Forensic Investigation and Comparison of Blood Sample Referring to Thalassemia

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Abstract

Identification of an individual is one of the significant features of forensic investigation. The present study was conducted to study the difference between normal and thalassemic blood. The study was conducted on 40 blood samples aim to compare control and disease wet blood samples by using different methods of microscopy. The comparison was done based on the morphology of the RBCs and red blood cell count. A (monolayer) thin blood film is prepared on a slide with a wet blood sample and observed under a compound microscope. Red blood cell count is performed with the help of Neubauer's chamber. Observation shows the changes in the morphology of red blood cells in the thalassemic population as compared to the control population, moreover based on total RBCs count the number red blood cell count found higher in the thalassemic population as compared to the control. Both morphology and Count significantly differed from the control population. Thus, the present method can be used in the daily routine at forensic science casework.

Keywords: *Thalassemia, Forensic, Investigation, Neubauer's chamber, RBC morphology*

Introduction

Blood Disorders

Blood disorders affect one or more than one part of the blood and thus prevent the blood from doing its regular job that is transportation, regulation, and protection. Blood disorders can be inherited or other causes include some other diseases, side effects of some medicines and also can happen due to a lack of important nutrients in your diet.

Blood disorders mainly affect the three main components of the blood which are erythrocytes, leukocytes, and thrombocytes. These disorders affect the oxygen-carrying capacity of the blood, immunity and also the clotting property to heal the wounds.

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Thalassemia is one of these disorders which generally affect the shape and size of blood cells.

Thalassemia

Thalassemia is a red blood cell disorder which is a rare genetic disease caused by the markedly decreases in accumulation of one of the globin subunits of hemoglobin. Hemoglobin is an iron-rich protein in red blood cells that carries the oxygen to different parts of the body, it also carries the carbon dioxide which a waste gas from the body to the lungs. Thalassemia is an anemic disease and the person can have both mild and severe anemia. Thalassemia is of two types one is alpha thalassemia and another is beta-thalassemia. Hemoglobin contains four protein chains i.e. two alpha globins and two beta globins. The two major types of thalassemia are named after a defect in this protein chains[1].

Alpha Thalassemia

Alpha thalassemia is the disease that is the most common hemoglobin genetic defect which is caused due to the reduction in the alpha globin's chains or due to the absence of alpha-globin chains[2]. The disease

is prevalent in tropical and subtropical regions. But recently this disease is spreading throughout the world because of massive population migration. This disease is occurred most frequently due to the deletion in both or one of alpha-globin and least commonly due to non-deletion abnormality[2]. Symptoms: The person suffering from alpha thalassemia will show abnormal hemoglobin, microcytic anemia, and abnormality in the immune system, gallstones, cognitive impairment.

Types of alpha thalassemia:

HbH disease is the mild form and results from a defect in one alpha-globin and the presence of only one functioning alpha-globin gene. Due to this, there is the presence of excess beta chains and they form beta 4 tetramers. HbH is unstable and they are prematurely breakdown in spleen hence consequently hemolysis formed.

Hb Bart hydrops fetalis syndrome: the second type of alpha thalassemia in this disease there is an absence of all four alpha globins alleles[2]. It is moderately insoluble and thus accumulates in the red blood cells. Its presence indicates that one or more of the four genes that produce alpha globin's chains are dysfunctional, causing alpha thalassemia. Fetal hydrops fetalis is characterized by an accumulation of fluid in at least two fetal compartments and cardiac failure. This condition is so severe that the foetuses are either stillborn or die soon after death[3].

Beta Thalassemia:

Beta thalassemia is a hereditary disease, which is characterized by abnormalities that occur in synthesis or accumulation of the beta chains of hemoglobin which results in severe anemia to clinically asymptomatic individuals. Anemia begins at the 3-6th month of age. It is caused by the reduction or absence of the synthesis of beta globins chains of the hemoglobin tetramer, as we know that hemoglobin tetramer contains two beta chains and two alpha chains, thus the mutation in beta chains cause beta thalassemia[4]. A large number of imperfect red cells destroyed in bone marrow, giving rise to ineffective erythropoiesis, a prominent feature of disease[1]. Beta thalassemia is further of three types that is thalassemia major, thalassemia intermedia and thalassemia minor also known as beta-thalassemia carrier state[4]. The person suffering from beta-thalassemia major shows the symptoms of severe anemia within two years of life and they require a regular blood transfusion. The person with this disorder shows various features such as diabetes mellitus, parathyroid, thyroid, liver fibrosis, etc.

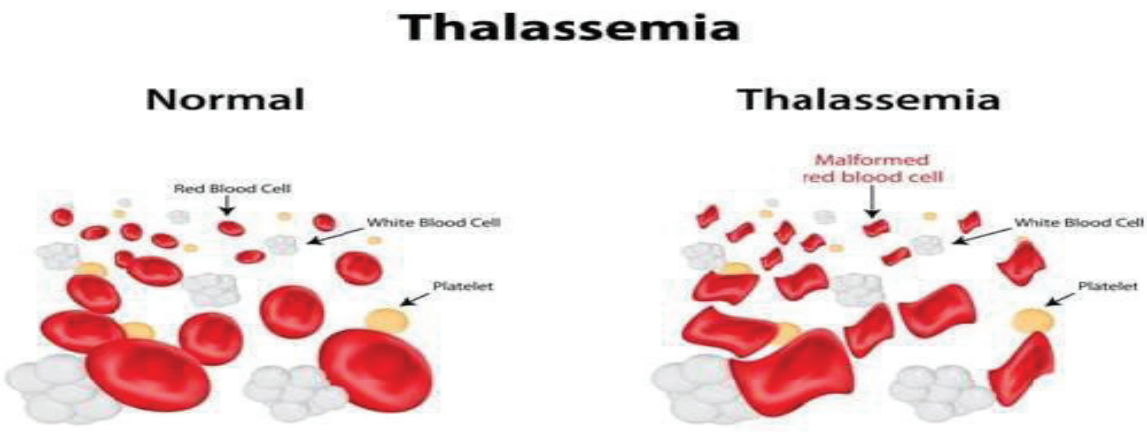


Fig 1. Showing Difference between control and thalassemia population

In forensic science the blood act as very important evidence, blood residue acts as a weapon which helps the investigator in the identification purpose, reconstructs a criminal action and links the suspect to the crime. Various studies show that thalassemia is a disease in which there is changes occur in the morphology of the red blood cells. The shape, size, color of the cells show clear changes that can be identified with the help of microscopy. Thus in forensic science, it is easy to determine if blood is present in the crime scene then that blood is from a normal person or thalassemic patient.

Hence, this study was designed on thalassemia patients to identify their blood using a normal microscope and compare the shape of RBC with the control population to distinguish between thalassemic

and non-thalassemic patients. All these help to narrow down the search of the area by taking inclusion and exclusion, identification of the suspected person which ultimately provides a lead in the investigation and helps the investigator to link the person with the crime.

Materials and Method

Chemicals: Ethylene diamine tetraacetic acid (EDTA) used as an anticoagulant, Ethanol, 1% HCL, Leishman's Stain, Buffer, Distilled water, Saline water.

Glass Ware: Glass slides, Coverslips, Dropper, Test tubes, Beakers

Instruments: Compound Microscope of Microcater instrumentation, Haemocytometer (Neubauer Chamber)

Sample collection

A total of 40 samples of blood were collected out of which 20 were collected from normal people and 20 were collected from the patients suffering from thalassemia. Samples were collected from the laboratories of hospitals (left after hematological test) and all the samples are stored in EDTA vials and labeled properly. Only waste blood samples were collected from the laboratories situated in hospitals in the Chandigarh region.

Procedure for Microscopy.

1. Blood films are prepared by placing the drop of blood on one end of the slide with the help of a micropipette.

2. The blood is dispersing over the slide's surface to form a monolayer. The slide was left for a few minutes to air dry. The dried slide was fixed by immersing into methanol for 3-4 minutes. Afterward, the slide was stained with Leishman stain, 2-3 drops of dye were put on the slide and wait for left for 3-4 minutes.

3. The slide was diluted using the buffer for 7-8 minutes. Buffer is used to maintaining the pH so that cells do not get destructed. The slide was washed and observed under a microscope for morphology.

Procedure for Neubauer Chamber

1. A 1:200 dilution of blood was prepared.

· A 20 microlitre of whole blood placed on the tube.

· RBC diluting fluid was mixed and shaken with mechanical shaker for 2-3 minutes.

2. 10 microlitres of blood filled to each slide of the counting chamber. Once the counting chamber is filled with the sample allow the cells to settle for 2-3 minutes before counting.

3. Count of cells: Counting was done under a microscope

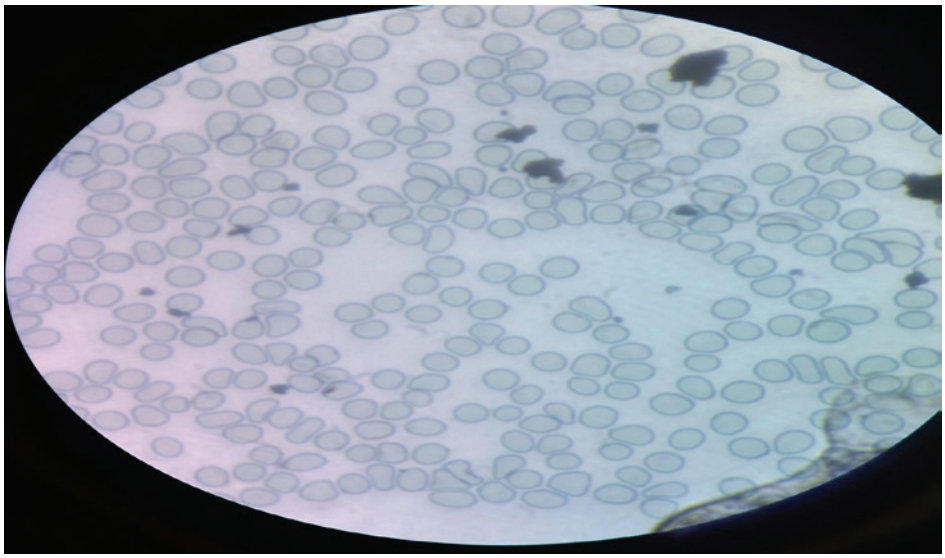


Fig. 2 The shape of RBC in normal Blood at 100X

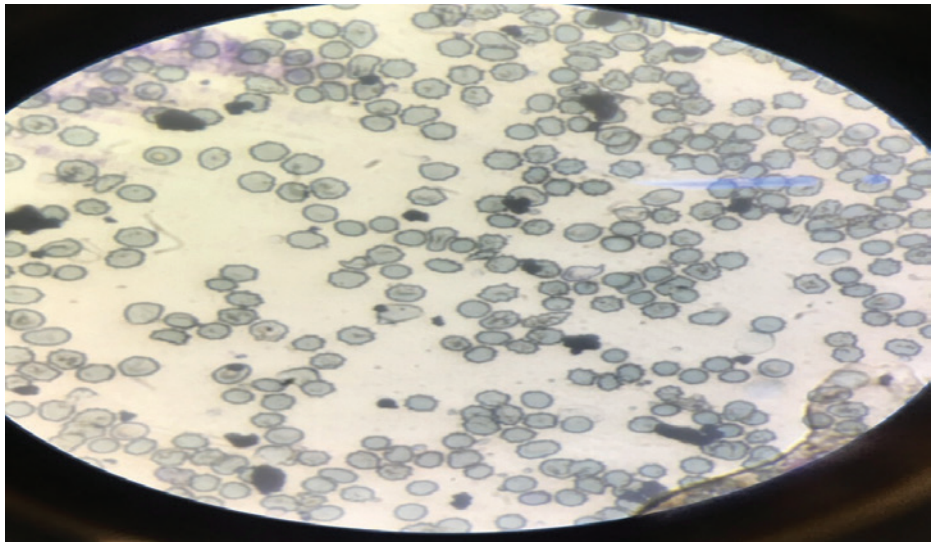


Fig. 3. The shape of RBCs in Thalassemic blood 100X

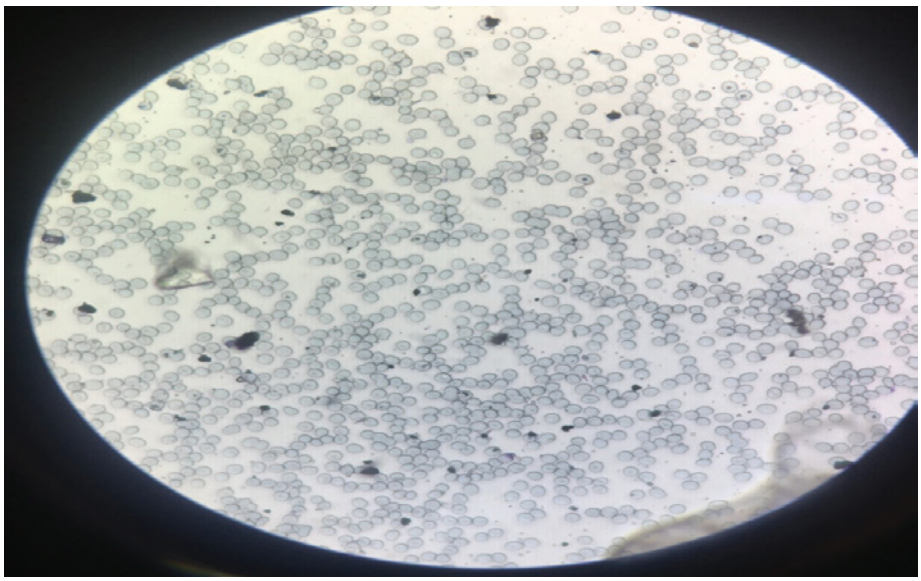


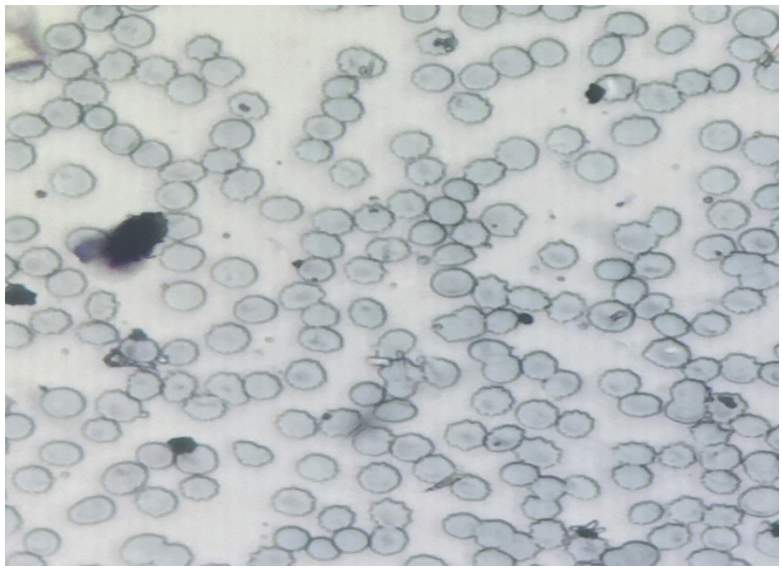
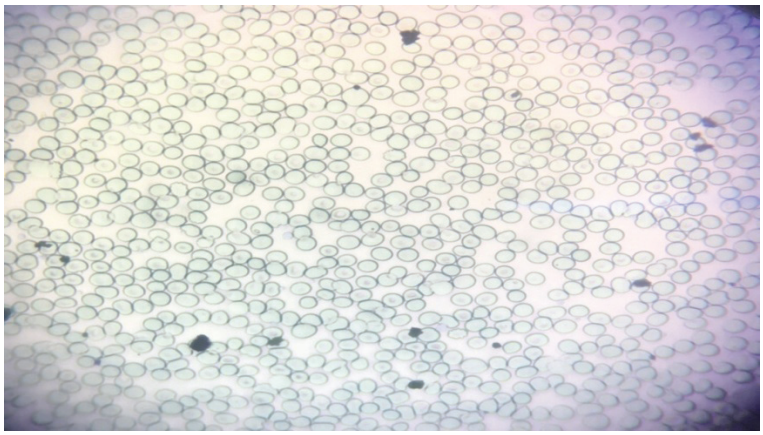
Fig. 4. The shape of RBCs in Thalassemic Blood at 40 Red

Blood Cell count

Table 1. Variation of red blood cell count between normal and thalassemic blood

Samples	**Red Blood cell Count (cells per microliter)**
Normal Blood	4.5 million
Thalassemic blood (S1)	7.2 million
Thalassemic blood (S2)	7 million
Thalassemic blood (S3)	7.2 million
Thalassemic blood (S4)	7.5 million
Thalassemic blood (S5)	6.8 million
Thalassemic blood (S6)	6.9 million

Table No. 2: Showing Comparison in the Shape of Cells

S. 1	The Shape of RBCs in Thalassemic Blood Sample
	
N.1	**The shape of RBCs in Normal Blood Sample**
	

Result

Blood samples were taken both thalassemia and normal population and observed under the microscope and the changes in the blood were observed which are explained further.

On observing the blood sample of thalassemia patients, different shapes of erythrocyte were found. Out of which the hypocromic, microsite shapes were observed in greater number, other shapes like targeted, acanthocyte, basophilic, schistocyte, barr cell were also observed in the average number and limon cell were observed in the least number during the study wherein the control sample no such shape change observed and normal or basic changes were observed.

On comparing the blood sample on basic of cell count different level of cell volume or cell count was observed both in thalassemic and control population. The level of RBCs was found to increase in the thalassemic population as compared to the control population. The volume of erythrocyte observed in the control population was 4-5 million/microliter of blood

where 6.8-7.2 million/microliter was observed in the thalassemic population. Hence thalassemia patients are richer in the count of corpuscles than control.

At the end of the observations, it results that the hypothesis made during the research is an alternative hypothesis.

Discussion

A total number of 40 samples were taken, 20 samples of normal blood and 20 samples of thalassemic blood. The study was designed in such a way that each sample was studied by using different methods and comparison is done between the normal blood and thalassemic blood. Two methods are used to study the wet blood those are microscopy and red blood cell count.

According to various previous studies change in the morphological shape of erythrocytes were observed due to the formation of methemoglobin and precipitation of globin. Bunyaratvej et al. in 1985 performed a study on thalassemia patients an analyzed the shape of RBC especially in Beta-thalassemia and observed the change in the shape from normal (biconcave) RBCs to torocytes, acanthocytes[5]. Galanello and Origa in 2009, performed a study in beta-thalassemia patients, they also observed the morphological changes in the shape (speculated teardrop, elongated and nucleated) RBCs from normal morphology of RBCs[4]. Galanello and Cao in 2011, work on alpha thalassemia patient Alpha thalassemia is a very rare disease that affects the shape, size, color and total count of the RBCs. RBCs number was found to be increased[2]. Akrimim Jameeka Ali et al. in 2013 performed a study in a different type of anemia and study the effect on RBCs. They found 93% shape in RBCs was abnormal form the basic shape. Founded abnormal shapes were hypocromic microsites, target, acanthocytes, etc[6].

The finding from all the studies is consistence, all the studies indicated about the change in the morphological shape of the RBCs, in various from alike hypocromic microsites, target, acanthocytes, tear drop, elongated, nucleated and torocytes. All the existing research supports each other findings and predicts the same finding.

In the present study, abnormal shapes of RBCs were found in the thalassemia population where normal/basic shapes were found in the control population. The current study shows a different type of cell shape were found as hypocromic microsite, targeted, acanthocyte, basophilic, schistocyte, barr cell, limon cell. All these abnormal shapes of RBCs are already reported in above given previous studies, from the comparison and cell characterization were made (Bunyaratvej et al. in 1985)[5] (Renzo Galanello and Rafaella origa in 2009)[4] (Renzo Galanello and Antonio Cao in 2011)[2] (Akrimim Jameeka Ali et al. in 2013)[6] all previous study supports and validate the current study.

Studies show that total RBCs count is higher in thalassemic blood as compare to normal blood because due to the lack of hemoglobin body is not able to get enough amount of oxygen thus to overcome this situation body tends to make more and more red blood cells to carry more and more oxygen. Evan and Jehle in 1991, studied on anemic population and reported that anemia can be categorized based on mean corpuscular volume and red blood cell distribution[7]. Galanello and Rafella in 2009, worked on beta-thalassemia and reported the increase in the number of RBCs after splenectomy[4]. Again, Galanello and Cao, worked on alpha thalassemia and reported that same observation as in previous research, the increase in the count of RBCs[2].

In the current study, number or count and volume of RBCs were analyzed, the results observed were the same as observed in previous studies. The count and the volume of RBCs were found to increase in the thalassemic population form normal volume and comparison to the control population. The volume of RBCs was observed on an average of 4-5 million/microliter where in thalassemic population volume was 6.8-7.2 million/microliter, which is far greater than the control population. So, it will be easy for the comparison between normal and anemic patients.

All the results observed are found the same as in the previous study which indicates that the previous study supported the present study, moreover the presented method can be used as a tool for identification of individual as class characteristics. As the class characteristic help in narrow down the area of investigation, the presented method also helps to narrow down the area of investigation and research, rather than used sophisticated instruments/methods and tests to narrow down the investigation. According to J. Ford in 2013 RBCs morphology can be used for identification in hematology laboratory and microscopy can easily help in identification[8]. So, abnormal shape RBCs using microscopy can be used as a tool for personal

identification. Ja hyun, Yang and lee in 2012 stated that body fluids will give important insight into crime scene reconstruction by supporting a link between the donor and criminal act[9].

According to Colah, Italia, and Gorakshakan, a low rate of thalassemia was found in India, on an average 10000-12000 thalassemic children born in India where specifically in Maharashtra and Gujarat per 1000 birth 0.28 and 0.39 % was born with thalassemia[10]. Data is also available based on communities that reveal a minimum of 4% to a maximum 17% population was affected with thalassemia[10]. The less prevalence of thalassemia used as a tool for the identification of a suspect or narrow down area of investigation. Blood sample comparison sets criteria for inclusion and exclusion of individuals with different blood diseases and saves time and gear up the investigation. Such types of evidence play a crucial role in criminal trials and help to link the suspect with the crime by determining whether they having a common source or not[11]. (Thompson Williams).

Presented research can be used in analyzing of regular cases encounter with the forensic science investigation for further research and investigation.

Conclusion

The study shows that the comparison between the normal and thalassemic blood is possible based on the morphology/shape of the RBCs and total red blood cell count by using a wet blood sample. This study is helpful to compare the blood evidence found at the crime scene, based on this comparison it is possible to link the suspect with the crime and helps to narrow down the investigation.

Limitations: RBC count can only be done with the wet blood sample., Dry blood can also be used for investigation purposes.

Further Scope:

The size of the RBCs is also a factor that can also be used for the comparison; the size of red blood cells gets smaller and smaller in thalassemic blood.

The Colour of the RBCs can also be included as a factor for comparison.

Use of dry blood for all investigations.

Conflict of Interest: Authors shows no conflict of interest.

Source of Funding: Self/ no funding was provided by any agency.

Ethical Clearance: NA (waste blood used for study).

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