# Frequency Rate of Abnormal Morphologic Shapes of the Erythrocytes upon the Different Types of Anemia

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**Abstract- Anaemia is one of the blood diseases that are different forms of blood cells. These shapes indicated the type of disease. It is through the study of 10,000 image from 19 types of anaemia has been drawn 40 different shapes of the natural shape of red blood cells. The interesting findings in this study are included Iron deficiency anaemia (IDA) is the most common cases disease; it could reach to 39.5% out of total cases. The most important frequency shapes in IDA are hypocromic microsite (54-62%), Ellipsoids (12-15%), Normocytes (9-11%) and (target, dear drop) (7-9%). Southeast Asian anaemia (SEA) is most common in Malaysia and might be second place after iron deficiency anaemia. The most common shapes of anaemic erythrocytes are stomatocytes (45-64%), kinzocyte (40-45%) and Basophilic (5-8%). Thalassemia showed about 93% of shape of total abnormal RBCs shaped. The majority of abnormal shapes were the hypocromic microsites (54-67%), target (17-21 %) and basophilic cells (8-15%). Megoblastic showed most common shapes of ovalocytosis (41-45%), spherocytosis (29-33%). Haemolytic anaemia is the fourth type of anaemia was shown the erythrocytes look hyporocromic macrosytis, which showed 39% of total abnormal cells. The majority of abnormal shape showed in the Spherocytis (29-35%), Polychromatophilia (13-16%) and Ovalocytosis (9-15%). Red distribution width (RWD) showed highest in IDA, which caused a wide variation in red cell size.**

**Key words-** *anemia, image collection, feature extraction, distribution width (RWD),* use Euclidian distance, *shape cell.*

## INTRODUCTION

Anemia is defined as a significant reduction in the mass of circulating red blood cells. As a result, the oxygen binding capacity of the blood is diminished. Because blood volume is normally maintained at a nearly constant level, anemic patients have a decrease in the concentration of red cells or hemoglobin in peripheral blood. Haemoglobin and hematocrit levels vary with the age of the individual and, in adults, with gender. Anemic patients' values are more than one standard deviation below the mean values for their gender. However, because of the wide range in normal hemoglobin and hematocrit levels, it is often difficult to document mild anemia (Beutler and Waalen, 2006) Anemia also spelled anemia or anæmia; from Ancient Greek: anaimia, meaning lack of blood, is a decrease in number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood (Parmar et al., 2011; Saimak and Melissa, 2009). However, it can be included decrease oxygen-binding ability of each hemoglobin molecule due to deformity or lack in numerical development as in some other types of hemoglobin deficiency. Because hemoglobin (found inside RBCs) normally carries oxygen from the lungs to the capillaries, anemia leads to hypoxia (lack of oxygen) in organs. Since all human cells depend on oxygen for survival, varying degrees of anemia can have a wide range of clinical consequences (Parmar et al., 2011, Finch et al., 1968).

The blood sample is examined under a microscope to assess the size, shape, and color of the red blood

cells. Normal mature red cells can be described as round, elastic, non-nucleated, bi-concave and cells have an area of central pallor which covers about one-third of the cell. Normal mature red blood cells have an average diameter of 7.2 microns with a range of 6-9 microns (Kim et al., 2012).

The most common anaemia are Iron deficiency anaemia, megaloblastic anaemia, thalassemia, South East Asia anaemia, hemolytic anaemia, sickle anaemia, sideroblastic, Hereditary Spherocytosis, Elliptocytosis and stomatocytosis anaemia, anaemia of chronic disorders (Hermiston et al., 2002). There are two general approaches that could use to identify the causes of anaemia that involved kinetic and morphologic approaches. Morphological approach is also known as a peripheral smear or red blood cell morphology (Tefferi, 2003; Guyatt et al., 1992; Gjorup et al., 1986). The objective of this study was to determine the frequency rate and abnormal shapes of the erythrocytes upon the different types of anaemia.

## Materials and Methods

The red blood cells shape extraction system (RBSES) has two phases, included the conversion phase and processing phase. First phase aims to conversion the peripheral blood smear slide to images. The second phase aims to process images of anaemic cases to extract the frequency and abnormal shapes of cells for each case.

In conversion phase, Peripheral blood smear slides related hematological cases were obtained from haematology unite/ pathology department/ faculty of medicine/ Serdang Hospital. It is through the study of 200 haematology slides for 19 types of anaemia. anaemic cases that involved Iron deficiency anaemia (IDA 79), Thalassemia, 13). Haemolytic anaemia (HA 10), Southeast Asian anaemia (SEA 15) and Megoblastic anaemia (MA 8), while there were 75 cases for individual cases combined from more than one type.

A. 25 imagines were obtained of each slide from different cases. Morphologic abnormalities of peripheral red blood cells using microscopic examination with the oil immersion lens of well-prepare areas that red blood cells individual separated stained with Wright's stain. It has been used Olympus BX43 photo imaging microscope U-CAM D3 (Japan) in SEGI University.

B. The process phase consists by four stapes. After image collection, the imaging is the first segmented prepared in order to isolate the interesting part and remove noise and undesired components. The segmentation of interesting part of RBC is the first necessary step that aims at making the rest of the steps focus on the slide. Next step of the feature extraction process is applying to extract the useful information from segment blood cells, this information includes calculating size and frequency of each different abnormal cells. Then measure size of the cell depending on the number pixels edge cell. Finally, calculation red cell distribution width (RDW). Where RDW is a parameter that measures variation in red blood cell size. Finally, the classification can be operated according to the feature extracted by previous stage. In addition we use Euclidian distance methods to classify the given class of input abnormal image (Fig.1) presented this phase.

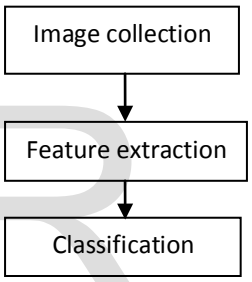



Fig 1: flow diagram of image processing

**Feature Extraction**

Feature extraction is involves simplifying the amount of resources required to describe a large set of data accurately. Our features mainly fall into two categories: shape based features and color texture based features. In this research focused in one of most important feature is the cell size. Cell size describes number of pixels in couture shape. Equation (1) descript of size feature. While equation (2) descript of frequency

$$M = \frac{Y^2}{D} \quad \dots 1$$

$$F = \frac{n}{N} * 100\% \quad \dots 2$$

where:

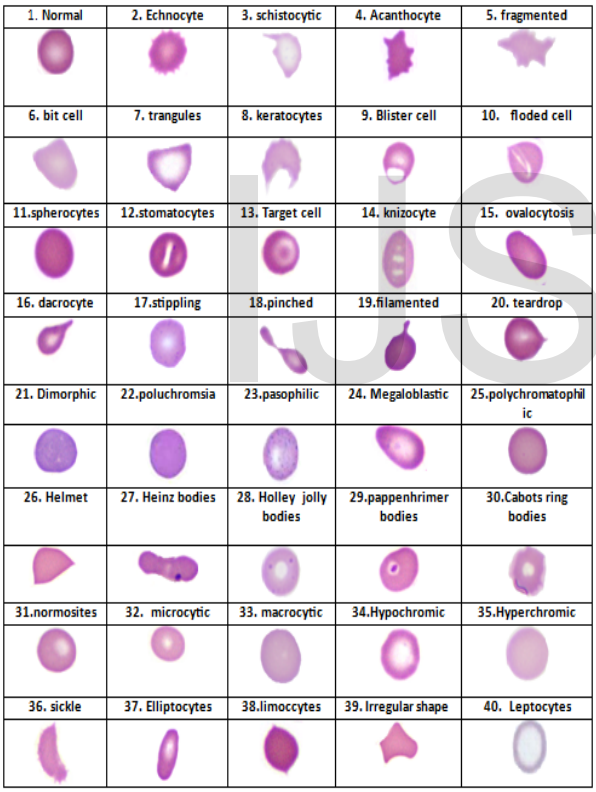
*M is number of boundary pixel*
*Y is number of circumference of shape pixel.*
*D is number of area of shape pixel.*

*n is number of type cell.*
*N is total number cell*

## Results

Anaemia is one of the blood diseases that are different forms of blood cells. These shapes indicated the type of disease. It is through the study of 10,000 image from 19 types of anaemia has been drawn 40 different shapes of the natural shape of red blood cells (Table 1). The interesting findings in this study was the most common hematological cases in Serdang hospital are included Iron deficiency anaemia (IDA), Thalassemia, Southeast Asian anaemia (SEA), Haemolytic anaemia, and Megoblastic anaemia.

Table 1: The common abnormal shapes of erythrocytes of different anemic cases




***Iron deficiency anaemia*** (IDA) is the most common cases disease, it could reach to 39.5% out of total cases, the RBCs showed 39 different types and shapes (Table (1). The most important frequency shapes in IDA are hypocromic microsite (54-62%), Ellipsoids (12-15%), Normocytes (9-11%) and (target, dear drop) (7-9%) while 7-13% of other forms (Table 2).

Table 2: The shape, size and frequency of each erythrocytes an Iron deficiency anemia

| No. | Cell type | Cell shape | Size cell (number of pixels) | frequency |
|---|---|---|---|---|
| 1 | Hypocromic microsite | | 29852 - 46469 | (54-62)% |
| 2 | Ellipsoids | | 25136 - 44486 | (12-15) % |
| 3 | Normocytes | | 30979 - 31730 | (9 -11) % |
| 4 | Teardrop | | 20082- 41006 | (7 -9) % |
| 5 | Target cell | | 30209 - 35352 | (7- 9) % |
| 6 | fragment | | 24916 - 36994 | (2- 4)% |
| 7 | Helmet | | 24916 - 40619 | (1-3)% |
| 8 | Bit cell | | 28814 -37334 | (1-3) % |
| 9 | Others | | 29987-36897 | (7%-13)% |

***Southeast Asian anaemia*** **(SEA)** is most common in Malaysia and neighboring countries, and might be second place after iron deficiency anaemia. This type has two different cases either individually case or united with another case such are IDA, Thlassemia and megoblastic. In any case of them, it contents 16 different abnormal shapes. The most common shapes and frequency of anaemic erythrocytes are stomatocytes (45-64%), kinzocyte (40-45%) and Basophilic (5-8%), while other frequency shows in table (3).

Table 3: The shape, size and frequency of erythrocytes in SEA

| N0 | Cell type | Cell shape | Size cell (number of pixels) | Frequency |
|---|---|---|---|---|
| 1 | Stomatocytes | | 31996 -33504 | (45 -64)% |
| 2 | Knizocyte | | 33949-43591 | (40 -54)% |
| 3 | Basophilic | | 32014 -33383 | (5-8)% |
| 4 | Target | | 55261 -62259 | (4 -8)% |
| 5 | Teardrop | | 31542 -34443 | (3 -5)% |
| 6 | Ovalocytosis | | 30475 -39366 | (2 -5)% |
| 7 | Eilliptocytes | | 31107 -37275 | (1 -3)% |
| 8 | Spherocytes | | 31399 -35875 | (1-3)% |

***Thalassemia*** is other type of anaemia, which showed the erythrocytes in different shapes and frequency. It about 93% of shape of total abnormal RBCs shaped in case (Table 1). The majority of abnormal shapes were hypocromic microsites (54-67%), target (17-21 %) and basophilic cells (8-15%), these are considering the keys indicators for the diagnosis thalassemia, and there other different shapes (Table (4).

Table 4: The shape, size and frequency of erythrocytes in thalassemia

| N0 | Cell type | Cell shape | Size cell (number of pixels) | Frequency |
|---|---|---|---|---|
| 1 | Hypocromic microsites |  | 31843-34887 | (54-67)% |
| 2 | Target |  | 32014-32145 | (17-21)% |
| 3 | Basophilic |  | 31786- 33654 | (8- 15)% |
| 4 | Acanthocytes |  | 28619-31841 | (8-12)% |
| 5 | Spherocytes |  | 31124-32671 | (7-13)% |
| 6 | Burr cells |  | 28404-37129 | (2- 7)% |
| 7 | Schistocytes |  | 27619-31490 | (3-6)% |
| 8 | Limon cell |  | 39365-40640 | (1-6)% |

***Haemolytic anaemia*** is the fourth type of anaemia was shown the erythrocytes look hyporocromic macrosytis, which showed 39% of total abnormal cells (Table 1). The majority of abnormal shape showed in the Spherocytis (29-35%), Polychromatophilia (13-16%) and Ovalocytosis (9-15%), also there other abnormal shapes (Table 5).

Table 5: The shape, size and frequency of erythrocytes in haemolytic anaemia

| N0 | Cell type | Cell shape | Size cell (number of pixels) | Frequency |
|---|---|---|---|---|
| 1 | Normocytec |  | 30644 --31834 | (30 -46)% |
| 2 | Spherocytes |  | 31124 --32671 | (29 -35)% |
| 3 | Polychromatophilia |  | 30606 --33813 | (13 -16)% |
| 4 | Ovalocytosis |  | 28611 --38023 | (9 –15)% |
| 5 | Irregular shape |  | 24946 --41460 | (9 –13)% |
| 6 | Burr cell |  | 31355 --32721 | (2 –6 )% |
| 7 | Acnathocyte |  | 29321 --33278 | (2 -4)% |
| 8 | Schistocytic |  | 22322 --29815 | (1 –2)% |
| | Limon cell |  | 23116 --37981 | (1-2)% |

***Megoblastic anaemia*** is the case showed the erythrocytes are large and contain one or more of the abnormal bodies such as Hinze or Holly jolly. The most common shapes were ovalocytosis (41-45%), spherocytosis (29-33%) and others cells show in table (6).

Table 6: The size, shape and frequency of erythrocytes in megoblastic anaemia

| N0 | Cell type | Cell shape | Size cell (number of pixels) | Frequency |
|---|---|---|---|---|
| 1 | Ovalocytosis |  | 29502-35858 | (41- 45)% |
| 2 | Spherocytosis |  | 29502-35858 | (29-33)% |
| 3 | Teardrop |  | 31587- 48936 | (14-18)% |
| 4 | normocytoses |  | 29763- 33783 | (13-17)% |
| 5 | Elliptocytes |  | 27618- 29905 | (2- 6)% |
| 6 | Acanthocyte |  | 30960 - 33601 | (1-3)% |

On another hand, sometimes there is a great similarity in the images shape of RBCs between Thalassemia and Iron deficiency anaemia, it looks of red distribution width (RWD). The RWD is high in IDA, which caused a wide variation in red cell size. In Thalassemia the red cells are strangely all in same size there is virtually no variation, however the RWD is low table (7).

The results of this study showed the RWD is high in IDA compared with thalassemia and other diseases that showed the variation in types of anaemia, while show rate of abnormal cells in each type of anaemia. It is worth mentioning that the size of blood cell may change from one state to another. For example, a cell from type Eillipocytes sizes up to 62259 pixels in ESA while in IDA up to 25136 pixels, also Tear drop size (Fig. 2). The variation of abnormal cells was also seen in the megoblasitic anaemia up to 48936 pixels and in IDA up to20082 pixels (Table 8).

## Discussion

The normal red blood cell is a biconcave disk, 6 to 9 µm in diameter and 1.5 to 2.5 µm thick. In the peripheral smear, red blood cells are anucleate and contain predominantly hemoglobin that is distributed to form a dense outer rim with a paler center that occupies approximately one third of the diameter of the cell. The hemoglobin imparts a uniform pink to orange-red color to the cytoplasm that is typically without inclusions. Normally all red blood cells are relatively uniform in size and shape. Numerous disease states affect the size, shape and hemoglobin content of red cells. Variation in size is referred to as “anisocytosis,” and variation in shape is termed “poikilocytosis.” Pathologic red cells may be larger or smaller than normal, may be abnormally shaped, or may contain inclusions (Perkin, 2009).

The results of this study showed there are about 40 different forms of cells with anaemia, and the majority of these forms are found in the IDA and Thalassemia. The diagnosis of the disease might be not depended on all these forms, but the most frequent forms. Therefore, each condition is different in shape and recurrence (RBC). For instance, the red blood cells in an Iron deficiency anaemia showed an anisocytosis appear dimorphic which a population hypochromic microcytic cells as well as population of normochromic, normocytic cells poikilocytosis is increased with occasional ellipocytes, tear drop and target cells present. Polychromasia appears increased and a rare nucleated red blood cell is seen on scanning. While the Megaloblastic anaemia cells as described Macrocytosis, Howell-Jolly Bodies nucleated erythrocytes and Anisocytosis. Hereditary stomatocytosis anaemia cells as described Stomatocytes and knizocyte (Gabriel and Przybylski, 2010, Carl, 1995). In another case, thalassemia can be inferred that red blood cells look hypochromic microcytic, target cell, spiculet, basophilic, ovalocyte, terdrop and envelope tables 2,3,4,5, and 6 displays shapes of cells that characterize each case that can be diagnosed on this basis. Sometimes there is a great similarity in the images shape of RBCs between thalassemia and iron deficiency anaemia, in this case of similarity in the peripheral blood smear.

The results of this study showed the RWD is high in IDA because of a wide variation in red cell size. In thalassemia the red cells are strangely all the same size and there is virtually no variation therefore the RWD is low. We found that iron deficiency with any other case satisfactory results in a high rate RWD. It is worth mentioning that the size of blood cell may change from one state to another. For example, a cell from type Eillipocytes sizes up to 62259 pixels in ESA while in IDA up to 25136 pixels. Also Tear drop size, these results were agreed with previous repots (Sultana et al., 2011; Aulakh et al., 2009)

The examination of blood films stained with Wright's stain frequently provides important clues in the diagnosis of anaemia. Diagnostic standpoint of poikilocytosis has no specificity, but the recognition of specific forms of poikilocytes as irregularly shaped cells often points to specific disorders. Spherocytes are round, densely staining red cells that lack central pallor and have a smaller than normal diameter. In stomatocytes, the area of central pallor is elliptical rather than round, giving the cell appearance of the opening of a mouth (stoma). Target cells (codocytes) have a centrally located disk of hemoglobin surrounded by an area of pallor with an outer rim of hemoglobin adjacent to the cell membrane giving the cell the appearance of a target. Leptocytes or wafer cells are thin, flat cells with the hemoglobin at the periphery of the cell. Sickle cells (drepanocytes) are elongated, sometimes crescent-shaped, erythrocytes with pointed ends. Elliptocytes (ovalocytes) range from slightly oval to elongated cigar-shaped forms. Teardrop erythrocytes (dacryocytes) are red cells with one end round and the other end more pointed (Gabriel and Przybylski, 2010, Carl, 1995). Acanthocytes have several usually 3 to 7 irregularly spaced blunted projections from the margin of the cells. Echinocytes are also cells with cytoplasmic projections, but in contrast to acanthocytes, the projections are typically evenly spaced on the cell surface, more numerous often 10 to 15, and frequently have sharper points.

Schizocytes (schistocytes) are fragmented erythrocytes appearing in a variety of morphologic forms such as small triangular erythrocytes, helmet cells, and normal-size erythrocytes with 2 to 3 pointed surface projections (keratocytes, or "horn cells"). Round erythrocytes with a single, elliptical or round surface defect are termed bite cells. Rouleaux formation is a phrase denoting the stacking of erythrocytes, generally in a curving pattern (Eliane et al., 2010; Giovanna and Stefano, 2011). Morphologic identification of inclusion bodies within erythrocytes can be helpful clinically. Howell–Jolly bodies are purple spheres, usually about 0.5 μm in diameter, presenting singly, or rarely multiply in the cytoplasm. Basophilic stippling of erythrocytes refers to numerous very small coarse or fine blue granules within the cytoplasm. When the stippled particles are due to iron granules (demonstrable by the Prussian blue stain), they are termed Pappenheimer bodies (Tefferi, 2003; Ciccoli, et al., 2012).

**Conclusion**

Anaemia is one of the types of blood disease which infects red blood cells. When red blood cells are being abnormal, they not take the form of described as round, but vary according to the causative. Each type of

anaemic cells showed different shape, color and size there are about 40 different forms of cells with anaemia, most these forms in satisfactory condition as an IDA and Thalassemia. The RWD is high in IDA compared with Thalassemia and other types of anaemia. The iron deficiency showed highest rate of RWD.

Table 7: Shows the variation in size of cells in each type

| Type anaemia / Cell | Rang of cell size | | | | |
|---|---|---|---|---|---|
| | Haemolytic | Thalassimia | Megoblastic | IDA | ESA |
| Normocytec | 30644 - 31834 | | 29763 - 33783 | 30979 - 31730 | |
| Polychromatophilia | 30606 -33813 | | | | |
| Spherocytes | 31124 -32671 | 31124 -32671 | 29502 - 35858 | | 31996 -33504 |
| Irregular shape | 24946 - 41460 | | | | |
| Burr cell | 31355 -32721 | 28404 -37129 | | | |
| Ovalocytosis | 28611 - 38023 | | | | |
| Acnathocyte | 29321 -33278 | | 30960 - 33601 | | 33949-43591 |
| Schistocytic | 22322 -29815 | 22619 - 40490 | | | |
| Limon cell | 23116 -37981 | 39365 - 40640 | | | |
| Target cells | | 32014 -32145 | | 30209 - 35352 | 32014 -33383 |
| Basophilic | | 31786 - 33654 | | | 31399 -35875 |
| Tear drop | | | 31587 - 48936 | 20082- 41006 | 30475 -39366 |
| Eillipocytes | | | 27618 - 29905 | 25136 - 44486 | 55261 -62259 |
| Hypocromic microsite | | | | 29852 - 46469 | |
| Fragment | | | | 22916 - 36994 | |
| Helmet | | | | 24916 - 40619 | |
| Bit cell | | | | 28814 -37334 | |
| Stomatocytes | | | | | 31542 -34443 |
| Knizocyte | | | | | 31107 -37275 |

Table 8: Shows the variation in frequency cells in each type

| Type anaemia / Cell | frequency | | | | |
|---|---|---|---|---|---|
| | IDA | ESA | Thalassimia | Haemolytic | Megoblastic |
| Normocytec | (9 -11) % | | | (30 -46)% | (13-17)% |
| Polychromatophilia | | | | (13 -16)% | |
| Spherocytes | | (1-3)% | (7-13)% | (29 -35)% | (29-33)% |
| Irregular shape | | | | (9 –13)% | |
| Burr cell | | | (2- 7)% | (2 –6 )% | |
| Ovalocytosis | | (2 -5)% | | (9 –15)% | (41- 45)% |
| Acnathocyte | | | (8-12)% | (2 -4)% | (1-3)% |
| Schistocytic | | | (3-6)% | (1 –2)% | |
| Limon cell | | | (1-6)% | (1-2)% | |
| Target cells | (7- 9) % | (4 -8)% | (17 -21)% | | |
| Basophilic | | (5-8)% | (8- 15)% | | |
| Tear drop | (7 -9) % | (3 -5)% | | | (14-18)% |
| Eillipocytes | (12-15) % | (1 -3)% | | | (2- 6)% |
| Hypocromic microsite | (54-62)% | | (54-67)% | | |
| Fragment | (2- 4)% | | | | |
| Helmet | (1-3)% | | | | |
| Bit cell | (1-3) % | | | | |
| Stomatocytes | | (45 -64)% | | | |
| Knizocyte | | (40 -54)% | | | |

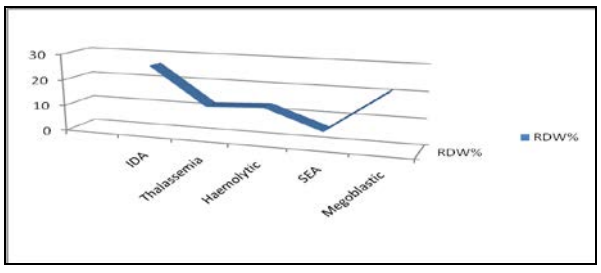



Fig 2: Shows the RDW of different type of anemia

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