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Review Article

Comparison between Giemsa, Harris Hematoxline & Eosin and Lieshman Stain in Peripheral Blood Picture

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Abstract

This was a Descriptive comparative study was performed the compare the morphology of peripheral blood picture when stained by giemsa lieshman and harris hematoxline and eosin. The study was performed in El-imam El-Mahdi University faculty of medical laboratory science, in Kosti town -white Nile state, Kosti is 315 km from Khartoum to south of Sudan, total area of 39701km and population about 159500. That lies south of Khartoum on western bank of the white Nile river. Kosti bridge linked between Kosti and Rabak which is the capital of the state, The town have the important Nile port in Sudan which linked between south Sudan and Sudan. Study duration From September to November /2017. The target population of this study was the student of Medical laboratory technology in university of elemam almahdi.50samples was collected to make 150 thin blood films, three films from each samples and stained with Giemsa, lieshman, H&E the comment of this films as the following:

In all type of stain the morphology WBCs, RBCs, and platelet were normal. the different in color of RBCs and cytoplasmic granules of WBCs. In Lieshman stain RBCs was stained by pale red color. In Giemsa stain RBCs was stained by pale gray color. In hematoxline stain RBCs was stained by strong red color. In hematoxline and eosin stain the white blood cell cytoplasmic granules was stained as the following: neutrophils is stained by fine pink, with light purple or violate color of nucleus, lymphocyte: blue cytoplasm with light purple color nucleus. Eosinophils: course red granules. Monocyte blue: gray cytoplasm with clear vacuolated nucleus. In Giemsa and lieshman stain white blood cell cytoplasmic granule was stained as the following: neutrophil is stained by fine purple with violate color of nucleus, lymphocyte: stain pale blue cytoplasm with condensed nuclear chromatin. Eosinophil: orange–red cytoplasmic granule. Monocyte: stained by blue–gray cytoplasm with clear nucleus. And Platelet in Giemsa stain is stained by purple color in Lieshman stain platelet was stained by purple –blue color and in hematoxline & Eosin was stained by orange red color. The type of stain was different in WBCs cytoplasmic granule, present of deposit and background of stain. In Giemsa stain: the cytoplasmic granule out of total 50 blood film 35 which represented 70% is valid appears and 15 represented 30% is not appear. The background out of total 50 blood sample 40 blood films is valid clear that represented about 80%, while the 10 film is not clear that represented about 20%. And deposit of stain 40 blood film had no deposit that represented about 80%, while the 10 blood film with deposit that represented about 20% from total blood film.

In Lieshman stain the cytoplasmic granule out of total 50 blood film 33 is valid appear that represented about 66%, while the 17 blood film is not appear that repress ended about 33%. The background 32 blood films is valid clear that represented 64% while the 18 is not clear that represented about 34% from total blood films. and deposit of stain 33 blood film is absent from deposit that represented about 66%, while the 17 blood film is present of deposited of stain that represented about 34% from total blood film. In hematoxline & Eosin stain the cytoplasmic granule from 50 blood film 50 is valid clear that represented about 100%. The background 50 blood film is valid clear that represented about 100% from total blood film. The deposit of stain 50 blood film had no deposit of stain that represented about 100% from total blood film.

Introduction

The stained blood film is one of the world s most widely and frequent used tests. Since its introduction in the late nineteenth century basic elements of the blood film preparation and analysis have change little modern technology improvement and refinement have enhanced the availability of good quality commercial Romanowsky stain. Automated and semi-automated slide makers [1].

Wright-Giemsa Stain Solution was developed by Romanowsky

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in 1891. He observed that this combination of dyes gave excellent selective staining of blood films. Also in1891, Giemsa modified Leishman's stain to provide better stain intensity and fine cellular detail. The stain, however, required an extended staining process. The Wright-Giemsa Stain Solution has been developed to incorporate the exceptional brilliance and resolution of cellular details obtained from Giemsa Stain with the rapid staining time of Wright's Stain [2].

Leishman's stain is applied in conventional staining techniques to uniformly stain chromosomes. These techniques leave centromers constricted, thus enabling the measurement of chromosome length, centromeric position, andarmratio. Slides can be easilydistained and banded by most banding procedures. Orceinstained chromosomes cannot be distained! Leishman's stain belongs, as Giemsa and Wright's stain, to the group of Romanovsky stains. It is considered as an easy to do technique which gives a fairly acceptable contrast. For the detection of malaria parasite Lieshman staining seems more sensitive than e.g. Field's stain [3].

Harris hematoxline Newcomer Supply Hematoxline Stain, Harris Modified is a ready to use high quality regressive hematoxline that does not require filtering, incompletely mercury-free and can be used in either manual or automated staining platforms. This modified Harris formulation contains glacial acetic acid for more precise and selective nuclear staining and ethylene glycol to increase solution stability and reduce surface precipitate.

The routine Hematoxline & Eosin (H&E) stain is used for screening specimens in anatomic pathology, as well as for research, smears, touch preps and other applications. Its two primary coloring agents stain all cellular material including nuclei (blue), and cytoplasmic elements (pinked). Popularity of this stain is due, in large measure, to its simplicity, ability to clearly demonstrate a wide variety of different tissue components, dependability, repeatability, and speed of us [4].

In this research we were modified the method H&E to stain peripheral blood picture.

Justification

Peripheral blood picture is important tool in diagnosis of hematological diagnosis disorder. So in order to perform this function, the blood film should be well prepared and stained. Many stain can be used for staining peripheral blood picture. eg.'Geimsa's, lieshman and have advantage and disadvantage in this study we will examine new type of stain and compare it with the routine hematological stain to choice the best for staining of peripheral blood picture.

Objective

General objective: To make comparison on peripheral blood picture when stained with giemsa, lieshman, Harrishematoxline and eosin stain**.**

Specific objective

1. To examine the affect of giemsa stain on peripheral blood film.

2. To examine the affect of lesishman stain on peripheral blood film.

3. To modified method of harris hematoxline and eosin examine the affect of this method on peripheral blood film.

Literature Review

Romano sky Stains

Romano sky stains are universally used in hematology. They are composed of Methylene blue, oxidative products of Methylene blue (Azure A, Azure B, Azure C and Thionin) and eosin dyes. Giemsa, a commonly used stain does not adequately stain red blood cells, platelets or white blood cell cytoplasm's when used alone. A second Romano sky stain is therefore often used in combination with Giemsa and contains azure dyes to intensify the staining of nuclear features and of azurophilic and toxic granulation. Wright, Wright-Giemsa and May-Grunewald Giemsa are commonly used combinations of Romanowsky stains. Rapid or "quick" stains, developed for 'stat' situations or for small laboratories, are adequate for assessing normal cell morphology [5].

Giemsa stain

Intended use: Giemsa and May Grunewald solutions are intended for use in staining blood film or bone marrow films. Solutions are for "*In Vitro* Diagnostic Use." Giemsa stain is a buffered thiazine-eosinate solution designed to provide coloration of blood cells similar to the original product described by Giemsa. It may be used separately or in combination with a May Grunewald Stain, also available from Sigma-Aldrich.

Preparation: To prepare 500 ml of Giemsa stain:

1. Geimsa powder 3.8 g
2. methanol alcohol 250ml
3. glycerole 250ml**.**

Storage: Store Giemsa at room temperature (18-26˚C) Reagent label bears expiration date [2].

Leishman's stain

Description: Leishman's stain is applied in conventional staining techniques to uniformly stain chromosomes. These techniques leave centromers constricted, thus enabling the measurement of chromosome length, centromeric position, and arm ratio. Slides can be easily distained and banded by most banding procedures. Orcein stained chromosomes cannot be distained! Leishman's stain belongs, as Giemsa and Wright's stain, to the group of Romanovsky stains. It is considered as an easy to do technique which gives a fairly acceptable contrast. For the detection of malaria parasite Lieshman staining seems more sensitive than e.g. Field's stain [6].

Storage: Room temperature Keep away from moisture.

Preparation of Leishman's Stain solution: Mix and dissolve 0.15 g of Eosin-Methylene blue in 100 ml Methanol dried at 56°C. When the stain is dissolved completely remove the solution from the heater. (Alternatively, dissolve at RT over night. Cover container with Para film in order to prevent contamination by moisture). After the solution reached RT clear the solution by using a dry what man paper filter. Collect filtered solution in a clean and dry brown glass bottle. Age the solution at least 2-3 days before using it the first time.

Store Leishman's Stain solution at RT in tightly sealed bottles, protected from light and heat. Do not store the solution near bottles

containing acid. If stored correctly Leishman's Stain solution is stable for approx. 3 months [6].

Harris Hematoxline

The word hematoxline is derived from the old Greek words Haimato (blood) and Xylon (wood), referring to its dark red color in the natural state, and to its method of manufacture from wood. Hematoxline is the most widely used histological stain. Its popularity is based on its comparative simplicity and ability to demonstrate enormous number of different tissue structures. It can be prepared in numerous ways. It stains nucleus blue/ black, with good intra nuclear details, while Eosin stains cytoplasm [7]. Hematoxline is extracted from the bark of the logwood tree (Haematoxylon campechianum). When oxidized it forms haematein, a compound that forms strongly colored complexes with certain metal ions, notably Fe (III) and Al (III) salts. Haematein (US spelling) or haematein is an oxidized derivative of hematoxline, used in staining. Haematein must not be confused with haematein, which is a brown to black Iron-containing pigment formed by decomposition of hemoglobin. In the Colour Index (but nowhere else), haematein is called haematein, a confusing word that wrongly implies that the compound is an amine. Haematein is anionic, having poor affinity for tissue, and is inadequate as a nuclear stain without the presence of the mordant. The useful mordants for hematoxline are salts of Aluminum, Iron and Tungsten.

The mordant/metal cations confer a net positive charge to dye-mordant complex and enable it to bind anionic tissue. Haematein is produced from hematoxline in two ways:

Natural oxidation (ripening)

1. By the exposure to air and light.
2. Slow process (3-4) month.
3. The resultant solution retains its staining ability for long time.
4. Ehrlich & Delafield's are examples.

Chemical oxidation: By using chemical oxidizing agent that converts the hematoxlineto hematein almost immediately [8].

Harris'shematoxline (Harris 1900): This alum hematoxline was traditionally chemically ripened with mercuric oxide. As mercuric oxide is highly toxic, environmentally unfriendly, and has detrimental and corrosive long-term effects on some automated staining machines, sodium or potassium iodide is frequently used as a substitute for oxidation.

Harris is a useful general-purpose hematoxylin and gives particularly clear nuclear staining, and for this reason has been used, as a progressive stain, in diagnostic exfoliative cytology. In routine histological practice, it is generally used regressively, but can be useful when used progressively. When using Harris shematoxylin as a progressive stain, an acetic acid-alcohol rinse provides a more controllable method in removing excess stain from tissue components and the glass slide. The traditional hydrochloric acid-alcohol acts quickly and indiscriminately, is more difficult to control, and can result in a light nuclear stain. A 5–10% solution of acetic acid, in 70–95% Alcohol, detaches dye molecules from the cytoplasm/ nucleoplasm while keeping nucleic acid complexes intact (Feldman & Dapson 1985) (Table 1).

Table 1: Preparation of solution.

Hematoxline	2.5 g
Absolute alcohol	25 ml
Potassium alum	50 g
Distilled water	500 ml
Mercuric oxide	1.25 g *or*
Sodium iodide	0.5 g
Glacial acetic acid	20 ml

Preparation of solution: The alum which has previously been dissolved in the warm distilled water in a 2-liter flask. The mixture is rapidly brought to the boil and the mercuric oxide the hematoxline is dissolved in the absolute alcohol, and is then added to de or sodium iodide is then slowly and carefully added. Plunging the flask into cold water or into a sink containing chipped ice rapidly cools the stain. When the solution is cold, the acetic acid is added, and the stain is ready for immediate use. The glacial acetic acid is optional but its inclusion gives more precise and selective staining of nuclei. As with most of the chemically ripened alum hematoxline, the quality of the nuclear staining begins to deteriorate after a few months. This deterioration is marked by the formation of a precipitate in the stored stain at this stage the stain should be filtered before use, and the staining time may need to be increased. For the best results, it is wise to prepare a fresh batch of stain every month, although this may be uneconomical unless only small quantities are prepared each time [9].

Eosin stock solution

1. Eosin Y 1 g.
2. Distilled water, 100 ml.
3. Mix to dissolve.

Application: Harris hematoxline solution is widely used in the areas of histology and cytology as a component of hematoxline-eosin staining. Basically, it reveals the nuclear structure in both nucleoproteins and nucleic acids in samples of human tissue.

Principle: Hematoxline itself is a compound without staining properties. It is the product of its oxidation, haematein, which lends coloring properties to the solution. Oxidation can be produced either spontaneously, or by inducement. The first option is slow because it involves natural oxidation through air and light The second, rapid or instantaneous method, involves the use of an oxidizing agent such as mercury or iodine, however, this reduces the durability of the stain because the haematein may also be oxidized. Therefore, the hematoxline solutions where oxidization is induced are less durable, but they do not require a maturation process in order to achieve adequate results; they can be used practically an instant after their preparation. In order to reduce the problems caused by using an oxidizing agent, increase the concentration of ethanol and decrease the pH of the solution. Both strategies were adopted in the original formulation of Harris Hematoxline solution. In some cases these modifications are insufficient to preserve the coloring activity after dying many samples, probably because the oxidizing agent has worn out the haematein and because of the elevated number of samples which require dying.

Main advantages

Better Formulation

1. Better contrast and more color fixing power.
2. Increased capacity to process a higher number of samples.

Mercury free

1. Contributes to the preservation of the environment.
2. Safer for the user [10].

Peripheral smear procedure

Principle: When automated differentials do not meet specified criteria programmed into the automated hematology instrument, the technologist/technician must perform manual differential count from a prepared smear. There are two types of blood smears: the wedge smear and the spun smear. The wedge smear will be discussed in this procedure. Smears are prepared by placing a drop of blood on a clean glass slide and spreading the drop using another glass slide at an angle. The slide is then stained and observed microscopically. A well-stained peripheral smear will show the red cell background as red orange. White cells will appear with blue purple nuclei with red purple granules throughout the cytoplasm. A well made, well distributed peripheral smear will have a counting area at the thin portion of the wedge smear which is approximately 200 red cells not touching. A good counting area is an essential ingredient in a peripheral smear for evaluating the numbers of and types of white cells present and evaluating red cell and platelet morphology.

Reagents and equipment

1. Glass slides (frosted)
2. Wooden applicator sticks
3. DIFF-SAFE (an apparatus designed to avoid removing the tube top)

Specimen collection and storage

1. EDTA specimen or EDTA Microtainer.
2. Smears are made from EDTA.
3. Microtainers within 1 hour of collection.
4. EDTA blood within 2 to 3 hours.
5. Check all Microtainers for clots with applicator sticks.

Quality control: A random slide is picked after it has been stained and technologist/technician checks the quality of the stain for the WBCs and RBCs, platelets, and the distribution of cells [11].

Procedure

1. Insert the DIFF-SAFE dispenser through this topper of the tube held in an upright position.
2. Turn the tube upside down and apply pressure at the frosted end of the slide. When the drop of blood appears, discontinue pressure.
3. Using a second slide (spreader slide), place the edge of the second slide against the surface of the slide at an angle between 30 and 45 degrees.
4. Bring the spreader slide back into the blood drop until contact is made with the drop of blood
5. Move the spreader slide forward on the slide, so a smear is made approximately 3 to 4cm in length. The smear should be half the size of the slide, with no ridges, and a "feather edge "should be toward the end of the smear.
6. Label the frosted end of the slide with the patient's last name and first initial, specimen number, and the date.
7. Allow the smear to air dry completely.
8. Proceed with staining. Manual Wright staining is not found often in the clinical laboratory setting. Most clinical laboratories have an automated.

Staining instrument attached to their automated CBC analyzer. If there is no automated stainer attached to the analyzer, there still is a separate staining instrument [11].

Staining the blood film: Prior to staining, cells must be fixed to the glass slide with acetone-free methanol, either alone or in solution with dye. Addition of a buffer solution to the dye changes the pH of the solution and ionizes the reactants to initiate the pH-dependent staining process. Acidic cellular elements such as nucleoproteins, nucleic acids and primitive cytoplasmic proteins, react with the basic dyes, Methylene blue and its oxidative products. These elements are basophilic and stain variations of blue. Basic cellular elements such as hemoglobin molecules and some cytoplasmic constituents in leukocytes, have an affinity for the acidic dye, eosin. These elements are acidophilic and stain orange-red. A neutrophil has neutral staining characteristics and stains blended shades of purple or pink, representing combinations of acidic and basic molecular groups. Azure dyes stain the primary or non-specific granules in most myeloid cells red-purple, hence, the term azurophilic granules [12].

Manual staining methods

Dip method (Rapid): The dip method is a quick staining method that uses a modified Wright-Giemsa stain buffered in methanol at pH 6.8. Slides are immersed in the stain in a coplin jar for a user-determined length of time. It is important that the Wright-Giemsa stain be kept tightly sealed in coplin jar when not in use and be replaced when water artifact appears in red cells (see: Causes and Corrections of Stain Deviations).

Note: Staining time may be increased for greater cellular detail as required.

Automated staining methods

Hema-Tek slide stainer - Miles scientific (platen-type stainer): The Hema-Tek slide stainer is a self-contained bench top slide stainer that uses a Hema-Tek Stain Pak. Three sensing switches are triggered sequentially to activate three solution pumps which deliver metered volumes of stain, buffer and rinse solution from the stain pack. A Wright-Giemsa Pak is most commonly used. Slides are advanced by two parallel conveyer spirals with the stain, buffer and rinse solutions pumped up between the blood film and platen.

Note:

1. Slides can be randomly added if required
2. the staining process takes approximately ten minutes
3. Time phases are constant
4. Pump volumes are user adjusted by control knobs
5. a stain/buffer ratio of 1:2 is desirable
6. Cleaning procedures and daily maintenance are vitally important to prevent stain deposit artifact

Hemastainer automatic slide stainer - Miles scientific (dip-type stainer): This is an automated staining instrument that stains up to fifty slides at one time. The slides are loaded into a slide basket and dipped into each of six stations in the staining process. Five individual, adjustable timers control the first five stations. The six stations consist of: methanol, stain, a second stain, buffered-water rinse, a phosphate buffer rinse and lastly, forced-air drying. The two stains commonly used in the stainer are May-Grunewald and diluted Giemsa.

Midas II automatic slides stainer- EM Diagnostic (dip-type stainer): This instrument is a completely automatic slide stainer that stains up to twenty slides at one time. The slides are loaded into a bucket and are cycled through six stations: methanol, stain, a second stain, water rinse, phosphate buffer rinse, followed by the drying station. The first five stations are on individual, adjustable timers. The Midas II stainer is similar in operation to the Hemastainer.

Note:

1. Slides cannot be added once the staining process is started.
2. The staining process takes approximately twenty minutes.
3. Produces good quality, reproducible staining [13].

Previous study

Giemsa stain: Clarke Dr. Gwendolyn - prephral Blood film: staining cell estimation and reviow, CPSAALQEP.MAY.2003 find that Red blood cells Salmon pink Nuclei of neutrophils Deep blue-purple Specific granules of neutrophils, granules of Light purple or violet lymphocytes, granules of platelets. Specific granules of Basophils Deep purple Specific granules of Eosinophils Orange Cytoplasm of lymphocytes Blue Cytoplasm of Monocytes Blue-grey (ground glass appearance).

Ramadas Nayak, Sharada Rai, Astha Gupta, Essentials in Hematology and clinical pathology first Edition 2012. Find that: The red blood cell was stained by red color; the white blood cytoplasmic granule was stained as following: Neutrophils are stained by fine purple cytoplasmic granule. Basophile was stained by dark stained granule. Monocyte was stained pale grayish blue cytoplasm. Lymphocyte was stained by pale blue cytoplasm and nuclear is course. The platelet was stained by purple color.

Dacia, Sir J.V., Lewis, S.M. *Practical Haematology* 7th edition, pages 77 to 81, Churchill Livingstone, 1991 find that: Stain blood film by Romano sky stain the red blood cell was stained by dark pink color, the white blood cytoplasmic granule was stained as following: lymphocyte stained blue color, monocyte stained by gray –blue color, neutrophil stained by pink-orange and purple granule, basophile stained by blue color and purple black granule, eosinophil stained by red color and orange granule. platelet was stained by purple color.

Lieshman stain: In Version: MM2/130108 find that: Red blood cell was stained by: light pink to brown Cores. lymphocyte was stained by: deep, dark blue to blue-violet Cytoplasm of lymphocytes: light blue Nuclei of neutrophil, polymorph nuclear leukocytes: a deep blue to blue-violet Granules of neutrophilic polymorph nuclear leukocytes: red Cores of eosinophil leukocytes: blue violet Granules of eosinophilic leukocytes: deep red Cores of basophilic leukocytes: blue violet.

Hematoxline and eosin stain: No previous study for uses this stain in hematological lab. This stain use as routinely in histopathology lab. We modified it and use in hematological lab for staining peripheral blood picture that give reliable result.

Materials and Methods

Study design

Descriptive study

Study area

The study was conducted in kosti town -while Nile state, kosti is 315 km from Khartoum to south of Sudan state area in 39701km and population about 159500 [2008].

Study population

The target population of this study volunteer of Medical student in university of elemam almahdi.

Study duration

From September to November /2017.

Inclusion criteria

Healthy individual without any hematological disorder.

Exclusion criteria

Any individual suffer from hematological disorder.

Ethical approval

Collecting sample is done by agreement of individual.

Sampling

Amount of sample we take 2ml from each health individual in EDTA anticoagulant.

Sample size

Sample collected from 50 health's individual.

Lab diagnosis

Giemsastain

Procedure

Giemsa May-Grunewald:

1. Dilute Giemsa Stain 1:20 with deionized water. For bluer coloration, water buffered at pH 7.2 may be used in place of deionized water.
2. Place slides in May-Grunewald Stain for 5 minutes.

3. Place slides in Working Phosphate Buffer or Trizma® Buffer (20-70 mol/L). PH 7.2, for 1.5 minutes.

4. Place slides in dilute Giemsa solution from step 1 for 15-20 minutes.

5. Rinse slides BRIEFLY in DEIONIZED water.

6. Air dry and evaluate.

Standard giemsa

1. Fix slides in methanol 5-7 minutes.

2. Air dry.

3. Dilute Giemsa Stain 1:20 with deionized water. Color can be varied by diluting in buffer.

4. Stain film for 15-60 minutes.

5. Rinse in deionized water.

6. Air dry and evaluate.

Quick stain giemsa

1. Place air dried blood film in undiluted Giemsa Stain for 1-2 minutes.

2. Place in deionized water for 2-4 minutes depending upon color preference.

3. Rinse in deionized water.

4. Air dry and evaluate.

Performance characteristics

Nuclei will be varying shades of purple. Cytoplasmic staining will be varying shades of blue to light pink. Fine reddish to lilac granules may be present in cytoplasm of some cell types. Basophiles will demonstrate dark blue black granules in the cytoplasm. Eosinophils will demonstrate bright orange granules in the cytoplasm. Red blood cells should be pink to orange. If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance [12].

Harris' Hematoxline

Staining Procedure:

1. Deparaffinize sections, 2 changes of xylem, 10 minutes each.

2. Re-hydrate in 2 changes of absolute alcohol, 5 minutes each.

3. 95% alcohol for 2 minutes and 70% alcohol for 2 minutes.

4. Wash briefly in distilled water.

5. Stain in Harris hematoxylin solution for 8 minutes.

6. Wash in running tap water for 5 minutes.

7. Differentiate in 1% acid alcohol for 30 seconds.

8. Wash running tap water for 1 minute.

9. Bluing in 0.2% ammonia water or saturated lithium carbonate solution for 30 seconds to 1 minute.

10. Wash in running tap water for 5 minutes.

11. Rinse in 95% alcohol, 10 dips.

12. Counter stain in eosin-phloxine solution for 30 seconds to 1 minute.

13. Dehydrate through 95% alcohol, 2 changes of absolute alcohol, and 5 minutes each.

14. Clear in 2 changes of xylem, 5 minutes each.

15. Mount with xylem based mounting medium.

Results Nuclei should be blue, cytoplasm pink to red [14-16].

This histological method is causes red cells destruction

Method of staining thin blood film

1. Fixed thin blood film by absolute methanol.

2. Covered blood film by Harrishematoxline 5 min.

3. Washed by DW.

4. Add Eosin for 10 second.

5. Wash by DW.

Leishman's Stain

Example Staining Protocol:

Blood staining according to Lieshman (covering technique):

1. Use smears that are as thin as possible and air-dried.

2. Fully cover the smears with Leishman's Stain solution. Stain for 2 minutes.

3. Add twice the amount of distilled water and mix by swirling. Incubate for at least 10 min

4. Rinse thoroughly with distilled water.

5. Dry the slides using blotting paper and air-dry.

6. (Optional) for fixing, include the slides in balsam. (Canada balsam genuine AppliChem product No.A0569) or neutral Malinol [6].

Equipment

1. Glass slides 75.25.

2. Spreader slides.

3. baster pupate.

4. Anticoagulant EDTA.

5. Syringes.

6. Oil.

7. ligh microscope.

Data analysis

Done by spass program

Data presentation

Result presented as tables.

Results

This was descriptive study was performed in EL-Imam El-mahdi University Faculty of medical laboratory science 50 blood sample was collected from healthy volunteer student in EDTA container. Thin blood film was made from this samples (three films) from each samples and stained with Giemsa, lieshman and H&E the comment of this films as the following. In all types of stain the morphology WBCs, RBCs, and platelet was normal. The different in color of RBCs and cytoplasmic granule of WBCs. In Lieshman stain RBCs was stained by [pale red color]. In Giemsa stain RBCs was stained by [pale gray color] and In hematoxline stain RBCs was stained by [strong red color]. In Lieshman stain RBCs was stained by [pale red color]. In Giemsa stain RBCs was stained by [pale gray color] and in hematoxline stains RBCs was stained by [strong red color].

In hematoxline and eosin stain the white blood cell cytoplasmic granules was stained as the following: neutrophils is stained by fine pink, with light purple or violate color of nucleus, lymphocyte: blue cytoplasm with light purple color nucleus. Eosinophils: course red granules. Monocyte blue: gray cytoplasm with clear vacuolated nucleus.

In Giemsa and lieshman stain white blood cell cytoplasmic granule was stained as the following: neutrophil is stained by fine purple with violate color of nucleus, lymphocyte: stain pale blue cytoplasm with condensed nuclear chromatin. Eosinophil: orange – red cytoplasmic granule. Monocyte: stained by blue–gray cytoplasm with clear nucleus.

Platelet in [giemsa stain is stained by purple color] in Lieshman stain platelet was stained by [purple –blue color] and in hematoxline & Eosin was stained by [coarse red color]. The type of stain is differing in WBCs cytoplasmic granule, deposit and background of stain.

In Giemsa stain the cytoplasmic granule from 50 blood film 35 is valid appear that represented about 70% while 15 is not appear that represented about 30% from total blood film, the background 40 blood film is valid clear that represented about 80%, while the 10 film is not clear that represented about 20%. And deposit of stain 40 blood film is absent from deposit that represented about 80%, while the 10 blood film present of deposit that represented about 20% from total blood film.

In Lieshman stain the cytoplasm granule from 50 blood film 33 is valid appear that represented about 66%, while the 17 blood film is not appear that represented about 33% from total blood film. The background 32 blood film is valid clear that represented 64% while the 18 is not clear that represented about 34% from total blood film. and deposit of stain 33 blood film is absent from deposit that represented about 66%, while the 17 blood film is present of deposited of stain that represented about 34% from total blood film.

In hematoxline & Eosin stain the cytoplasm granule from 50 blood film 50 is valid clear that represented about 100%. The background 50 blood film is valid clear that represented about 100% from total blood film. The deposit of stain 50 blood film is absent of deposit of stain that represented about 100% from total blood film

Table 2: White blood cell cytoplasmic granules: Out of 50 films.

	Giemsa	Lieshman	H&E
Clear	35 (70%)	33 (66%)	50 (100%)
Not clear	15 (30%)	17 (34%)	0 (0%)

Table 3: Deposit of Stain: Out of 50 films.

	Giemsa	Lieshman	H&E
Absent	35 (70%)	33 (66%)	50 (100%)
Present	15 (30%)	17 (34%)	0 (0%)

Table 4: Background: Out of 50 films.

	Giemsa	Lieshman	H&E
Clear	35 (70%)	32 (64%)	50 (100%)
Not clear	15 (30%)	18 (36%)	0 (0%)

Table 5: Time consumes & Cost: In about 30 ml of each stain.

	Giemsa	Lieshman	H&E
Time consumes	10 minutes	10 minutes	6 minutes
Cost	3.5 SDG	19.5SDG	40SDG

(Tables 2-5) (Appendixes).

Discussion

In this descriptive comparative study 50 blood samples were collected from volunteer healthy student thin blood film was made from this sample (three films) from each samples and stained with Giemsa, lieshman and H&E the comment of this films as the following in all type of stain the morphology WBCs, RBCs, and platelet was normal. the different in color of RBCs and cytoplasmic granule of WBCs.

In Lieshman stain RBCs was stained by [pale red color]. In Giemsa stain RBCs was stained by [pale gray color] and in hematoxline stains RBCs was stained by [strong red color].

In hematoxline and eosin stain the white blood cell cytoplasmic granules was stained as the following: neutrophils is stained by fine pink, with light purple or violate color of nucleus, lymphocyte: blue cytoplasm with light purple color nucleus. Eosinophils: course red granules. Monocyte blue: gray cytoplasm with clear vacuolated nucleus.

In Giemsa and lieshman stain white blood cell cytoplasmic granule was stained as the following: neutrophil is stained by fine purple with violate color of nucleus, lymphocyte: stain pale blue cytoplasm with condensed nuclear chromatin. Eosinophil: orange – red cytoplasmic granule. Monocyte: stained by blue–gray cytoplasm with clear nucleus.

Platelet in [Giemsa stain is stained by purple color] in Lieshman stain platelet was stained by [purple –blue color]and in hematoxline & Eosin was stained by [coarse red color]. The type of stain is differing in WBCs cytoplasmic granule, deposit and background of stain.

In Giemsa stain the cytoplasmic granule from 50 blood film 35 is valid appear that represented about 70% while 15 is not appear that represented about 30% from total blood film, the background 40 blood film is valid clear that represented about 80%, while the 10 film is not clear that represented about 20%. And deposit of stain 40

blood film is absent from deposit that represented about 80%, while the 10 blood film present of deposit that represented about 20% from total blood film.

In Lieshman stain the cytoplasmic granule from 50 blood film 33 is valid appear that represented about 66%, while the 17 blood film is not appear that represented about 33%from total blood film.

The background 32 blood film is valid clear that represented 64% while the 18 is not clear that represented about 34%from total blood film. and deposit of stain33 blood film is absent from deposits that represented about 66%,while the 17 blood film is present of deposited of stain that represented about 34% from total blood film. In hematoxline & Eosin stain the cytoplasmic granule from 50 blood film 50 is valid clear that represented about 100%. The background 50 blood film is valid clear that represented about 100% from total blood film. The deposit of stain 50 blood film is absent of deposit of stain that represented about 100% from total blood film.

In most of this study agreement of Clarke, Dr. Gwendolyn, the Peripheral Blood Film: Staining, Cell Estimation and Review, CPSA ALQEP: May, 2003.

Clarke, Dr. Gwendolyn, the Peripheral Blood Film: Staining, Cell Estimation and Review, CPSA ALQEP: May, 2003. Red blood cells Salmon pink Nuclei of neutrophils Deep blue-purple Specific granules of neutrophils, granules of Light purple or violet lymphocytes, granules of platelets. Specific granules of Basophile Deep purple Specific granules of Eosinophils Orange Cytoplasm of lymphocytes Blue Cytoplasm of Monocyte Blue-grey (ground glass appearance)

Cytoplasm of neutrophils Light pink Cytoplasm of platelets Purple-blue to lilac. This was descriptive study was performed in EL-Imam El-Mahdi University Faculty of medical laboratory science 50 blood sample was collected from healthy volunteer student in EDTA container.

Ramadas Nayak, Sharada Rai, Astha Gupta first Edition 2012, in this study agree with me, the red blood cell was stained by red color; the white blood cytoplasmic granule was stained as following:

Neutrophils are stained by fine purple cytoplasmic granule. Basophile was stained by dark stained granule. Monocyte was stained pale grayish blue cytoplasm. Lymphocyte was stained by pale blue cytoplasm and nuclear is course. The platelet was stained by purple color.

Barbara j Bain, Imelda Bates, Michael A Laffan and S.Mitchell Lewis eleventh Edition 2011 agree with me that stain blood film by romanowisky stain the red blood cell was stained by dark pink color, the white blood cytoplasmic granule was stained as following: lymphocyte stained blue color, monocyte stained by gray –blue color, neutrophil stained by pink-orange and purple granule, basophile stained by blue color and purple black granule, eosinophil stained by red color and orange granule. platelet was stained by purple color.

Leishman's Eosin-Ethylene blue Product No. A4277 agree with me the red blood cell was stained by light pink to brown Cores color, lymphocytes was stained by: deep, dark blue to blue-violet Cytoplasm of lymphocytes was stained by: light blue Nuclei of neutrophil, polymorph nuclear leukocytes: a deep blue to blue-violet, Granules of neutrophilic polymorph nuclear leukocytes was stained by: red Cores of eosinophil leukocytes was stained by: blue violet Granules of eosinophilic leukocytes was stained by deep red Cores of basophilic leukocytes was stained by: blue violet color.

Conclusion

The study concluded that the hematoxline and eosin are found to obtain reliable result for staining peripheral blood picture that compared with giemsa and lieshman stain by modified method and NEW. And we improved that Harris hematoxline is best stain with all situation like general appearance and cell color and morphology and background and present of deposit. On the other hand the best stain used for deferential count and estimated cell morphology.

Recommendations

1. More studies should be done.
2. More modification for staining method of H&E.
3. Examine the possibility of addition of eosin to harris hematoxline to make one step staining method.

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