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Original Research Article

Red Blood Cell Inclusions, Interpretation and Uses in Day to Day Practice: An Institutional and Tertiary Care Hospital Based Study

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Abstract

Introduction: Erythrocyte inclusions are elements that may be present in red blood cells (RBCs). The appearance, composition, and associated physiology of the inclusions are specific for each type of inclusion. Identification and reporting of these inclusions are important because their presence may indicate diseases or disorders. Typically, inclusion bodies are nuclear or cytoplasmic aggregates of stainable substance, usually proteins. The inclusion bodies in red blood cells are almost always indicative of some sort of pathology, and thus it is useful to understand each inclusion body that can occur within a red blood cell.

Aim:

1. To interpret inclusion bodies and correlate with smear findings.
2. To find out the uses of identifying them in day to day practice.

Material and Methods: Present study is a retrospective study for 3 months. All the cases which came with request for peripheral smear study in the clinical central laboratory were included in the study.

Results: There were total of 400 cases in 3 months, which included 130 microcytic hypochromic anemia, 33 macrocytic anemias, 30 dimorphic anemias and 207 normal smears. There were 4 cases of leukemia, 1 microangiopathichemolyticanemia and 42 eosinophelia. Of the 400 cases 58 presented with thrombocytopenia, 13 as pancytopenia and 7 showed parasites (malaria and borrelia). There were total of 32 (8%) cases which showed the RBC Inclusions, consisting of Howell jolly bodies-20 cases (62.5%), Basophilicstippling-10 (31.25%), cabot ring 01(3.125%)and Pappenheimer bodies -01 (3.125%).

Conclusion: Identification and reporting of these inclusions are important because their presence may indicate diseases or disorders. This article tells us the importance of smear study which is basic and a very important tool indiagnosing some conditions which would have been missed by CBC alone.

Keywords: Peripheral smear, RBC inclusions.

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Introduction

Inclusion bodies are those things you look at in cells and think, "Why is it here?" The appearance, composition, and associated physiology of the inclusions are specific for each type of inclusion. Identification and reporting of these inclusions are important because their presence may indicate diseases or disorders. Typically, inclusion bodies are nuclear or cytoplasmic aggregates of stainable substance, usually proteins. Many erythrocyte inclusions can be visualized on a Wright-stained smear; however, some erythrocyte inclusions can only be observed by using a special stain. For example, to confirm the presence of Heinz bodies, hemoglobin H bodies, or reticulocytes, smears must be prepared after staining an aliquot of fresh whole blood with a supravital stain such as new methylene blue or brilliant cresyl blue.

The inclusion bodies in red blood cells are almost always indicative of some sort of pathology, and thus it is useful to understand each inclusion body that can occur within a red blood cell.

Howell Jolly bodies: Appear as a very dark purple (described as a basophilic area) spot within the cytoplasm of the red blood cell. They are in fact, basophilic nuclear remnants, or remnants of DNA. Typically, they are round to oval shaped, 1um across and are usually restricted to only 1 per cell, although there can be multiple amounts. They are associated with nuclear maturation abnormalities such as megaloblasticanemia. They are also seen in some hemolyticanemia, and after splenctomy or in functional Asplenia [1,2]. they may be numerous in case of celiac disease in which there is splenic atrophy and coexisting folate deficiency [3].

Basophilicstippling (punctuate basophilia): Erythrocytes with bluish black granular inclusions distributed throughout the entire volume of the cell. Granules may vary in size and distribution from small and diffuse to coarse and punctuate. The granules which are of aggregated ribosomes, are sometimes associated with mitochondria and siderosomes. It is believed that basophilic stippling is not present in living cells, instead stippling are produced during preparation of the blood smear or during staining process. Cells dried slowly or stained rapidly may demonstrate fine diffuse stippling as an artifact. Pathologic basophilic stippling is more coarse and punctuate and is indicative of disturbed rather than increased erythropoisisseen in many diseases like Lead poisoning and other heavy metal poisoning, thalassemia, megaloblasticanemias, infections, liver diseases, and other disorders of hemoglobin synthesis [4,5,6].

Cabot rings: They are literally loops, rings or figure 8 type structures that are located within the cytoplasm of the RBC. They may look like beads on a string. They are typically colored red-purple under the Wright Stain. They are quite rare, they are microtubular remnants of mitotic tubules that are involved in mitosis; indicate some abnormality in the production of red blood cells [7].

Diseases Associated with Cabot Rings:

- Myelodysplastic Syndrome
- MegaloblasticAnemia

Both these conditions show abnormalities in production of red blood cells.

Pappenheimer Body: Are secondary lysosomes, variable in their composition of iron and protein, or mitochondria with iron micelles. Apper as small irregular basophilic deposits in erythrocytes and normoblasts and stain with both Romonowsky and Prussian blue stains [8]. Romonowsky stains visualize pappenheimer bodies by staining their protein matrix of the granules; whereas Prussian blue stain is responsible for staining the iron portion of the granule. They are never distributed in

large numbers throughout the cell as in punctuate basophelia. However a single cell may show both punctuate basophelia and papperheimer bodies. With perl's stain, the former granules are pink, whereas the later are blue [9].

Pappenheimer bodies are pure iron deposits unlike Heinz bodies which contain Heme. They should not be confused with late with late reticulocytes. Prussian blue stain, which is not taken up by reticulocytes, is helpful in differentiating the two. Pappenheimer bodies can also cause a false elevation of platelet counts when performed with electronic counters.

Diseases Associated with Pappenheimer Bodies:

- Splenectomy
- Due to inability of spleen to remove pappenheimer laced cells.
- Hemolytic Anemia
- Sideroblastic Anemia
- Megaloblastic Anemia
- Hemoglobinopathies

Heinz Body: Heinz bodies do not stain with Romonowsky stains but can be visualized with Supra vital stains or with phase microscopy in the living cell. "Bite cells" also referred to as Degmacytes, where a portion of a red blood cell is phagocytosed due to the presence of an inclusion body within the cytoplasm of the cell. This inclusion body is the Heinz Body. Heinz bodies appear as 2 – 3 micronround masses lying just under or attached to the cell membrane. They are composed of aggregated denatured hemoglobin.

Typically, during oxidative damage to hemoglobin, an electron is transferred from the hemoglobin to oxygen, resulting in the formation of a reactive oxygen species (ROS). This ROS can lead to severe damage within the cells, and can even cause lysis of the entire cell.

The ROS denatures portions of the hemoglobin, causing them to precipitate and produce Heinz Bodies, which becomes an antigenic agent. Thus, macrophages detect the antigen and remove the damaged portions of the cell, its damaged membrane and the denatured hemoglobin (now called the Heinz Body).

Diseases Associated with Heinz Bodies [10]:

1. Enzymopathies - Glucose - 6 - phosphate dehydrogenase Deficiency (G6PD Deficiency), Nadph Deficiency.

2. Chronic Liver Disease

3. Alpha Thalassemia Normal adult hemoglobin is composed of two alpha and two beta chains. Alpha thalassemia patients have partial or complete defects in alpha globin production, leading to a relative abundance of beta globin chains in the cell. These excess beta globin chains aggregate to form HbH, which has decreased solubility and precipitates in the red blood cell cytoplasm. This is not direct damage to hemoglobin per se, but rather a perturbation in the quaternary structure of hemoglobin.

Thus, HbH is merely a moderate to severe form of alpha-thalassemia that consists of hemoglobin precipitating within the cytoplasm of the red blood cells in high amounts due to the insoluble nature of beta-chain heavy hemoglobin molecules.

Because the HbH aggregates are precipitated and are non-functional, HbH is considered a subtype of Heinz Bodies.

4. Hyposplenism and Splenectomy

Difference between Heinz Body and Howell-Jolly Body:

Table 1:

Howell Jolly bodies	Heinz bodies
Stains with Romonowsky stain	Does not stain with romonowskystain
Small round, dark purple bodies form in variable positions around the cell	Stained with supra vital stain, purple shaped bodies of varying sizes, usually closes to the inner surface of the cell membrane.
	Clue on peripheral smear: Bite cells.

Reticulofilamentous substance:

Reticulocytes: 0.5% to 2.5% in adults and 2% to 6% in infants.

Art factual aggregation of ribosome's, not visible on Wrights stained smear: Supra vital stain must be used and filaments appear as deep blue reticular network.

Reticulocyte count, corrected reticulocyte count and reticulocyte production index to be calculated in cases of anemia.

C crystals

Hexagon shaped / rhomboid shaped crystalline structures in erythrocytes seen in Hb C and HbSC disease. HbCis abnormal hemoglobin in which

substitution of a glutamic acid residue with a lysine residue at the 6th position of the β-globin chain has occurred.

Malarial Parasites:

Round to oval ring shaped intracellular parasites in RBCs, found in Malaria. Differences between various specious of Malaria is given below.

Table 2:

	Plasmodium falciparum	Plasmodium Vivax	Plasmodium Malariae	Plasmodium Ovale
RBC size	Not enlarged	Enlarged	Not enlarged	enlarged
RBC shape	Round, sometime crenated	Ameboid	Elongation	Fimbriation
Stippling	Rarely, Maurer's cleft seen	Schuffners dots	Ziemanns dots	James' dots or James' stippling
Pigment	Black or dark brown	Golden brown granules	Dark brown	Black to brownish black
Ring	Fine ring, 1- 2 chromatin dots	Thick ring, often irregular, one chromatin dot	Thick ring ,one chromatin dot	Rings large and coarse , one chromatin dot
Trophozoit	Ring enlarged , slightly irregular	Irregular ameboid	Band forms are characteristic of these species	Round, compact
Schizont	Medium sized rarely seen in smear (19-32 merozoits)	Large; 12-18 merozoits, arranged irregularly	Small, 9-10 merozoits arranged in rosette	Medium sized 8-14 merozoits arranged irregularly
Gametocyte	Cresent shaped	Spherical, compact	Similar to vivax but smaller	Like vivax but smaller

Various types of RBC inclusions, morphological features and conditions associated with these inclusions are tabulated below.

Table 3:

Terminology	Description	Associated diseases
Howell Jolly bodies	Small round bodies composed of DNA usually located in the red cells: usually occur singly, rarely more than 2 per cells; stains dark purple with wrights stain	Post splenctomy Megaloblastic anemia's. Some hemolyticanemia's, functional Asplenia, severe anemia.
Basophilic stippling	Round/irregularly shaped granules of variable number and size distributed throughout the red cells; composed of aggregates of ribosome's (RNA); stain bluish black with wrights stain	Lead poisoning (coarse stippling) anemia associated with abnormal hemoglobin synthesis; thalassemia. Myelodysplastic syndrome.
Cabot rigs	Appear as figure eight, ring, incompletering; thought to be composed of the microtubules of the mitotic spindle; stain reddish violet with wrights stain.	Severe anemia Dyserythropoisis
Pappenheimer bodies	Iron containing bodies usually found at periphery of the cell; visible with Prussian blue stain and with wrights stain.	Sidroblastic anemia; Thalassemia,other severe anemias
Heinz bodies	Composed of denatured /precipitated hemoglobin; not visible on wrights stained smear; with supravital stain appears as purple shaped bodies of varying size, usually close to cell membrane; can also be observed with phase microscopy on wet preparations.	G6PD deficiency Unstable hemoglobin disorders Oxidizing drugs and toxins; post splenctomy
Reticulo filamentous substance	Artifactual aggregation of ribosomes, not visible on wrights stained smear; supravital stains must be used, appear as deep blue reticular network.	Normal reticulocytes
Hemoglobin H inclusions	Composed of precipitated chains of beta hemoglobin; stains with supravital stain (blue green dots)	Hemoglobin H disease
C crystals	Variable sized RBC crystalline (hexagon/ rhomboid crystals) dark blue purple inclusions	Hemoglobin C disease Hemoglobin SC disease
Malarial parasites	Round to oval ring shaped intracellular parasite in RBCs	Malaria

Aim:

1. To interpret inclusion bodies and correlate with smear findings.
2. To find out the uses of identifying them in day to day practice.

Materiala and Methods

Present study was a retrospective study for the duration of 3months.The study was conducted after obtaining ethical committee clearance. All the cases which came with request for peripheral smear study in the clinical central laboratory were included for the study.

Study was done under following headings

Total number of samples for Complete blood count, No of samples for peripheral smear study, No of smears showing inclusions, interpretation and causes for inclusions. Uses for identifying them. And any other rare or additional findings associated with inclusions were also noted down.

Results

The present study included a total 400 cases in 3 months. Among the 400 cases 130 (32.5%) were Microcytic Hypochromic Anemia, 33 (8.25%) were Macrocytic Anemias, 30 (7.5%) were Dimorphic Anemias and rest 207 (51.75%) were showing Normal blood picture. (Table1).

Table 1: Type of blood picture

Diagnosis – Basic type of blood picture	Number of cases Total (400)	Percentage %
Microcytic Hypochromic Anemia	130	32.5%
Macrocytic Anemia	33	8.25
Dimorphic Anemia	30	7.5
Normocytic Normochromic Blood Picture	207	51.75

There were 4 (1%) Leukemia cases, 1 Microangiopathichemolyticanemia, 42 (10%) Eosinophilia, Of the 400 cases 58 (14.5%) cases presented with thrombocytopenia, 13 (3.25%) with features of Pancytopenia. There were 7 (1.75%) infectious diseases diagnosed consisting of Plasmodium Vivax, Falciparum and Borrelia Reccurrintis. There were one each cases of Thalassemia and Sickle cell Anemia. (Table 2).

Table 2: Abnormal Smear Findings

Abnormal findings noted in the above cases	Total number of cases (400)	Percentage %
Leukemias Acute Myeloid Leukemia – 1 Chronic Myeloid Leukemia -3	4	1%
Microangiopathic Hemolytic Anemia	1	0.25%
Eosinophelia	42	10%
Thrombocytopenia	58	14.4%
Pancytopenia	13	3.35%
Infecctions Plasmodium Falciparum Plasmodium Vivax Borrelia Recurrentis	2 4 1	1.75%
Hemolytic Anemias Thalasemia - 1	2	0.5%
Sickle cell Anemia– 1	1	0.25%

There were total of 32 (8%) cases which showed the RBC Inclusions, consisting of Howell jolly bodies, Basophilic stippling, cabot ring and Pappenheimer bodies. (Table 3).

Table 3: Type and number of RBC inclusions

Types of RBC inclusions seen	Number of cases (32)
Howell Jolly bodies	20 (62.5%)
Basophilic Stippling	10 (31.25%)
Cabot rings	01 (3.125%)
Pappenheimer bodies	01 (3.125%)

List of conditions which showed the RBC inclusions (Table 4)

Table 4: RBC Inclusions and associated diseases

1. Howell jolly Bodies	**20**
Macrocytic Anemia	13 (65%)
Megaloblastic Anemia (showing triad – oval macrocytes, hypersegmented neutrophils, howell Jolly inclusions)	05 (25%)
Hemolytic Anemia	1 (5%)
Post Splenctomy	1 (5%)
2. Basophilic Stippling	**10**
Thalassemia	1(10%)
Severe Megaloblastic Anemia	3 (30%)
Sickle cell Anemia	1 (10%)
Leukemias	2 (20%)
Artifactual	3 (30%)
3. Cabot Ring Megaloblastic Anemia	1
4. pappenheimer Bodies Sickle Cell Anemia	1

Discussion

Howell Jolly Bodies -out of 20 cases , 13 were seen in Macrocytic Anemia, 5 were seen in smears which showed classical triad of Megaloblasticanemia

(oval macrocytes, hypersegmented neutrophils, and howell jolly bodies), 1 case of thalasemia and 1 case of post splenctomy showed the inclusions (Fig. 1).

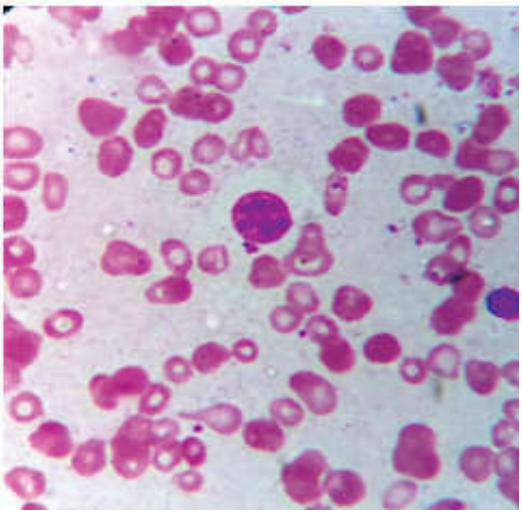

Fig. 1: Hypersemented neutophil and Howell jolly body inclusion

The presence of macro-ovalocytes having an MCV > 115 fl, anisocytosis, poikilocytosis and hypersegmented neutrophils suggests a megaloblastic disorder associated with a nutritional deficiency, i.e., vitamin B12 or folate deficiency. Round macrocytes are commonly seen in a variety of chronic illnesses, and round target-appearing macrocytes are characteristic of liver disease such as hepatitis, obstructive jaundice, and acute and chronic alcoholism with liver disease. For patients who present with disordered immaturity, hypogranulated or hyposegmented neutrophils, and cytopenias, a bone marrow examination is necessary to rule out or confirm a primary bone marrow disorder such as a myelodysplastic syndrome or leukemia [11].

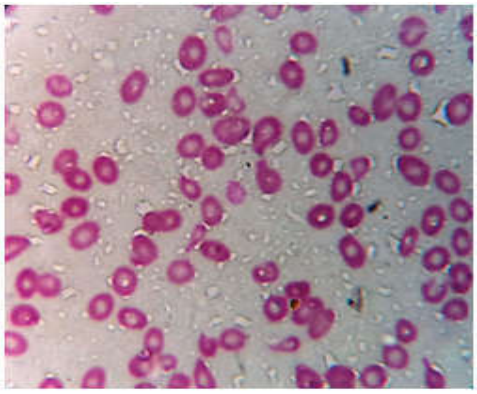

Fig. 2: Fine basophilic stippling (staining artifact)

Basophilic stippling – of the 10cases, 3 were seen in severe megaloblastic anemias. One each case of thalasemia, sickle cell anemia and leukemia showed the stippling. Basophilic stippling is indicative of disturbed erythropoiesis .Two normal smears showed artifactual fine stippling (due to precipitation of RNA during staining) and one case of Borrelia recurrentis showed fine RBC stippling which is mostly artifactual. (Fig. 2). Cabot ring – In our case we saw a figure of 8 shaped Cabot ring in a case of Megaloblastic anemia, which also showed howell jolly bodies, oval macrocytes and hypersegmented neutrophils.(Fig. 3).

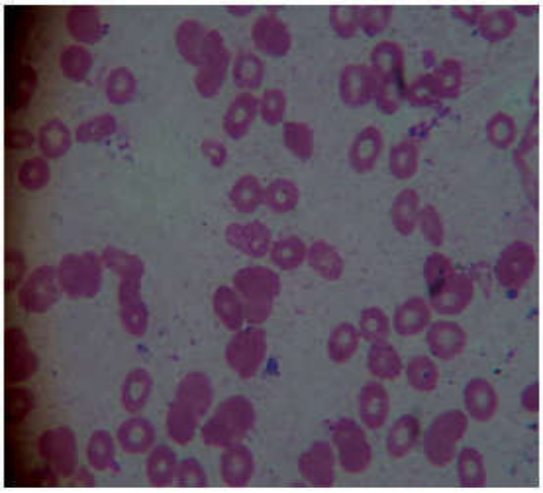

Fig. 3: Cabot ring (figure of 8)

Pappenheimer Bodies - was seen a case of Sickle cell anemia, on Romonwsky stain,smear showed anisopoikilocytosis with few sickle cells and purplish irregular aggregates of granules in the RBCs composed of ferric iron, demonstrated by pearls Prussian blue stain. (Fig. 4 and 5).

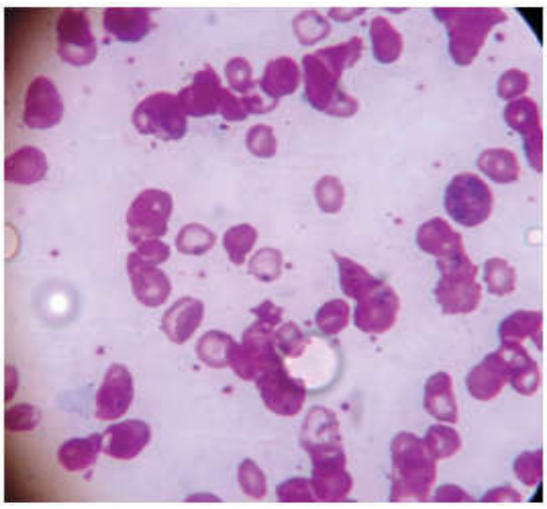

Fig. 4: Pappenheimer Bodies – purplish irregular aggregate of granules. (leishmans stain)

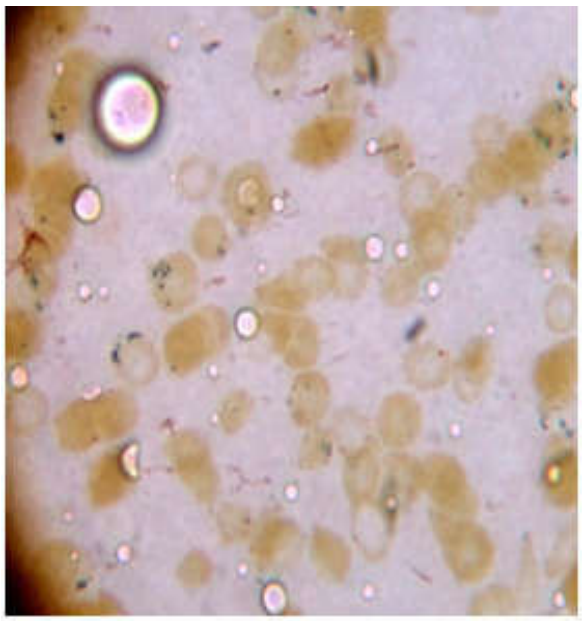

Fig. 5: Pearls Prussian blue stain: bluish green iron granules in the RBC'S

It was postulated that the spleen removed inclusion-containing red cells [12] or promoted metabolism of the iron [13]. In our case the cause of Pappenheimer bodies in sickle cell anemia could be because of Functional Asplenia or due to Iron overload occurring because of repeated blood transfusions.

Conclusion

Erythrocyte inclusions are elements that may be present in red blood cells (RBCs). The appearance, composition, and associated physiology of the inclusions are specific for each type of inclusion. Identification and reporting of these inclusions are important because their presence may indicate diseases or disorders. Since inclusion bodies in red blood cells are almost always indicative of some sort of pathology, and thus it is useful to understand each inclusion body that can occur within a red blood cell. This article tells us the importance of smear study which is basic and a very importanttool indiagnosing some conditions which would have been missed by CBC alone.

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