Analysis of blood smears

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The aims of this experiment are to study the morphology and characteristic of blood; distinguish the disproportion of blood when suffering from different diseases and identified the differences between animal and human blood. To achieve the aims, smears of horse blood are prepared and compare with human blood. Then the prepared human blood smears are observed under electron microscope.

INTRODUCTION

Blood performs a lot of important functions within the body; it contributes homeostasis to the body and playing major role in defence system by phagocytises activity. On an average male adult who weights 70kg has a blood volume of about 5 litres, about 1/12th of the body weight. Blood consists 55% of blood plasma 45% of hematocrit in men, 58% blood plasma and 42% of hematocrit in women. Hematocrit packed with erythrocytes, leukocytes and platelets (Sherwood 2010).

Erythrocytes are the most abundant blood cells with about 4-6 millions/mm3 in blood. Erythrocytes are commonly known as red blood cells. In mammalian, erythrocytes are free of nucleus to allow more room for haemoglobin and are biconcave in shape. Hence, vertebrate's erythrocytes have a nucleus. Haemoglobin is the main contained in erythrocytes; it carries oxygen to the tissues, collects and transports the unwanted carbon dioxide away, conveys nutritive substances like amino acids, sugars and mineral then gathers the waste materials that want to eliminated through the renal filter, carries hormones, enzymes and also vitamins to their sites of action (Sherwood 2010).

Leukocytes or white blood cells are much less abundant than red blood cells but bigger in size. They responsible for the defence of organism or eliminate harmful foreign material and make up the immune system of the body. The density of leukocytes in the blood is 5000-7000/mm3. There are two categories of leukocytes which are granulocytes and agranulocytes. Granulocytes is due to the presence of granules in cytoplasm and agranulocytes is the absent of granule in the cytoplasm. The granules are difference in different types of granulocytes and make it easier to distinguish among them. The granulocytes distinguish themselves as neutrophil, eosinophil and basophil. Agranulocytes distinguish themselves as lymphocytes and monocytes. Beside of the granules, shape of the nucleus help in recognition of leukocytes (Underwood 2004).

The proportion of neutrophil amongst leukocytes is about 50-70%. Its main function is phagocytes bacteria and always present in large amount within the pus of wound. Unfortunately, these cells dead after phagocytes due to

unable to renew the lysosomes that used in digesting microbes. Well, eosinophils only 2-4% amongst leukocytes, they attack parasites and phagocytes antigen-antibody complexes. Basophil is 0. 5-1%, it secrete anti-coagulant and vasodilator substances as histamines and serotonin. It takes part in phagocyte activity but the main function is secreting substances that mediate the hypersensitivity reaction. Lymphocyte own 20-40% proportion of leukocytes, its little cell that compact with round nucleus. Lymphocytes populate the lymphoid tissues (Bajanowski 1997), lymphoid organs (thymus, spleen, lymphoid nodules, and palatine tonsils) as well as the lymph that circulate in the lymphatic vessel (Underwood 2004).

Monocytes cooperate in immune defence although they are only 3-8% of leukocytes volume and it’s the precursors of macrophages (Sherwood 2010). They are large blood cells, which mature in the bone marrows before enter to the blood circulation and they only stay for 24-36 hours then will migrate into the connective tissue, where they become macrophages and move within the tissues. Monocytes migrate very rapidly to site if presence of an inflammation and intense phagocytory activity. Beside phagocytory activity, monocytes involve in secreting lysozime, interfereons and other defensive substances (Underwood 2004).

Platelets or thrombocytes are fragments of cells in the blood with diameter about 2-3μm; hence they are much smaller than erythrocytes. Their density in the blood is only 200000-300000/mm3. They are responsible for blood clotting to prevent blood loss from broken vessels. The blood vessel constricts to reduce blood flow and loss. Platelets then aggregate at the point of the broken vessel and produce a plug to stop blood loss. To this

purpose, they aggregate and release serotonin to reduce the diameter of lesion vessel and slow down the haematic flux to promote the blood coagulation (Sherwood 2010).

Plasma is the most abundant liquid component of blood with a yellowish colour. It makes up approximately 55% of total blood volume. Plasma is alkaline and it functional to maintains the pH of the blood at approximately 7. 4. It also maintains the osmotic balance of body cells. The composition of plasma is 90% water and 10% of dry matter like glucose, lipids, protein, glycoprotein, hormones, amino acids and vitamins (Sherwood 2010).

The morphology and characteristics of blood will be study by preparing the horse blood smears samples that with and without stained. Blood smears stained by haematoxylin and eosin are easier to identify under microscope during this experiment. Blood smears of different pathologies will be investigated and identified by taking noted the numbers of cells present, shape and sizes of different types of cells and remark with drawing.

METHODS

First part of this experiment involved preparation of horse blood smear samples. A small drop of horse blood is placed at one end of a slide and placed a cover slip at the edge of the blood then dragged gently through the slide in order to produce a thin blood smears. The blood smear needs to be essentially thin until the blood is hardly visible; this is to ensure that individual cells were easily determined. If the smear appear red that mean it is not thin enough or too thick, this may be hard to observe through the

microscope and do the cells count as packed cells is hard to see clearly under microscope.

Second slides are prepared by using exactly the same way as the first one. Both slides placed immediately into a container containing ethanol for 2 minutes. Ethanol is a colourless substance and used as a fixative, it helps to preserve cell smear samples so that cells do not denature. It does not damage the cells at all, just helps to maintain them for analysis. After the use of ethanol, the slide then dried by just slanting it on a piece of tissue. Dap and rubs are not allowed, as it will destroy the thin film of smear. The unstained smear was considered ready for analysis. It was placed a side waiting for investigation progress.

The second remaining slide then stained with haematoxylin and eosin. Haematoxylin is widely used in medical diagnosis; it is a blue substance stain that used to stained nuclei of cells into blue or purple colour. The nuclear staining is followed by other structures of the cells bodies with eosin stain that stain the granules of the cytoplasm in shades of red, pink and orange. Stained process performed by dipped the slide in a staining container containing haematoxylin for 2 minutes and rinse gently with water followed by dipped in another staining container containing eosin for 30 seconds and again rinse gently with water.

A drop of mountant is applied on the smear and then covered with a glass coverslip. Mountant is a medium used for mounting a slid for microscopy purposes. The staining times varied slightly because the specimen was leave

in the haematoxylin longer when the colour looks pale or pink and leave in the eosin for longer when it looks very dark blue.

Both slides are completed and viewed under microscope. Unstained and stained smears were then observed under the microscope initiate by x10 magnification to find the cells and upgraded to higher definition of x40 magnification for details observation. Observation started with stained smears followed by unstained smears, as stained smears is easier to determine the cells. Both smears were drawn accordingly and labelled all the particular structures of interest. Commend are made upon on how the stained smear differs from unstained smear.

In second part of the experiment, human blood smears are observed. Stained human blood smears taken from patients who suffer from no known pathology, sickle cell anaemia, eosinophilia, acute lymphocytic leukaemia and iron deficiency anaemia were observed. A textbook includes of brief description and expectation of what to see from the pathological blood smears are provided during the practical.

In this session, each slide provided is observed under microscope. Always started with x10 magnification and moved to x40 magnification while drawing. First, normal human blood smear is observed in order to identified elements in normal blood, then go onto the pathology smears and compared found morphology that identified in horse blood in part A. The cellular elements of each smear were drawn, labelled and recorded any differences observed in pathological smears when compared to normal blood smears. The relative numbers of each cell type are counted.

RESULTS:

Part A: Horse blood smears

Figure 1 illustrated stained horse blood smear under microscope of x40 magnification. The blood cells are stained with haematoxylin and eosin. One monocyte, one small lymphocyte, one neutrophil and bundle of erythrocytes (red blood cells) are seen. Nuclei of the leukocytes were purple-blue in colour due to the haematoxylin staining and the cytoplasm of the leukocytes appeared pink due to eosin staining. The erythrocytes are more abundant compared with leukocytes. Renown, erythrocytes are boconcave disc that absent of nuclei and mitochondria.

Figure 2 shows the unstained horse blood smear. The blood cells appeared to be transparent and hard to determine the differences between the erythrocytes and most of the leukocytes except monocyte, as it is greater in size.

Part B: Human blood smears

Figure 3 shows the human blood smear with no known pathology. Erythrocytes, leukocytes (neutrophils, monocytes, lymphocytes, basophil) and platelets are presented. The smear make out with more abundant of erythrocytes (R. B. C) than leukocytes and they all appeared healthy. The leukocytes were blue-purple in colour surrounded by numerous erythrocytes that were pinkish in colour. Most of the leukocytes seen are neutrophils; this proves the theory stated leukocytes making up with 50-70% of neutrophils. The neutrophils were intermediate in size, lymphocyte was smaller and monocyte was larger. Their cytoplasm appeared pink in colour. The nucleus of neutrophil lobed with clumps of chromatin.

Figure 4 shows human blood smear with sickle cells anaemia. Abnormal red blood cell morphology and sickle cells are seen.

Figure 5 shows human blood smear with eosinophilia deficiency. Abnormal or sickle red blood cells appeared. Eosinophil and monocytes are broken. Several of smudge cells presented.

Figure 6 shows the blood smear for acute lymphocytic leukaemia. The erythrocytes are not as densely pack as in the smear of human blood with no pathology. This observation clearly illustrated the presented of several lymphocytes in the smear and it appeared larger than erythrocytes. Where acute lymphocytic leukaemia is a blood cancer where the body produces a large numbers of lymphocytes.

Figure 7 shows the smear for human iron deficiency anaemia. The erythrocytes are pallor in appearance. Some of the erythrocytes were larger in size. Besides that, smudge cells and different types of leukocytes seen in this smear. There are lymphocytes that small in size and also neutrophils.

DISCUSSION

Many diseases, disorders, and deficiencies can distinguish by observation of blood cells distribution and appearances (Bain 2005). Disproportionate numbers of leukocytes, presence of immature leukocytes, too high or too low of platelets counts, and deformed of red blood cells are all signs of serious diseases. Somehow, blood smear provides the primary evidence of a specific diagnosis. Monocytes of horse blood smear in fig. 1 are greater in size compare with human blood smear in fig. 3. The comprehensive kinetic force between erythrocytes of horse blood is stronger and produced closely

attached long chain of erythrocytes. The erythrocytes in fig. 1 and 3 appeared normal, uniformed in size and do not have a nucleus as most other cells do. They are round and flattened like a donut with a depression in the middle. Due high density of haemoglobin presented inside the erythrocytes (Sherwood 2010), they appear pink to red in colour with a pale centre. While there are some erythrocytes in fig. 4, 5, 6 and 7 had significant different in shape and irregularities that indicate severe problems.

The histological section with stained are more visible and can be noted that the nuclei of the cells appeared purple-blue with stained of haematoxylin (Bain 2005); cytoplasm appeared pinkish with stained of eosin. Unstained leukocytes are colourless and hard to determine as they lack haemoglobin (Bain 2005). The stains enhanced the illustration of the leukocytes and make it easier to distinguish. Granulocytes and agranulocytes were differentiated by observed their cytoplasm. Granulocytes are neutrophil, eosinophil and basophil that has granule in their cytoplasm and its cytoplasm is visible when staining, while agranulocytes are lymphocytes and monocytes that absents of granule in their cytoplasm where their cytoplasm appeared transparent although stained.

Neutrophils are cells that have cytoplasm with pink granules, intermediate in size with lobed clumped nucleus, can be identified by observing their nuclei; their nuclei are segmented into 2-5 lobed of different shapes. They composed majority of leukocytes and function to phagocytosis . Eosinophils will easily recognize with their large, red-orange granules. Unfortunately, they aren’t found from the smears because they are generally low in number. Eosinophils most often become elevated in number when the

individual are facing with allergies or parasitic infections. Basophils (figure 1) had large black granules and least often seen from the smears as they are only 1% of leukocytes. Increased numbers of basophiles are not often encountered but may be elevated in certain leukaemia, chicken pox, ulcerative colitis, or after an immunization. Monocytes are the largest cell amongst leukocytes with diameter of 12-20 µm and are often referred as phagocytes. They engulf particles such as cellular debris and bacteria. LHYPERLINK " javascript: optionsdisplay('../../../glossary/lymphocyte. html')" ymphocytes are smaller and have a homogeneous cytoplasm and a smooth, round nucleus. These cells are responsible for the production of antibodies or immunoglobulin (Bajanowski 1997). There are two types of lymphocytes, B and T cells and they mediated within each other. B cells induce production of antibodies; T cells destroy specific cells (Bajanowski 1997).

Figure 4 illustrated human sickle cell anaemia. Sickle cell anaemia (SCA) affects millions of people worldwide (Charlotte 2010). SCA is disorders of erythrocytes that caused difficulty to haemoglobin molecules when delivers oxygen to cells throughout the body (Peterson 2009). The change of the amino acid results in haemoglobin that responds to the oxygen deficiency by stacking filaments and clustering in red blood cells containing the mutated protein in such a way that their shape is distorted (Sherwood 2010).

Eosinophil usually hardly noticeable in blood smears indicates the response of the body to abnormal cells, parasites, or substances that cause an allergic reaction. Donor of the blood smear illustrated in figure 5 may have eosinophilia disorder as broken eosinophil is presented. Eosinophilia is

commonly happened to people who have asthma, hay fever, food allergic or parasitic infections such as intestinal worms (Sherwood 2010).

In the acute lymphocytic leukemia sample shown in figure 6, there was a noticeable increase in the number of lymphocytes seen. The erythrocytes are pallor and lymphocytes appeared larger than erythrocytes and this is due to a disease of lymphoid cells causing uncontrolled production of lymphocytes (Underwood 2004). Acute lymphocytic leukaemia is a disease where the physical changes take place within the cell (McClain 1990), a reduced count of red blood cells with a raised level of leukocytes. This may leads to an accumulation of blast cells in the bone marrow and causes bone marrow failure (McClain 1990).

All the red blood cells in the iron deficiency anaemia sample appeared pale in colour. This usually caused to people with poor diet that contains little iron especially vegetariansbecause the main dietary source of ion is red meat. Besides that, diseases of the small intestine such as gluten intolerance can reduce its ability to absorb iron (Sherwood 2010).