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(Abnormal Red Blood Cell Shapes In Different Diseases)

A Graduation Report Project Submitted to the Council of the Medical Techniques Collage as a Partial Fulfillment for Achieving BSc in Medical Laboratory Techniques.

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بسم الله الرحمن الرحيم

صدق الله العظيم

Dedication

To each one of our families, thank you for being there for us, your encouragement and support, we wouldn’t be here without you, and for that, we thank you all sincerely

To our friends and colleagues, those last four years were a bless, and may it continue further beyond.

Acknowledgments

We would like to thank our entire committee from superiors and teachers of our medical analysis department, our professors, the head of the department and all of those whose been there for us, who advised, taught, and supported us ever since we've enrolled here at the Alkitab University, we truly benefited greatly from our daily interactions with them during the past four years, thus thank you, truly.

To our supervisor, Dr. Zirar Saleem, whose never hesitated to give his all to us, we are forever in his dept for his guidance and encouragement to us, and for his patience while discussing the graduation project.

To our diseased professors and lecturers whom we miss during these years, may our prayers reach them.

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Chapter One: Introduction

Red blood cells (RBCs) also referred to as red cells, red blood corpuscles (in humans or other animals not having nucleus in red blood cells), are the most common type of blood cell and the vertebrate's principal means of delivering oxygen to the body tissues—via blood flow through the circulatory system. RBCs take up oxygen in the lungs, or in fish the gills, and release it into tissues while squeezing through the body's capillaries. [1]

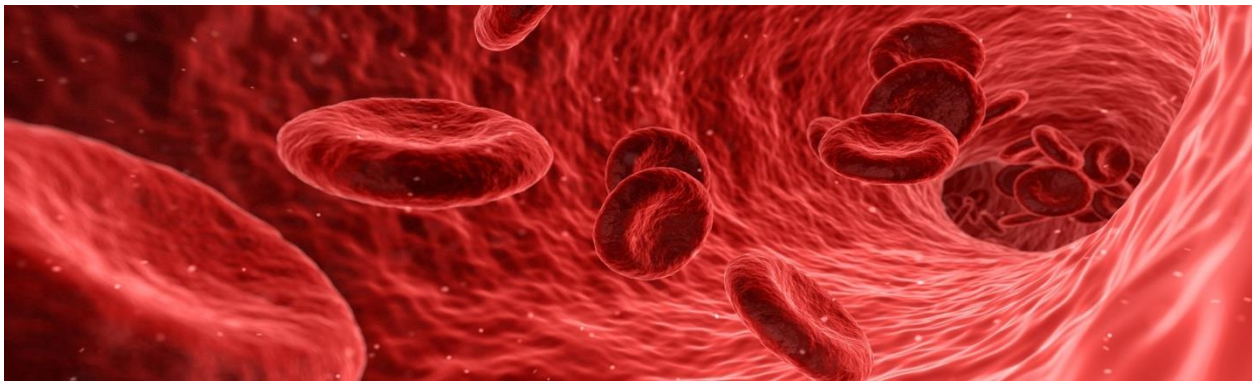

Figure 1: 3D Computer image of a blood vessel showing RBCs

The cytoplasm of erythrocytes is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the red color of thecells and the blood. Each human red blood cell contains approximately 270 million of these hemoglobin molecules. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network. [2]

Red blood cells are the most abundant type of blood cells in the human body. The count of these vital cells is often the first step done in analyzing a patient's pathological condition. Normal RBC's are biconcave in shape with a central pale area, and any deviation in size, shape, volume, structure or color represents an abnormal cell. Such abnormalities are detected by viewing the blood-smear images through a microscope, a time consuming and error-prone method. [3]

Chapter Two : Abnormalities

While Normal red blood cells are round, flattened disks that are thinner in the middle than at the edges, there are abnormalities that might happen to the shape of the RBCs, referred to as Poikilocytosis, which is the term for abnormally shaped red blood cells in the blood. Poikilocytes may be flat, elongated, teardrop-shaped, crescent-shaped, sickle-shaped, or may have pointy projections, or other abnormal features. It is necessary to know about the different types of poikilocytosis and common causes of early diagnosis and treatment. [4]

In this chapter, we will talk about these types of RBC abnormalities.

2.1-Echinocytes:

These are a form of red blood cell that has an abnormal cell membrane characterized by many small, evenly spaced thorny projections. A more common term for these cells is burr cells. [5]

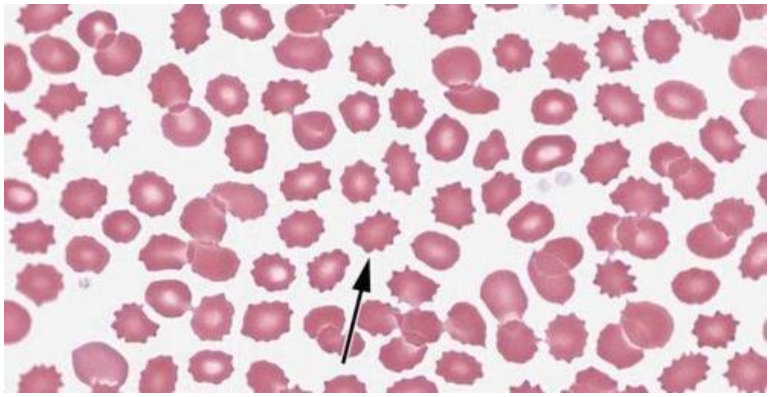

Figure 2: Echinocytes (burr cells) in a peripheral blood smear.

Echinocytes are associated with the following conditions [6]:

- Uremia and chronic kidney disease
- Liver disease (e.g., cirrhosis)
- pyruvate kinase deficiency
- hypophosphatemia
- hyperlipidemia
- Phosphoglycerate kinase deficiency
- Disseminated malignancy
- Myeloproliferative disorders
- Vitamin E deficiency

2.2- Schistocytes:

Schistocytes are typically irregularly shaped, jagged, and have two pointed ends.

Several microangiopathic diseases, including disseminated intravascular coagulation and thrombotic microangiopathies, generate fibrin strands that sever red blood cells as they try to move past a thrombus, creating schistocytes. [7]

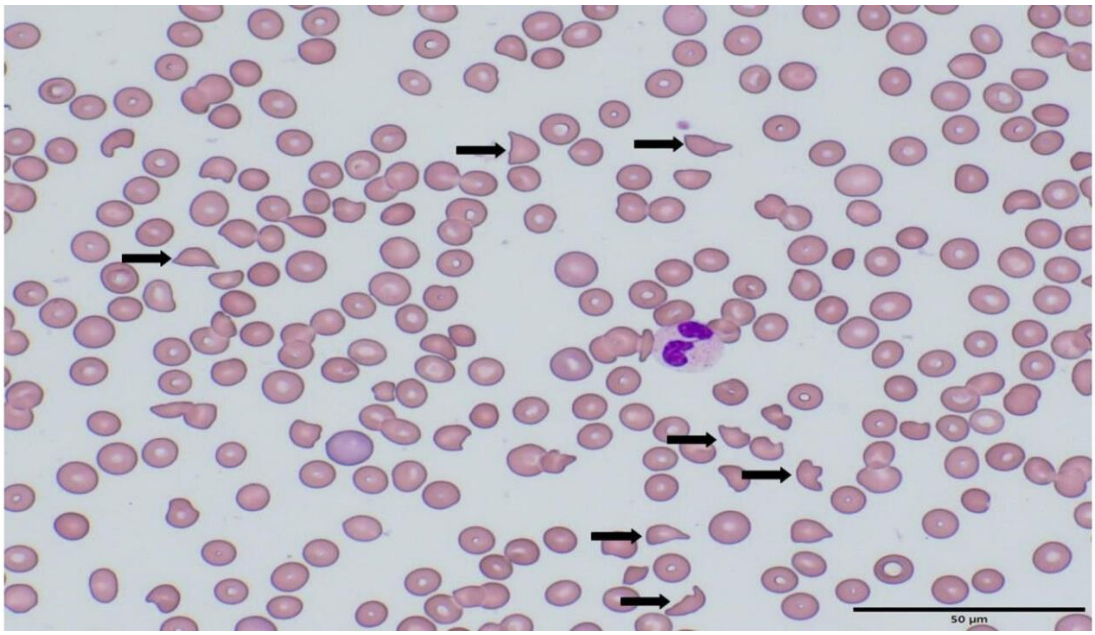

Figure 3: Numerous schistocytes in thrombotic thrombocytopenic purpura (TTP) patient, Kumamoto Medical Center, Kumamoto 860-0008, Japan.

Schistocytes are often seen in patients with hemolytic anemia. They are frequently a consequence of mechanical artificial heart valves and hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, among other causes.

Excessive schistocytes present in blood can be a sign of micro-angiopathic hemolytic anemia (MAHA). [7]

2.3-Acanthocytes:

These are abnormal red blood cells with spikes of different lengths and widths unevenly positioned on the cell surface. These unusual cells are associated with both inherited and acquired diseases. But most adults have a small percentage of acanthocytes in their blood. [8]

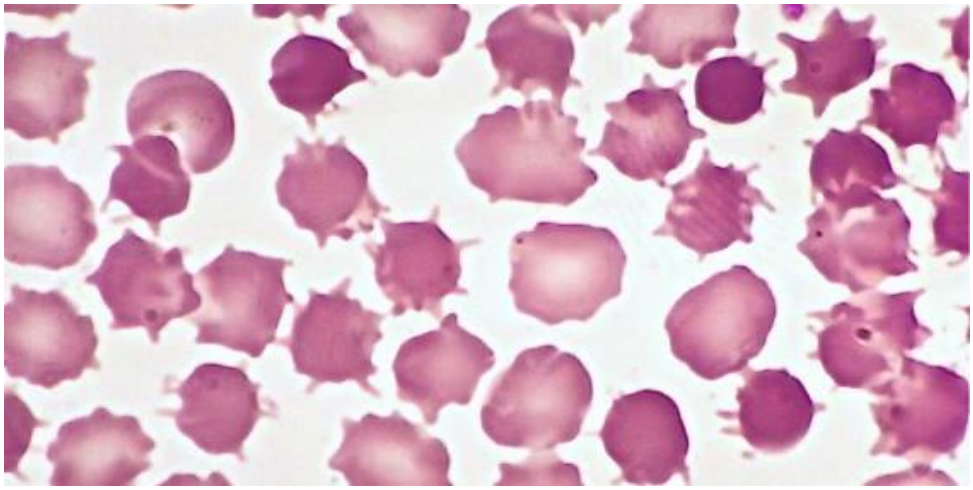

Figure 4: Acanthocytes – this blood smear taken from patient with Abetalipoproteinemia

Acanthocytes are found in people with the following conditions [8]:

- severe liver disease
- malnutrition
- hypothyroidism
- abetalipoproteinemia (a rare genetic disease involving an inability to absorb some dietary fats)
- Splenectomy (spleen removal)
- Anorexia nervosa

2.4-Degmacyte (Bite cell):

Are an abnormally shaped mature red blood cell with one or more semi-circular portions removed from the cell margin, known as "bites". These "bites" result from the mechanical removal of denatured hemoglobin during splenic filtration as red cells attempt to migrate through endothelial slits from splenic cords into the splenic sinuses. Bite cells are known to be a result from processes of oxidative hemolysis, such as Glucose-6-phosphate dehydrogenase deficiency, in which uncontrolled oxidative stress causes hemoglobin to denature and form Heinz bodies. Bite cells can contain more than one "bite." The "bites" in degmacytes are smaller than the missing red blood cell fragments seen in schistocytes. [9]

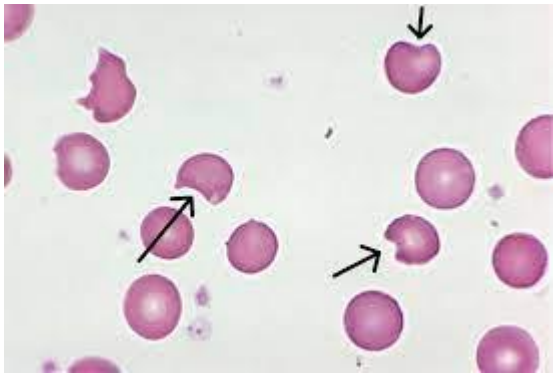

Figure 5: a sample showing Degmacyte cells which are indicated by the arrows, in comparison to normal RBCs.

Degmacytes usually appear smaller, denser, and more contracted than a normal red blood cell due to the bites. The appearance of the "bites" in red blood cells may vary in number, smoothness, and size. [10]

2.5-Keratocytes:

Keratocytes are erythrocytes with a blister-like vesicle, which may rupture, leaving a "bite-shaped" defect in the cell outline or one or two horn-like projections on the same side of the cell. This process may occur more than once for a given cell, resulting in very irregular shapes. [11]

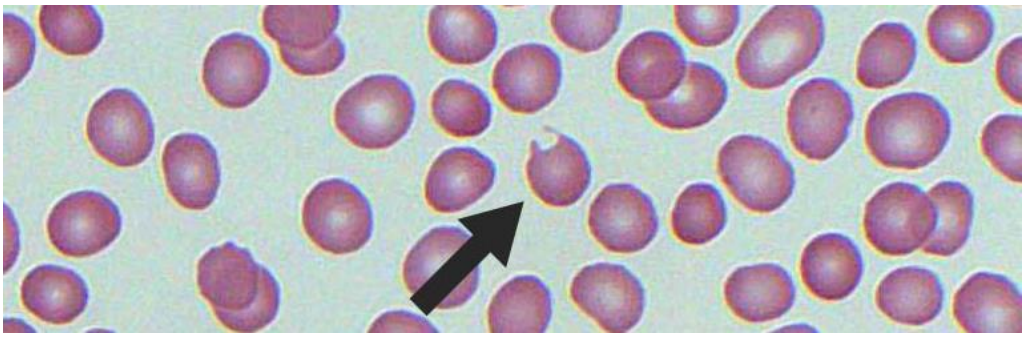

Figure 6: Keratocytes in a blood smear, indicated by the arrow.

Low numbers of kerato-cytes may be seen in various situations and may not have any clear clinical significance. keratocytes can indicate the following [11]:

- **Fragmentation injury:** Keratocytes will usually accompany schistocytes and acanthocytes in this setting, Associated conditions include causes of microangiopathic hemolysis (disseminated intravascular coagulation, vasculitis, hemangiosarcoma) and mechanical fragility, e.g. iron deficiency anemia.
- **Oxidant injury:** Here keratocytes may accompany eccentrocytes, pyknocytes, and possible Heinz bodies, depending on the oxidant.
- **Liver disease:** In cats, keratocytes can be seen in increased numbers in liver disease, e.g. hepatic lipidosis. The mechanism is unclear and could be related to mechanical fragility from alterations in phospholipid or cholesterol composition of the red blood cell membrane (membrane rigidity) or disseminated intravascular coagulation.

2.6- Spherocytes:

They are erythrocytes which have assumed the form of a sphere rather than the normal discoid shape. As a result, they appear on routine blood films as cells that are smaller and more dense than normal red blood cells of the species, and have a reduced area of central pallor. [11]

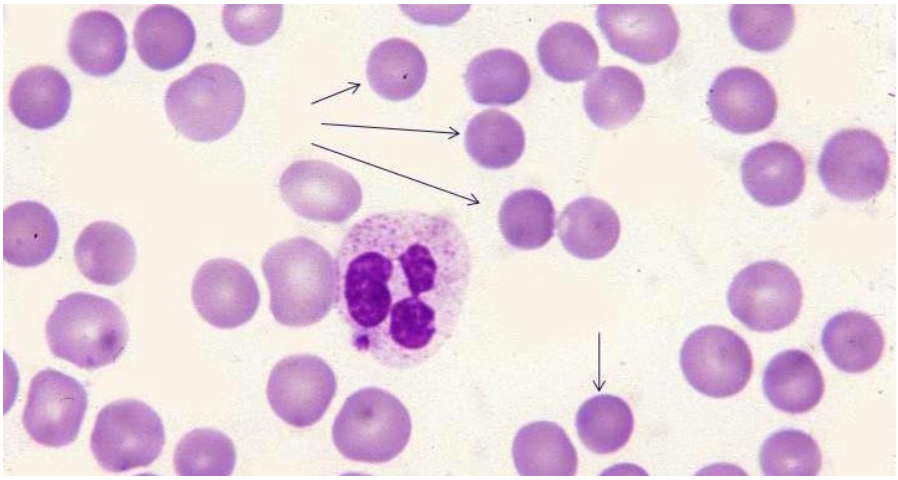

Figure 7: Hereditary spherocytosis showing numerous spherocytes, some of which are indicated by an arrow.

There are several causes of spherocyte formation and numbers do matter to some extent. Moderate to marked spherocytosis is diagnostic of immune-mediated hemolytic anemia (IMHA). Low numbers of spherocytes can be seen in conditions other than IMHA, therefore the presence of spherocytes (especially if in low numbers) is not always indicative of IMHA. [11]

Other conditions that spherocytes are associated with are:

1-**Inherited red blood cell abnormality**

2-**Transfused or stored red blood cells:** Stored red blood cells in blood bags will lose surface area with storage and, when transfused, will appear as spherocytes in blood smears from the recipient.

2.7-Eccentrocytes:

Eccentrocytes are RBCs that appear in a peripheral blood smear to have their hemoglobin shifted to one side of the cell. This abnormality, which is confined to the RBC membrane and cytoskeleton, is induced by oxidative damage. Often (but not always), they are seen in association with Heinz bodies, which provide evidence of an oxidant effect on hemoglobin. [12]

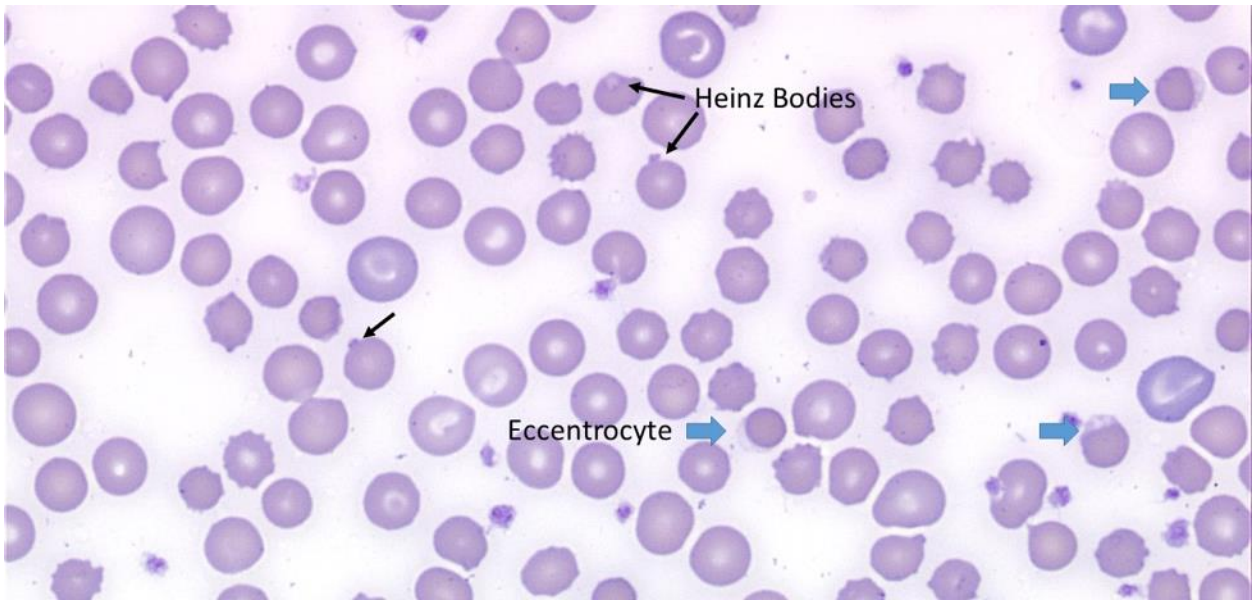

Figure 8: Peripheral Blood Smear sample showing Eccentrocytes in addition to Heinz bodies

Eccentrocytes are seen in the following conditions:

- Oxidant-induced hemolytic anemia
- Endogenous oxidants
- Inherited enzyme defects

2.8-Elliptocytes:

Elliptocytes are elongated red blood cells. There are three types: Type I is a slightly oval-shaped cell (used to be called ovalocyte), type II is a more rounded to oval shaped cell and type III is an elongate elliptical cell. Distinction between these three types is not of clinical relevance; however some forms occur more frequently in some diseases. An occasional elliptical or oval erythrocyte may be seen as a non -specific finding in a variety of settings. In some cases, smear-making technique and/or plasma viscosity may be contributing factors in their in-vitro formation. [13]

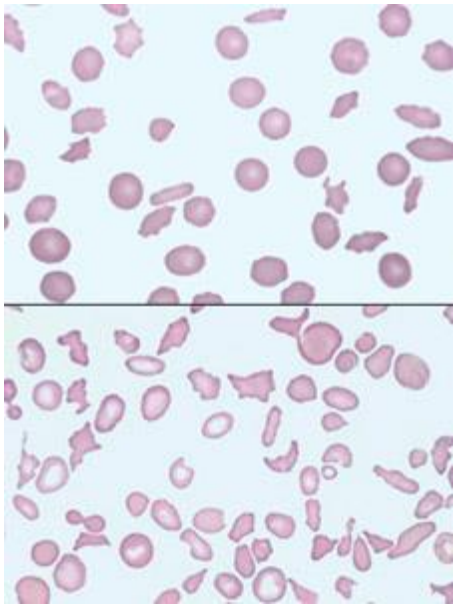

Figure 9:
Upper panel: Non-anemic patient with relatively few altered red cells.
Lower panel: Severe poikilocytosis including holly-leaf forms, ovalocytes and elliptocytes.

Elliptocytes can indicate underlying diseases, which are listed below:

- Liver disease
- Myelofibrosis
- Inherited/congenital red blood cell abnormality

2.9- Stomatocytes:

Stomatocytes are red cells in which the central biconcave area appears slitlike in dried films. In "wet" preparations, the stomatocyte is a cup-shaped red cell. [14]

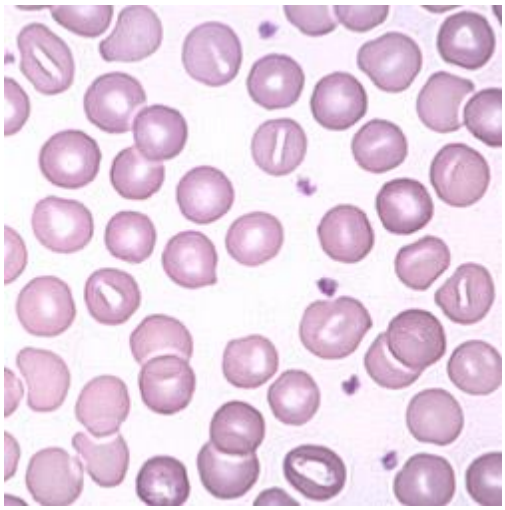

Figure 10: Numerous stomatocytes in the blood of a chondrodysplastic Alaskan malamute dog.

Stomatocytes are associated with the following conditions [14]:

- **Acquired Stomatocytosis**

Stomatocytes have been noted in diverse acquired conditions, including neoplasms, cardiovascular and hepatobiliary disease, alcoholism, and therapy with drugs, some of which are known to be stomatocytogenic in vitro. In some of these conditions, the percentage of stomatocytes on the peripheral blood smear can approach 100%.

- **Hereditary Stomatocytic Disorders**

Stomatocytes are erythrocytes with a central slit or stoma instead of a circular area of pallor when examined on dried smears; they are uniconcave rather than biconcave, giving them a bowl-like appearance.

2.10-Codocytes:

Codocytes, also known as target cells or leptocytes, are red blood cells that have a “lump” of hemoglobinized cytoplasm within the area of normal central pallor, causing them to resemble a “bullseye” target. In optical microscopy these cells appear to have a dark center (a central, hemoglobinized area) surrounded by a white ring (an area of relative pallor), followed by dark outer (peripheral) second ring containing a band of hemoglobin. However, in electron microscopy they appear very thin and bell shaped (hence the name codo: bell). Because of their thinness they are referred to as leptocytes. [15]

On routine smear morphology, some people like to make a distinction between leptocytes and codocytes- suggesting that in leptocytes the central spot is not completely detached from the peripheral ring.

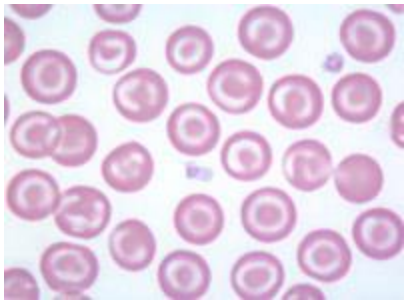

Figure 11: Numerous target cells in the blood of a dog with liver disease.

Increased numbers of normochromic target cells can be a useful diagnostic indicator of pathologic conditions resulting in a balanced increase in cholesterol and phospholipid in the red blood cell membrane. The most common disorder associated with normocytic normochromic target cells is liver disease; In addition to alpha-thalassemia and beta-thalassemia, Hemoglobin C Disease, Iron deficiency anemia.

2.11-knizocyte:

Knizocytes are triconcave RBCs with a "ridge," a "bridge" separating the three concavities (in scanning electron micrograph), or a strip of hemoglobin crossing the clear central area (in standard staining). They are very rarely observed in routine practice even if their percentage was estimated once 0.6% ± 0.5 in healthy controls.[16]

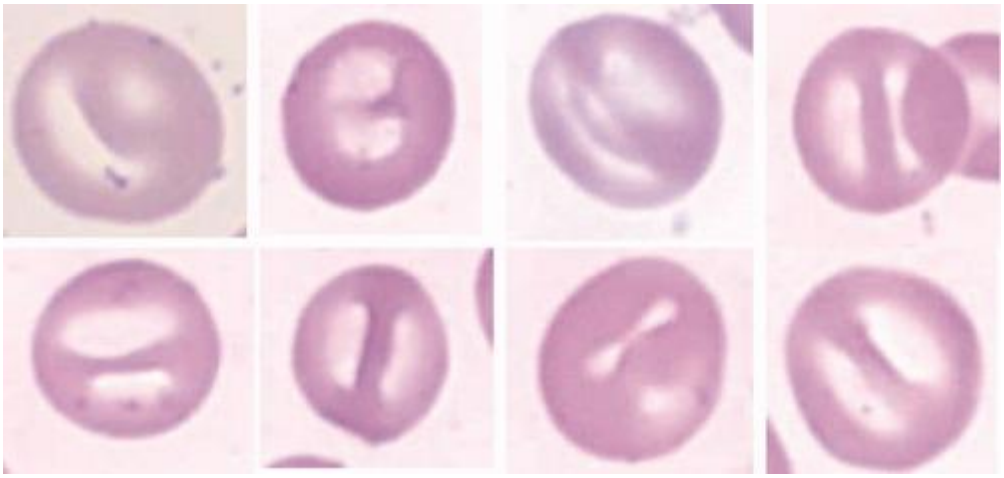

Figure 12: Mosaic image of knizocytes (May-Grünwald-Giemsa 1000×)

Knizocytes are associated with the following conditions: [17]

- Newborns: Regularly seen, where they are considered as relatively young RBCs with impaired membrane deformability usually among stomatocytes, spherocytes, and erythrocytes with spicules and protrusions (echinocytes/acanthocytes)
- Adults: they are mainly observed in the context of anomalies of the cholesterol metabolism related to any acute liver dysfunction.
- Chronically: knizocytes and/or target cells are observed in patients with familial lecithin/cholesterol acyltransferase deficiency, Knizocytes are also frequently observed in chronic liver diseases as chronic hepatitis or cirrhosis (alcoholic or postviral causes) where they can account for up to 15% of the red cells.

2.12- Dacrocytes

A dacrocyte is a type of Poikilocyte that is shaped like a teardrop. A marked increase of dacrocytes is known as dacrocytosis. [18]

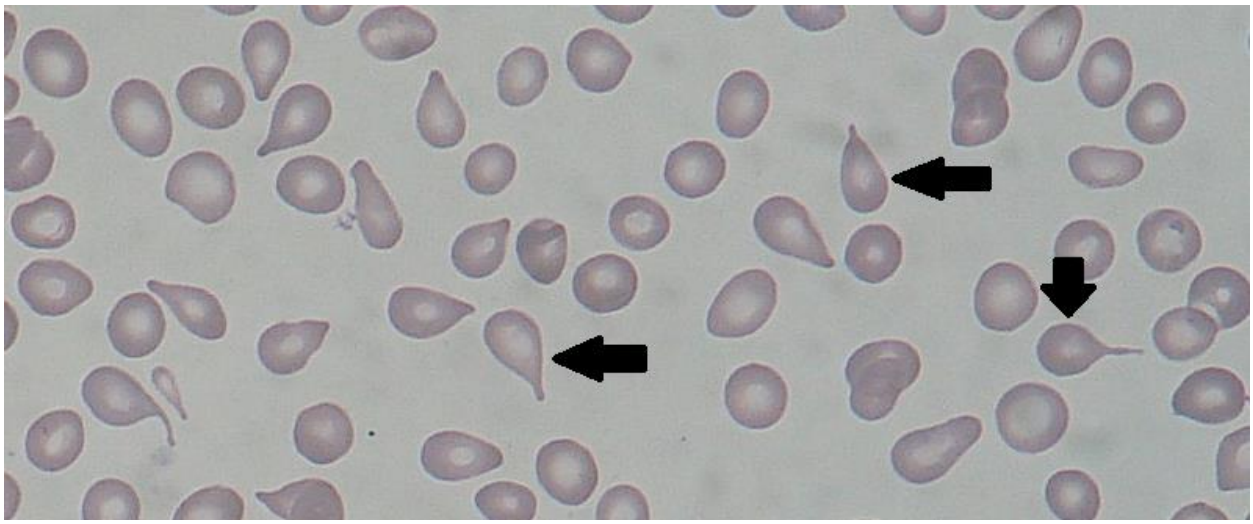

Figure 13: Teardrop Cells (Dacrocytes) in a peripheral blood using May-Grunwald Giemsa (MGG) stain blood smear

These tear drop cells are found primarily in diseases with bone marrow fibrosis, such as [18]:
primary myelofibrosis, myelodysplastic syndromes during the late course of the disease.

Rare causes are myelofibrosis associated with post-irradiation, toxins, autoimmune diseases, metabolic conditions, inborn hemolytic anemias, iron-deficiency anemia or β-thalassemia.

2.13-Basophilic stippling:

Basophilic stippling, also known as punctate basophilia, is the presence of numerous basophilic granules that are dispersed through the cytoplasm of erythrocytes in a peripheral blood smear. They can be demonstrated to be RNA. [19]

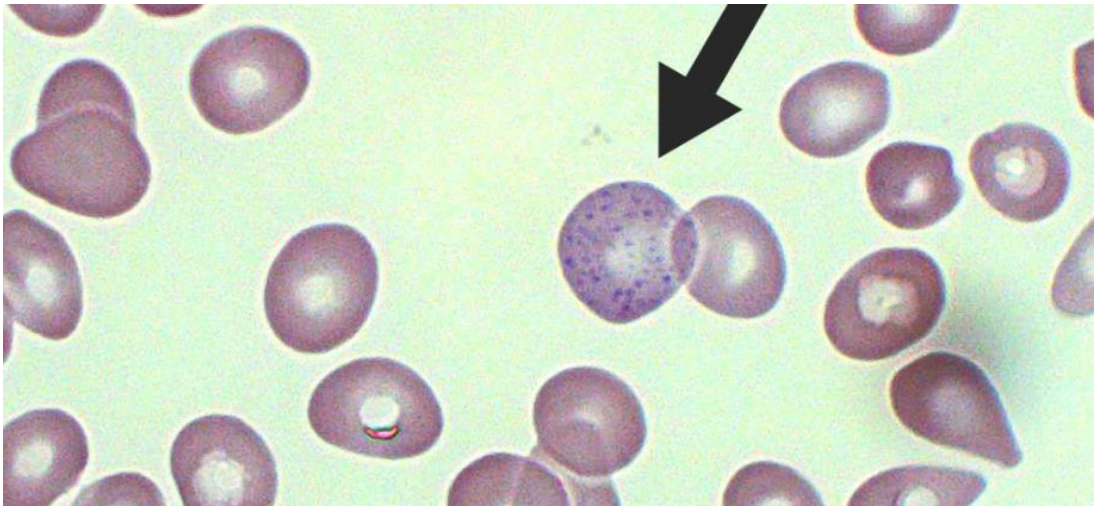

Figure 14: Wright-stained peripheral blood smear, showing basophilic stippling

Basophilic stippling, also known as punctate basophilia, is one example of several clinically significant erythrocyte inclusions identified on peripheral blood smears.

They are seen in the following conditions:

- Thalassemia
- Severe megaloblastic anemia
- Heavy metal poisoning
- Sickle-cell anemia
- Sideroblastic anemia
- Congenital dyserythropoietic anemia
- Alcoholism
- Leukemia
- Erythroleukemia
- Hemorrhage, e.g. from gastrointestinal tract

2.14-Heinz bodies:

Heinz bodies, also referred to as Heinz-Ehrlich bodies, are inclusions within red blood cells composed of denatured hemoglobin. They are not visible with routine blood staining techniques, but can be seen with supravital staining. The presence of Heinz bodies represents damage to hemoglobin and is classically observed in G6PD deficiency, a genetic disorder that causes hemolytic anemia. [20] .

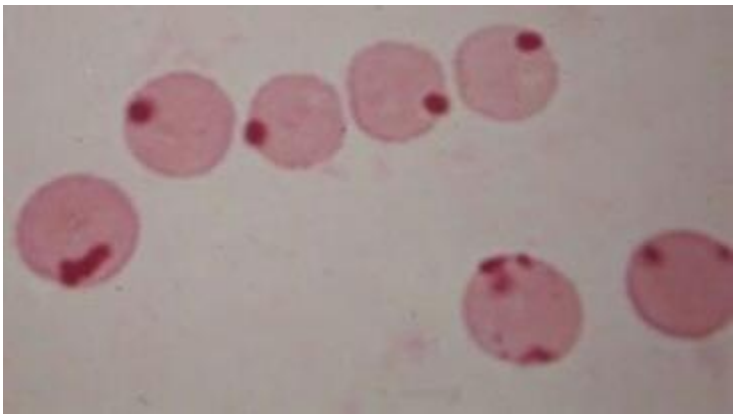

Figure 15: Blood sample showing Heinz bodies

Heinz bodies are formed by damage to the hemoglobin component PINmolecules, usually through oxidant damage by administered drugs, or from an inherited mutation (i.e. change of an internal amino acid residue).

As a result, an electron from the hemoglobin is transferred to an oxygen molecule, which creates a reactive oxygen species (ROS) that can cause severe cell damage leading to premature cell lysis.

Damaged cells are cleared by macrophages in the spleen, where the precipitate and damaged membrane are removed, leading to characteristic "bite cells". The denaturing process is irreversible and the continual elimination of damaged cells leads to Heinz body anemia.

There are several pathways leading to the hemoglobin damage.

- NADPH deficiency
- G6PD deficiency
- Heinz bodies can also be found in chronic liver disease.
- Alpha-thalassemia.

2.15-howell jolly bodies:

A Howell—Jolly body is a cytopathological finding of basophilic nuclear remnants (clusters of DNA) in circulating erythrocytes. During maturation in the bone marrow, late erythroblasts normally expel their nuclei; but, in some cases, a small portion of DNA remains. Its presence usually signifies a damaged or absent spleen, because a healthy spleen would normally filter this type of red blood cell. [21]

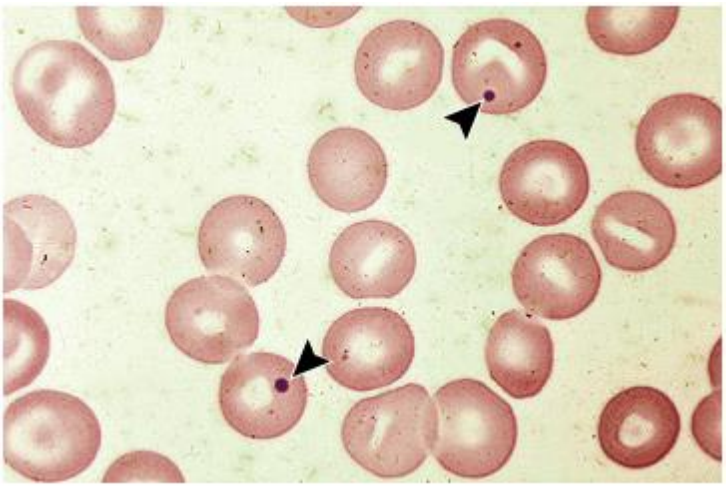

Figure 16: Peripheral blood smear shows 2 RBCs that contain Howell-Jolly bodies (arrowheads)

These bodies are seen with markedly decreased splenic function. Common causes include asplenia (post-splenectomy) or congenital absence of spleen. Spleens are also removed for therapeutic purposes in conditions like hereditary spherocytosis, trauma to the spleen, and autosplenectomy caused by sickle cell anemia.

Other causes are radiation therapy involving the spleen, such as that used to treat Hodgkin lymphoma.

Howell—Jolly bodies are also seen in amyloidosis, severe hemolytic anemia, megaloblastic anemia, hereditary spherocytosis, and myelodysplastic syndrome (MDS). The bodies can also can be seen in premature infants.

2.16-Pappenheimer bodies:

Pappenheimer bodies are abnormal basophilic granules of iron found inside red blood cells on routine blood stain. [22] They are a type of inclusion body composed of ferritin aggregates, or mitochondria or phagosomes containing aggregated ferritin. They appear as dense, blue-purple granules within the red blood cell and there are usually only one or two, located in the cell periphery. [23]

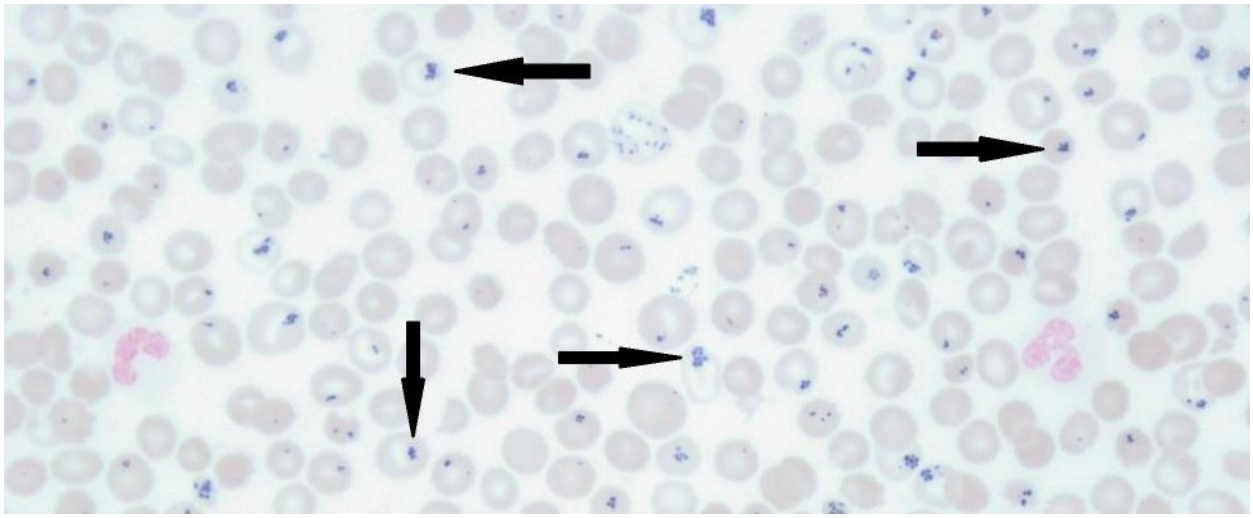

Figure 17: An iron stained peripheral blood smear with pappenheimer bodies present (indicated with arrows). Perls Prussian Blue. 50x oil immersion. From MLS Collection, University of Alberta,

Cell containing Pappenheimer bodies is a siderocyte. Reticulocytes often contain Pappenheimer bodies

They are mostly observed in diseases such as:

- Myelodysplastic syndrome (MDS).
- Sideroblastic anemia.
- Hemolytic anemia.
- Lead poisoning.
- Sickle cell disease.

2.17-Cabot rings bodies:

Cabot rings are thin, red-violet staining, threadlike strands in the shape of a loop, they are found on rare occasions in red blood cells (erythrocytes). And believed to be microtubules that are remnants from a mitotic spindle, and their presence indicates an abnormality in the production of red blood cells. [24]

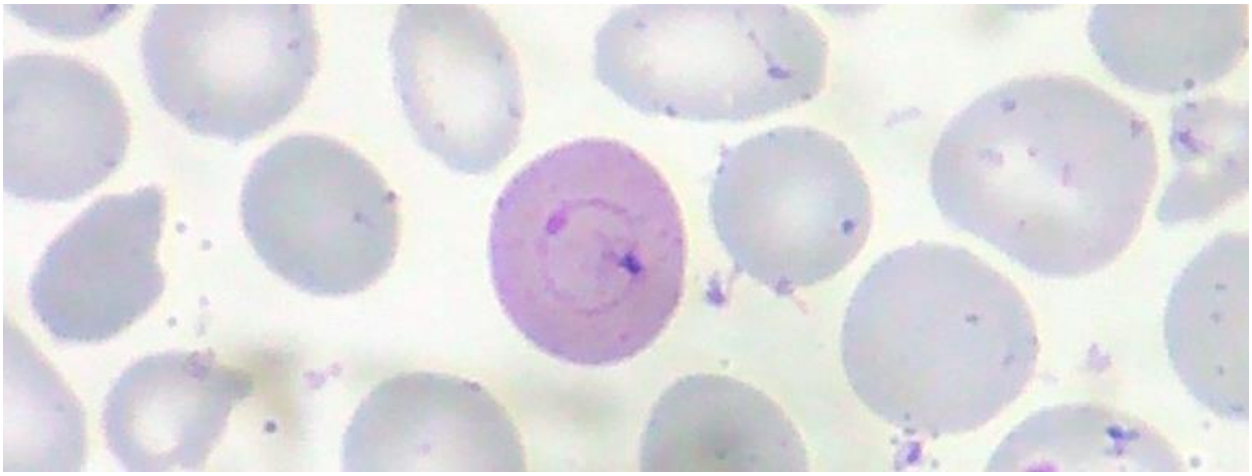

Figure 18: RBC showing Cabot rings bodies, using Wright's stain.

Cabot Rings, considerably rare findings, when present are found in the cytoplasm of red blood cells and in most cases, are caused by defects of erythrocytic production and are not commonly found in the blood circulating throughout the body.

Cabot rings have been observed in a handful of cases in patients with:

- Pernicious anemia.
- Lead poisoning.
- Certain other disorders of red blood cell production (erythropoiesis).

2.18-Macrocytic RBCs:

Red blood cells that are larger than the normal ones are called macrocytes which are defined by a mean corpuscular volume (MCV) of greater than 100 femtolitres, they are also called megalocytes which means (big cell). [25]

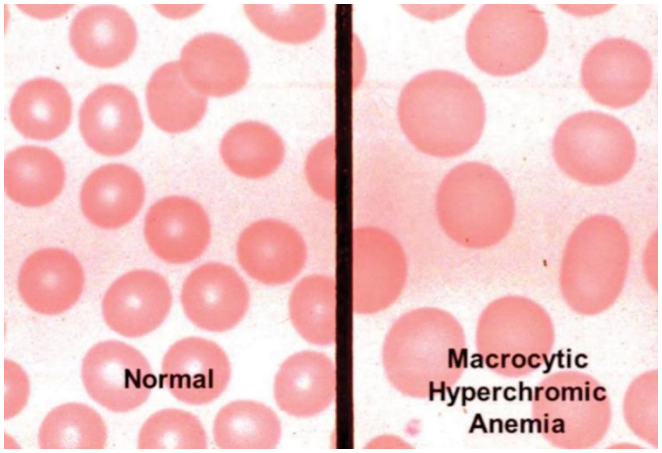

Figure 19: Comparison between normal RBCs (left) and macrocytic RBC (right).

In humans, most commonly (especially when the increase in size is mild, and just above normal range) the cause is bone marrow dysplasia secondary to alcohol abuse and chronic alcoholism. [25]

Gastrointestinal diseases that may cause macrocytosis include celiac disease (severe sensitivity to gluten from wheat and other grains that causes intestinal damage) and Crohn's disease (inflammatory bowel disease that can affect any part of the gastrointestinal tract).

They are also associated with the following conditions:

- Megaloblastosis (vitamin B 12 deficiency)
- Hypothyroidism
- Chronic obstructive pulmonary disease (COPD)
- Liver disease
- Pregnancy (most common, and requires no treatment as the person affected will return to normal post-partum)

2.19-Hypochromic RBCs:

Red blood cells that have less color than normal when examined under a microscope are called hypochromic RBCs. This usually occurs when there is not enough of the pigment that carries oxygen (hemoglobin) in the red blood cells. [26]

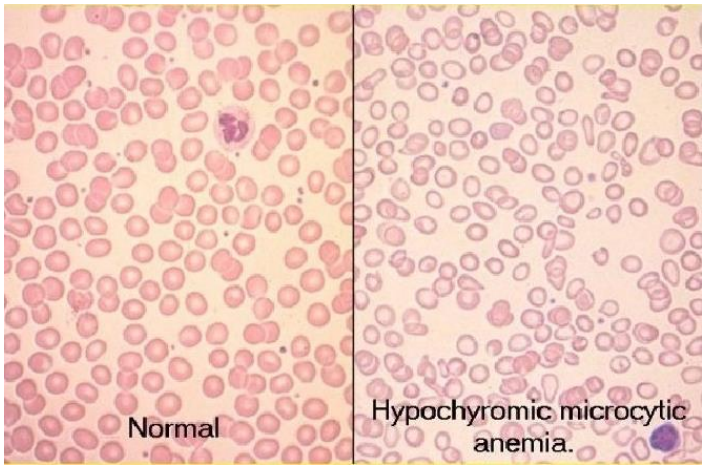

Figure 20: Comparison between normal RBCs (left) and hypochromic RBC (right) .

2.20-Hyperchromic RBCs:

Red blood cells that have more color than normal when examined under a microscope are called hyperchromic RBCs. [26]

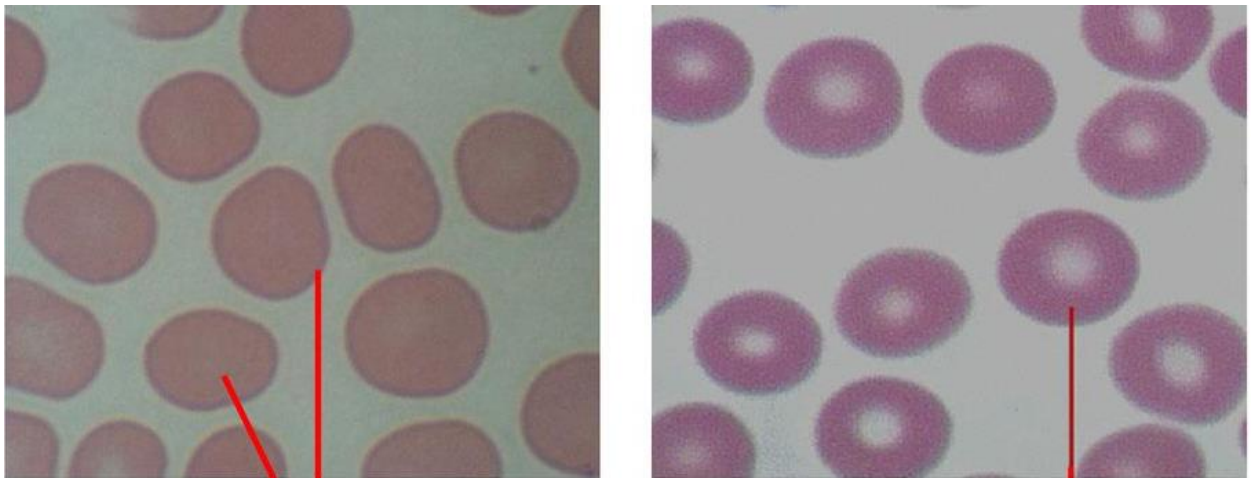

Figure 21: Comparison between and hyperchromic RBC (left) and normal RBCs (right)

They are also associated with Iron deficiency anemia which may be caused by a genetic condition known as congenital spherocytic anemia.

2.21-Sickle cell RBCs:

Are red blood cells that shaped like sickles or crescent moons. These rigid, sticky cells can get stuck in small blood vessels, which can slow or block blood flow and oxygen to parts of the body. [27]

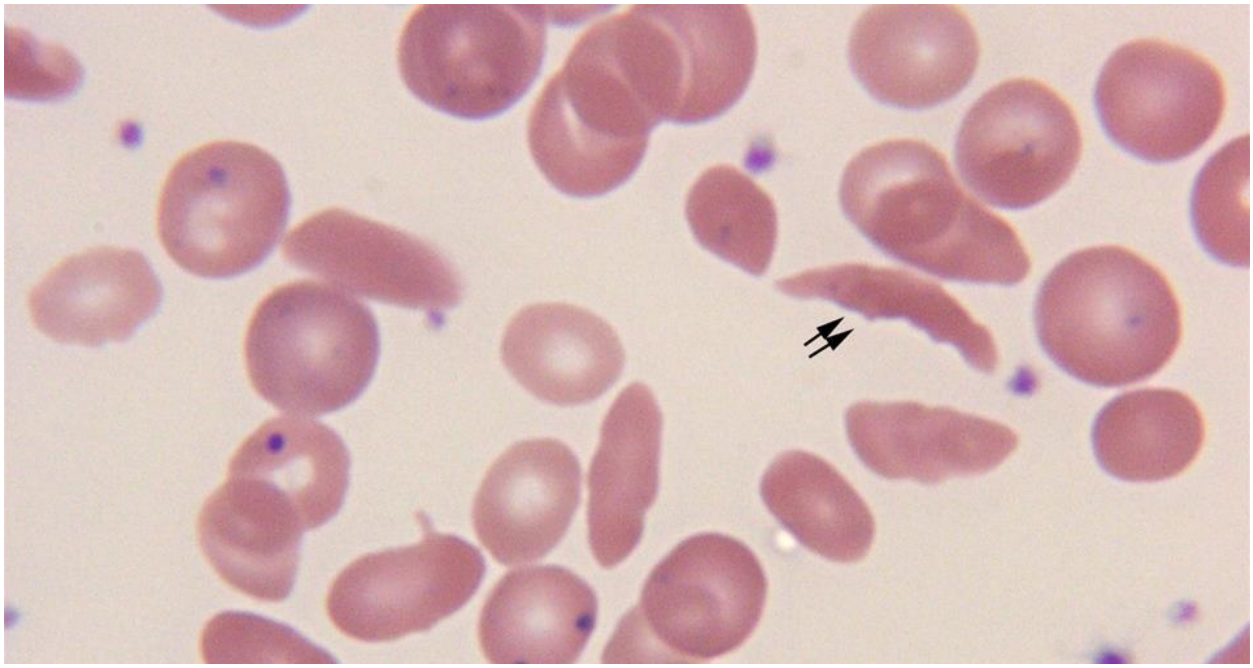

Figure 22: A blood sample showing crescent-shaped sickle cells

They are associated with the following conditions:

- Hemoglobin SS disease
- Hemoglobin SD disease
- Hemoglobin SC disease
- S-beta thalassemia
- Sickle cell anemia

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