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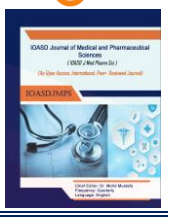

Role of Cytochemical staining in Haematology

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ABSTRACT

Historically, the diagnosis and classification of acute leukaemia involved morphologic review of blasts in the peripheral blood and bone marrow smears and cytochemical staining. Cytochemical stains, which are enzymatic colorimetric reactions that occur in the cells of interest, were necessary to assign and confirm myeloid and lymphoid lineage. In the current WHO 2008 Classification of leukaemia, immunophenotyping and cytogenetic analysis have largely replaced cytochemical staining in the characterization of acute leukaemia. Nonetheless, cytochemical testing remains a useful adjunct assay for the proper classification of acute leukaemia in a number of diagnostic settings.

Key words: Cytochemical staining, Leukaemia, Auer rod.

Original Research Article

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INTRODUCTION

Cytochemical staining is the technique to identify diagnostically useful enzymes or other substances in the cytoplasm of haemopoietic cells, usually by development of colour reactions. It is the staining method for bone marrow and peripheral blood smear for diagnosis and classification of different types of leukaemia. In countries like India role of cytochemical staining cannot be ignored as it is time saving, cost effective and easy to perform and yet an effective tool for diagnosis and classification of leukaemia especially rural and sub urban area where flow cytometry and immunochemistry is not easily available. It is mainly helpful in diagnosis and differentiation of different types of leukaemia by staining peripheral blood smear and bone marrow smear.

Purpose of cytochemical stain

- To characterize blast cell in acute leukaemia as myeloid or lymphoid.
- Classification of leukaemia.
- Identification of Auer rod in acute leukaemia.
- Identify early granulocytic and monocytic cells in acute leukaemia.
- Identification of unusual lineage- mast cells, basophil.
- For differentiation of leukaemoid reaction and Chronic myeloid leukaemia (CML)
- Confirmation of hairy cell leukaemia.

Principle of Cytochemical staining

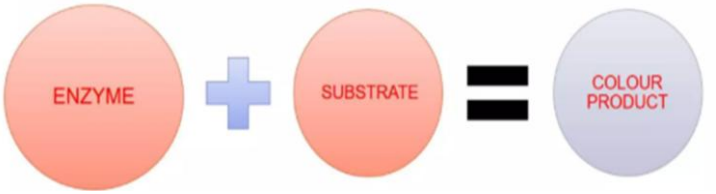

Figure-1: Enzymes or other substances such as glycogen, lipid present in the cytoplasm of haemopoietic cells reacts with substate present in the cytochemical stain and produce the colour reactions which can be seen in microscopically.

Types of cytochemical staining

Enzymatic	Non enzymatic
1.Myeloperoxidase	1. Periodic acid Schiff
2.Esterase	2. Sudan Black B
a) Specific	3. Toluidine Blue
b) Nonspecific	4. Perl's stain
3.Phosphatase	
a) Acid phosphatase	
b) Leucocyte alkaline phosphatase	

Myeloperoxidase stain

Myeloperoxidase is present in the primary azurophilic granules of neutrophils, eosinophils and monocytes and activity is increased with maturation and no activity is found in red blood cells and lymphocyte.

Principle

Myeloperoxidase splits H_2O_2 in the presence of chromogenic electron donor (e g – DAB) and forms an insoluble reaction product.

The reaction product is stable, insoluble and non-diffusible.

Use

Differentiate between myelogenous and monocytic leukaemia from acute lymphoblastic leukaemia (ALL).

Interpretation

- Red brown granules are found in neutrophil and myeloid precursor cells.
- Finely granular staining found in monocyte.
- Negative staining found in lymphoblast, plasma cells and basophil.

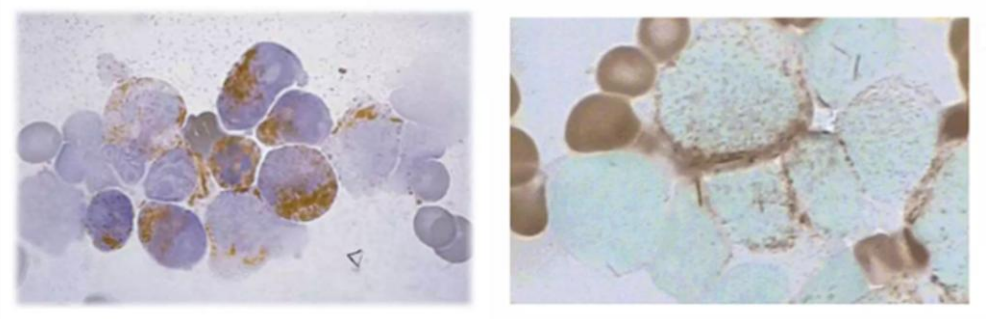

Figure 2: Picture shows myeloperoxidase positive granules in myeloblast and Auer rod.

Sudan Black B

Sudan Black B is a lipophilic dye that stains intra cellular phospholipids and other lipids.

Purpose

To differentiate between AML and ALL.

Principles

Sudan black B is fat soluble, it stains fat particles (Sterols, phospholipid and neutral fat) which is present in the primary and secondary granules of myelocytic and monocytic cells.

Result

- Positive result show as black and granular.
- Nuclei are blue in colour.

Interpretation

- Myelogenous cells show coarse staining granules with faint staining pattern for myeloblast and increase staining with maturation.
- Auer rod is stained positive.
- Monocytic cells show fine scattered granules.
- Negative staining in lymphocytes.

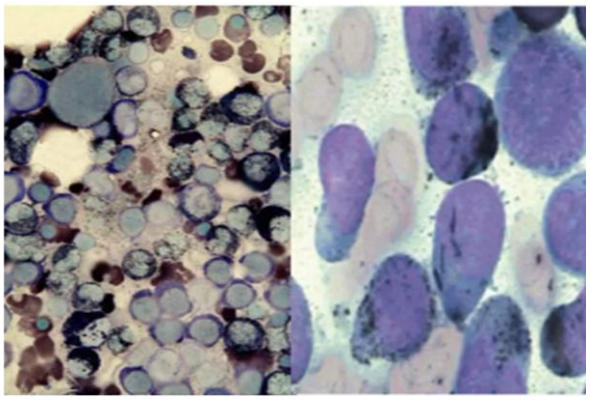

Figure-3: Sudan Black B positive myeloblast; Sudan Black B positive Auer rod in myeloblast

Leucocyte Alkaline Phosphatase (LAP)

LAP is present in the cytoplasm of neutrophil, eosinophil, B lymphocyte, osteoblast, endothelial cells. This activity quantified by scoring 0 to 4 grading depending on the intensity of the staining pattern, where cells with no staining classified as 0 grade and cells with intense staining score 4. This activity has been quantified by individual scoring of 100 consecutive neutrophils or band form.

Normal LAP score is15-130.

Purpose

To differentiate between leukaemoid reaction and CML.

LAP score elevated	**LAP score decrease**
• Leukaemoid reaction.	CML
• Newborn baby	Sickle cell anaemia
• Pregnant women	PNH

Generally, leukemoid reaction are associated with infection or inflammation which trigger neutrophils to produce more alkaline phosphatase with increase LAP score but in case of CML no such trigger or stimuli present so LAP score reduced in CML (except blast crisis)

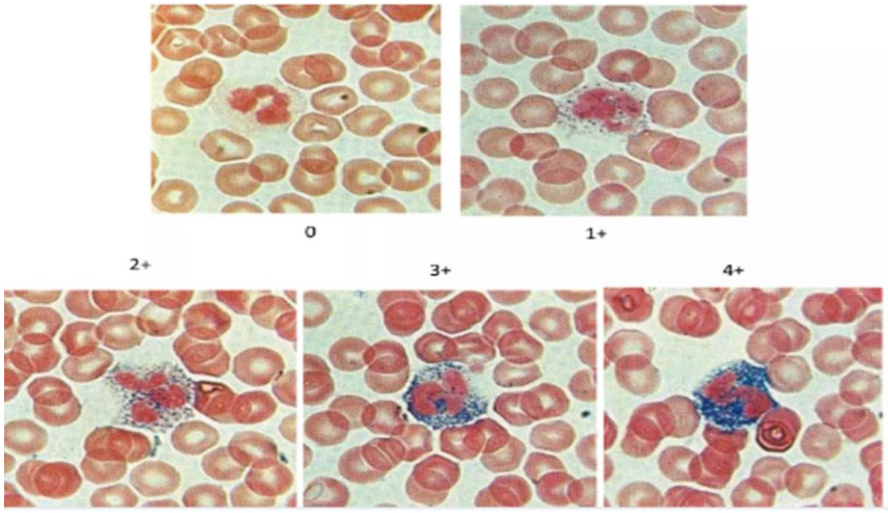

Figure-4: 0-No staining, 1- mild staining, 2- moderate staining, 3- Intense staining with cytoplasmic activity, 4- Intense staining with no cytoplasmic activity.

Acid Phosphatase

Principle

Acid Phosphatase enzyme present in myelocyte, lymphocyte, monocyte, plasma cell and platelets in these cells acid phosphatase activity is inhibited in the presence of L tartrate and produce no colour while in hairy cell leukaemia acid phosphatase will not be inhibited and give positive reaction.

Purpose

To differentiate T cell leukaemia and hairy cell leukaemia which are tartrate resistant.

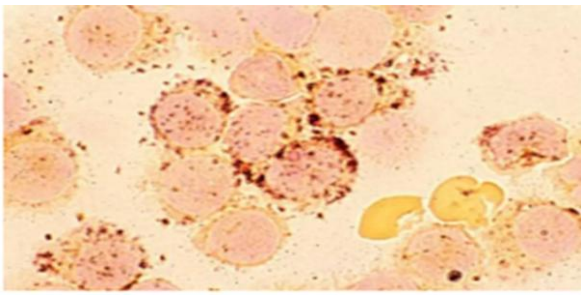

Figure-5: Hairy cell leukaemia with TRAP stain, show red granular cytoplasmic staining

Periodic Acid Schiff

Principle

Periodic acid Schiff (PAS) stain indicates the presence of intracellular glycogen and neutral mucopolysaccharides.

Purpose

To differentiate between AML (diffuse positivity) and ALL (Block positivity).

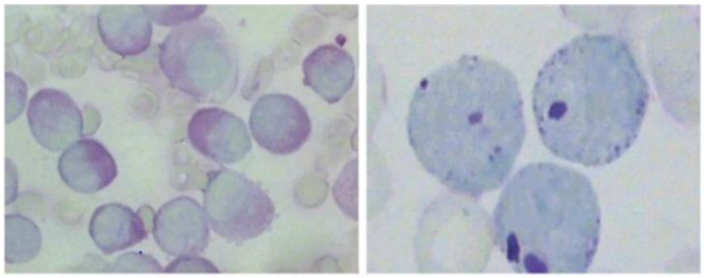

Figure- 6: Diffuse PAS positivity Block PAS positivity

Toluidine Blue

Toluidine blue reacts with acid mucopolysaccharides in the granules of basophil and mast cells to form metachromatic complex.

Purpose

Useful for enumeration of Basophils and Mast cells.

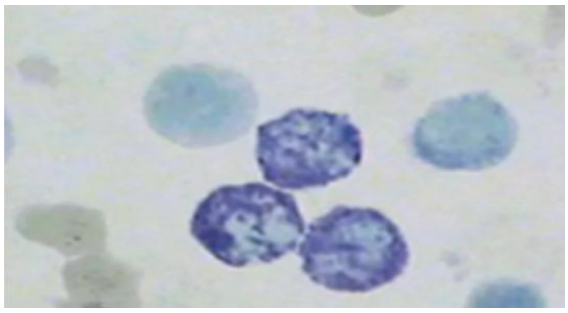

Figure-7: Toluidine blue positive basophil in CML

Esterases

There are 2 types of esterase stain are present- specific and non-specific esterase. Specific esterase is Naphthol AS-D chloroacetate esterase (CAE), these identify cells of granulocytic series and negative for monocyte, lymphocyte. Non-Specific esterase is both α-naphthyl butyrate and α- naphthyl acetate. Non-specific esterase is positive for monocyte, and they are negative for granulocytic series. α- naphthyl acetate is sensitive whereas α- naphthyl butyrate is more specific stain.

In suspected case of acute myeloid leukaemia double esterase stain is used where specific esterase will stain myeloblast and non-specific esterase will stain monoblast.

Principle

Esterase enzyme present in leucocyte hydrolyses the substrate esters and the product formed react with diazonium salt and produce coloured component.

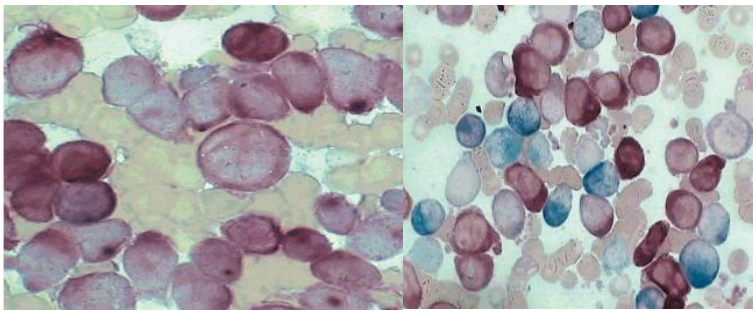

Figure-8: Non-specific esterase in monocytic leukaemia; Double esterase staining- Chloroacetate esterase (blue) and non-specific esterase (brown) positive cells.

Perl's Iron stain

Siderotic granules are found in the cytoplasm of developing cells in bone marrow in the form of ferric ions. Ferric iron stored in the tissue in the form of non-heme iron such as ferritin, hemosiderin, which react with potassium ferricyanide of Perl's stain and form blue colour pigment (hydrated ferric ferrocyanide complex).

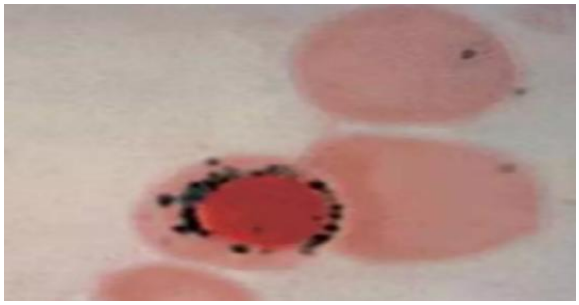

Figure-9: Perl's stain

Uses

- To assess iron reserves in bone marrow.
- Hereditary hemochromatosis.

CONCLUSION

In the developing world role of cytochemical stains cannot be ignored as it is cost effective, time savings and easy to perform in differentiating different types of leukaemia.

Financial and other competing interest: None.

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