myeloperoxidase, in both normal and leukemic cells. Therefore the SBB reaction may be used to discriminate the less differentiated type of myeloblastic leukemia (M1 class of the FAB classification) from the acute lymphoblastic leukemia (figures 1 and 3). The SBB stain is not as specific as the myeloperoxidase reaction, although it appears to be more sensitive. Its sensitivity is characterized by the fact that the percentage of SBB positive cells in acute myeloblastic leukemia may occasionally be higher than the percentage of myeloperoxidase positive cells. In rare cases of myeloblastic leukemia the myeloperoxidase reaction by light microscopy may be entirely negative while the SBB stain is positive. As in the myeloperoxidase reaction, more than 5% SBB positive blast cells are required in order to classify a case of acute leukemia as myeloblastic. Since the SBB stain is not an enzymatic reaction, blood and bone marrow smears may be used even if the slides have been stored at room temperature for several weeks.

Naphthol ASD Chloroacetate Esterase Reaction

The specificity of NASD chloroacetate esterase in the myelocytic series is similar to that of myeloperoxidase. The reaction, however, is less sensitive than the myeloperoxidase reaction and the SBB stain. This is probably due to the fact that this enzyme appears in the granulocytic series later than the myeloperoxidase. Therefore this reaction is not of help in the differential diagnosis of the M1 class of the FAB classification from the acute lymphoblastic leukemia. It is of great significance though, for the differential diagnosis of granulocytic sarcoma (chloroma) from histiocytic lymphoma on tissue sections, since this reaction can be applied in formalin fixed and paraffin embedded material. The cells of the monocytic series are NAS-D chloroacetate esterase negative. Therefore this reaction may also be used simultaneously with the NASDA esterase, for the differential classification of the hypergranular myelocytic leukemia (M3), the myelomonocytic leukemia (M4) and the monocytic variant of the Ms class of acute leukemia in the FAB classification (table 1).

Naphthol ASD Acetate Esterase Reaction

This esterase reaction demonstrates various degrees of cytochemical activity in most normal [63, 66] and leukemic cells. The cells of the monocytic series, however, are characterized by a strong NASDA positivity, which is extensively inhibited by sodium fluoride (NaF).

No evidence for NASDA inhibition by NaF exists in other normal or leukemic cells. Therefore this is a reaction of choice for the differential diagnosis of acute monocytic or monoblastic leukemia (Ms class of the FAB classification) from other types of acute leukemias (figures 4C-6C). The NASDA reaction occasionally is weaker in acute monocytic or monoblastic leukemia, but it always decreases significantly after the addition of NaF to the incubation media. Rare cases of monoblastic leukemia may be entirely negative for the NASDA reaction.

Periodic Acid-Schiff (PAS) Stain

The PAS reaction has been extensively used for the differential diagnosis of acute lymphoblastic from acute myeloblastic leukemias. The presence of a granular and/or globular pattern of PAS positivity against an entirely negative cytoplasmic background in a blast cell

population seems to be characteristic for ALL. This pattern of positivity is present in approximately 50 percent of the ALL cases. The remaining ALL cases are either PAS negative or they may contain a few fine scattered PAS positive cytoplasmic granules of no diagnostic significance. PAS positivity in the form of scattered cytoplasmic granules against a weak positive cytoplasmic background may also be found in cases of acute myeloblastic, myelomonocytic, and monocytic leukemias. These findings indicate that the PAS positivity in a blast cell population should be interpreted with great caution (table 1). Acute leukemias that present no morphologic evidence of granulocytic differentiation and that are myeloperoxidase, SBB negative and exhibit no NaF sensitive NASDA esterase reaction should be classified as ALL whenever they display a strong granular and/or globular PAS staining pattern. This group of PAS positive ALL, according to certain authors, has a better prognosis than the ALL, in which the PAS stain is negative or weakly positive. Granular or globular PAS positivity may also be found in the cytoplasm of the abnormal erythroblast in erythroleukemia cases (M6 class of the FAB classification).

Cytochemical Enzyme Markers Contributing in the Recognition / Leukemic Lymphocytic Cell Subpopulations According to Their Immunologic Phenotype

The parallel or combined use of immunologic markers and cytochemical methods in lymphocytic cells resulted in the demonstration of important observations in normal and leukemic lymphocytes regarding the pattern and degree of positivity of the following enzymes: acid phosphatase (AcP), a-naphthyl acetate esterase (aNAE) and f3-glucuronidase (f3-GLU) (table 1-2). Although the biologic significance of these differences is not yet clearly understood, they may be of diagnostic or prognostic value in certain subgroups of lymphocytic leukemias. The PAS reaction appears to be of no practical diagnostic significance in the classification of the lymphoproliferative disorders in relation to their immunologic phenotype.

Acid Phosphatase Reaction

This cytochemical reaction is positive in almost all hematopoietic cells, leukemic or not. Its pattern of positivity however is different in the various hematopoietic series. In the lymphocytic cells the end product of the enzyme reaction is granular and/or focal globular. The results of acid phosphatase positivity in normal B- and T lymphocytes are conflicting —. In the leukemic lymphocytic cells, however, a T immunologic phenotype is almost always combined with a focal globular pattern of acid phosphatase reaction (table 3); This pattern of AcP positivity has been found in the T acute lymphoblastic leukemia (T ALL) (figure 8) independently of the morphologic characteristics of the blast cells [16, 72], the T prolymphocytic leukemia (figures 10C and 11C) and in the T chronic lymphocytic leukemia (T CLL) (figures 13 and 14) [40, 57, 58]. In contrast, lymphoblasts from common ALL, from null ALL, from non-T, non-B ALL, from pre-T ALL, and from pre-B ALL are less frequently acid phosphatase positive (table 1-3). B leukemic lymphocytic proliferations are usually acid phosphatase negative. In cases of B chronic lymphocytic leukemia, however, acid phosphatase positivity may occasionally be found in a scattered granular form.

a-Naphthyl Acetate Esterase (aNAE)

This reaction identifies cells of the monocytic series whenever a strong diffuse and granular pattern of positivity is present. In the last few years it has been recognized that aNAE activity is also present in most normal T-lymphocytes with an intense focal globular paranuclear type of reaction using the standard or lower pH. The normal B lymphocytes are either aNAE negative or they may contain a few fine cytoplasmic granules. aNAE reaction is considered the best cytochemical enzyme marker for the discrimination of normal B- and T-lymphocytes. An intense focal globular paranuclear type of aNAE reaction, similar to that of normal T lymphocytes, has been observed in Tall (figure 9), pre-T ALL and T prolymphocytic leukemia (table 1-3). Common ALL may also be aNAE positive. Null ALL is aNAE negative, but in some cases the blast cells may contain scattered cytoplasmic granules of enzyme positivity. T CLL may be aNAE positive (figures 13 and 15) or negative. In B chronic lymphocytic leukemias, the lymphocytes are either aNAE negative or they may be positive with a granular reaction pattern.

The a-naphthyl butyrate esterase reaction at pH 8, appears to have the same specificity as the aNAE reaction, and therefore it may also be used as a marker for the discrimination of T-lymphocytic cells.

3-Glucuronidase (3GLU)

This lysosomal enzyme has been cytochemically studied in normal human blood lymphocyte subpopulations with variable results. In the normal T-lymphocytes a higher percentage of f3GLU positive cells was demonstrated as compared to normal B-lymphocytes, where the percentage of f3GLU positive cells was lower. When the f3GLU activity was expressed as degree of positivity (score), no significant difference was found between B and T normal blood lymphocytes. It appears that f3GLU cannot be used as a cytochemical marker for the discrimination of normal B- from T-lymphocytes. f3GLU activity is frequently reduced in the lymphocytes of B CLL, although in approximately 15% of the immunologically documented B CLL cases, increased enzyme activity has been reported. Strong f3GLU activity has been observed in all cases of T CLL in T prolymphocytic leukemia (figure 12) and in the circulating cells of the Sezary syndrome. In the B prolymphocytic leukemia, a decreased f3GLU activity was found. ~GLU reaction is usually positive in both T ALL and non-T, non-B ALL. However, this enzyme is not much help in the differential classification of the subgroups of acute lymphoblastic leukemia. Although an intense focal globular AcP, aNAE, and f3GLU reaction pattern is usually characteristic of T lymphocytic cell proliferation, this pattern of positivity is not pathognomonic of T leukemic cells since it may also be observed in common, null, and non-T, non-B ALL and in cases of acute myeloblastic leukemia.

Cytochemical Enzyme Markers for the Diagnosis of Certain Chronic Leukemias

The enzyme reactions that have been successfully used for the diagnosis of certain chronic leukemias are the neutrophil alkaline phosphatase (NAP), for the differential diagnosis of chronic myelogenous leukemia from myelofibrosis with myeloid metaplasia and the acid phosphatase resistant to tartrate (AcPT), for the diagnosis of hairy cell leukemia (table 1-2).

Neutrophil Alkaline Phosphatase

The exact subcellular location (secondary or tertiary granules) of this enzyme is not precisely known. It appears in the granulocytic series after the stage of the neutrophilic myelocyte. Alkaline phosphatase is not cytochemically demonstrable in other hemopoietic blood or bone marrow cells. The activity of this enzyme is expressed in score after the addition of the individual degree of positivity in 100 polymorphonuclear neutrophils. The NAP score for blood neutrophils of normal individuals ranges from 25 to 110. The NAP activity is almost zero in the neutrophils of chronic myelogenous leukemia (CML). This finding is characteristic, although not pathognomonic for CML, since it may be found in other conditions, such as viral infections, paroxysmal nocturnal hemoglobinuria, sideroblastic anemia, and idiopathic thrombocytopenic purpura. A normal or elevated NAP score may be seen in CML during bacterial infection, remission, and blast cell crisis. An increased NAP score is frequently found in patients with leukemoid reaction, pregnancy, myelofibrosis with myeloid metaplasia, polycythemia vera, and in idiopathic thrombocytopenia. The NAP score is a simple cytochemical reaction for the differential diagnosis of chronic myeloproliferative disorders (table 1-2).

Acid Phosphatase Resistant to Tartrate

This isoenzyme of AcP (isoenzyme 5) is present in the cells of hairy cell leukemia (HCL). It is characterized by a preservation or even 'increase of AcP positivity in hairy cells after the addition of tartrate, whereas AcP is entirely inhibited by tartrate in all normal hematopoietic and leukemic cells. In a small proportion of HCL cases, however, AcP activity may be weak or absent, or when present, it may be entirely or partially inhibited by tartrate. AcPT positivity has been reported in rare cases of B prolymphocytic leukemia. An acid phosphatase positive reaction resistant to tartrate is considered as an isoenzyme marker, specific, though not pathognomonic, of hairy cell leukemia (table 2).

2. Materials and Methods

Sterile speculum, Sterile cotton swab, Glass slides, diamond stylus, ethanol, methanol and acetone, absolute homogeneity, toluidine blue staining kit. Alpha-naphthalene propanoic acetate esterase kit, naphthyl AS-D chloroacetate esterase, acid phosphatase, PERIODIC-ACID SCHIFF (PAS), all of Manufacture, Sigma- 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 (38). Attending random cluster sampling, Immediately after sampling, and drying the slides fixation solution, all the samples were fixed (39).

Preparation of samples

Peripheral blood samples were taken every 11 samples were prepared slides, all slides were coded by a diamond pen, the dried solids 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 (40), 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-naphthalene propanoic acetate esterase, Acid phosphatase, periodic acid-Schiff with the books of commercial construction company Sigma-Aldrich was used (41).

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 cell 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 (42).

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 100x 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 magnification of 100X with a calibrated optical 100 and microscope was harvested imaging software was harvested and Software at a base 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 grapefruit 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 27ml of acetone and methanol (5ml) 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 a salt 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 5min. were washed with deionized water for 3 minutes. Naphthol AS-D chloroacetate solution by dissolving a capsule Naphthol AS-D chloroacetate in 2ml dimethyl form aldehyde 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-naphthalen propanoic 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 one 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 2 ml naphthalen propanoic 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. naphthalen propanoic acetate solution by dissolving a capsule naphthalen propanoic acetate in 2 ml of monomethyl etherglycol 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% form aldehyde 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 5.0 ml 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 5 min 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 in to a glass jar poured, and well in 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 15min of incubation for 2 minutes were well washed with distilled water and bring to the dry slides, and then microscopic we evaluate the samples (43).

3. 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 them or phology 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 in efficient 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 and Table 1).

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 chloroacetate 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.

Table 1. Cytochemical markers for the differential diagnosis and classification of acute leukemia

| Type of acute leukemia | Reactions | | | | | |
|--------------------------------|-------------|--------------------------------------|-----|-------|-------------|--------|
| Traditional terms | FAB classes | Myeloperoxidase and/or Sudan black B | NCA | NASDA | NASDA + NaF | PAS |
| Myeloblastic | M1 | + /+++ | +- | + | + | - /+++ |
| Myeloblastic with granulocytic | M2 | +- | +- | + | + | |
| Promyelocytic | M3 | | ++ | + | + | ++ |
| | | +++ | | | | |
| Myelomonocytic | M4 | | ++ | ++ | + | + /+++ |
| | | + /+++ | | | | |
| Monocytic and monoblastic | M5 | | - | +++ | +- | - /+++ |
| | | - /+++ | | | | |
| Erythroleukemia | M6 | | - | - | - | - |
| | | - | | | | /+++ |
| Lymphoblastic | L1, L2, L3 | - | - | +- | +- | - |
| | | | | | | /+++ |

Table 2. Summary of the contribution of cytochemical markers in the differential diagnosis and classification of leukemic proliferations

| Cytochemical reaction | For the discrimination of |
|--|---|
| Myeloperoxidase and Sudan black B | Acute myeloblastic |
| NASD chloroacetate esterase/ PAS | Acute lymphoblastic leukemia |
| NASD acetate esterase/NASD acetate esterase inhibited by sodium fluoride | Acute monocytic and myelomonocytic leukemias/Acute myeloblastic, lymphoblastic, and immunoblastic leukemias |
| Acid phosphatase a-Naphthyl acetate esterase f3-Glucuronidase | T from null and B acute or chronic lymphocytic leukemias |
| Neutrophil alkaline phosphatase | Chronic myelogenous leukemia from Other chronic myeloproliferative disorders |
| Acid phosphatase resistant to tartrate | Cells of hairy cell leukemia from Other lymphoproliferative disorders |

Table 3. Cytochemical enzyme positivity of leukemic lymphocytic cells according to their immunologic phenotype

| lymphocytic cells | | Enzyme reaction | | |
|-------------------|----------------------|------------------|---------|---------|
| | | AcP ⁿ | aNAE* | ~GLUt |
| ALL | common | +- | + | - |
| | null | +- | - | - |
| | non-T, non-B | +- | +- | ++ |
| | pre-T | ++ | ++ | - |
| | pre-B | + | - | - |
| | T (ER +) B (SIg+) | ++ /+++ | ++ | ++ /+++ |
| PL | T (ER +) B (SIg+) | ++ | +++ | +++ |
| | | +- /+ | +- | +- |
| CLL | T (ER +) B (SIg+) | +++ | +- /+++ | +++ |
| | | +- | +- | +- |

Table 1: Cytochemical characteristics in acute lymphoblastic leukemia

| 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 |

Figures:

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

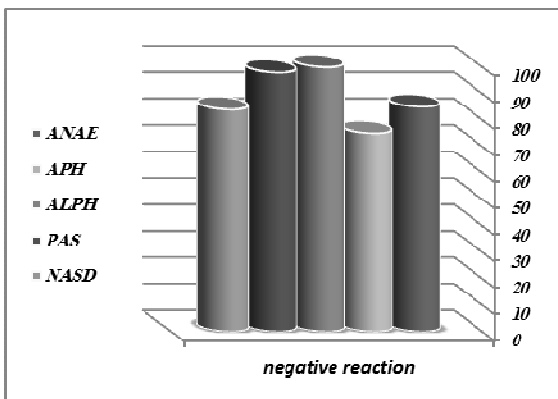
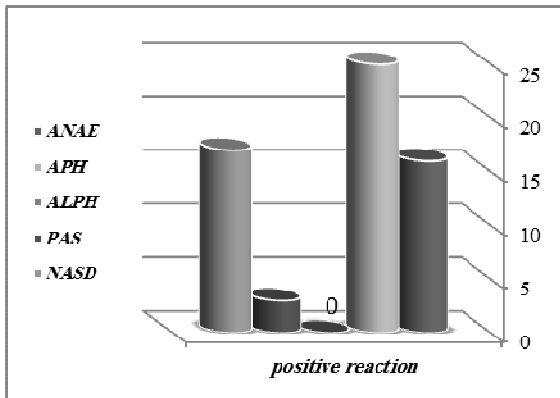


Figure 2. *Alph* a-naphthalen propanoic acetate esterase staining (ANAE): Granulated positive T - lymphocytes and monocytes.

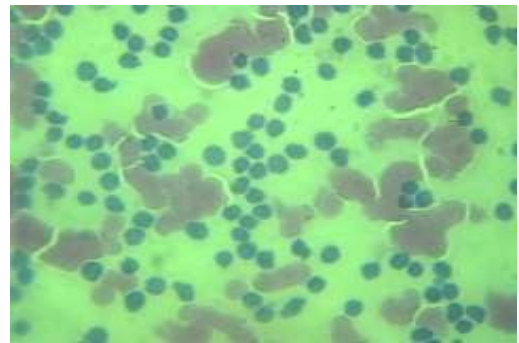
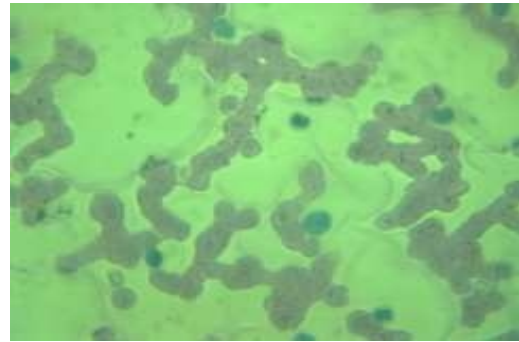
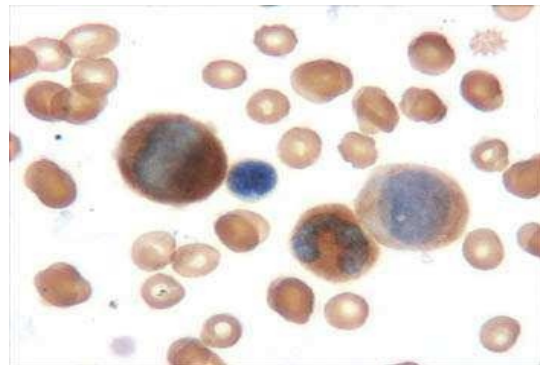


Figure 3. Periodic acid shift (PAS):L: two blast cells with PAS have been extremely positive, one round and the other against the background of positive cytoplasmic granules



4. Conclusions

The cytochemical reactions described are the most frequently used for the diagnosis and differential diagnosis of leukemia. Their application usually results in the precise classification of most leukemic cases (tables 1-1 and 1-2). In rare cases of acute leukemia, however, the demonstration of the platelet peroxidase at the electron microscopy level or their investigation for the presence of metachromatic cytoplasmic granules may be necessary in order to establish the diagnosis of acute megakaryoblastic and acute basophilic leukemias, respectively. 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 (44). 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 (45).

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