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Research Article

A STUDY ON OSMOTIC FRAGILITY OF ERYTHROCYTES OF TUBERCULOSIS PATIENT'S BLOOD

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ABSTRACT
The paper presents the data on percent lysis of hemoglobin in Tuberculosis patient's blood. Osmotic fragility is the ability of RBC's to resist rupture (lysis) in hypotonic saline. The degree of hemolysis can be estimated by measuring the colour intensity of the supernant in a spectrophotometer at 540nm and comparing it with 100%. The task is to examine the relationship between the extent of hemolysis and osmolarity of the medium in which the erythrocytes are suspended. The percent lysis of erythrocytes of tuberculosis patient's blood is significantly more than that of normal. It means erythrocytes of tuberculosis patients are more fragilie than normal.

Key Words*: Tuberculosis, Erythrocytes, Spectrophotometer, Osmotic Fragility, Percent of Lysis*

INTRODUCTION

Tuberculosis (TB) remains the single largest infectious disease causing high mortality in humans, leading to 3 million deaths annually, about five deaths every minute. Approximately 8-10 million people are infected with Mycobacterium tuberculosis every year (World Health Organization, 1999). It is one of the oldest known diseases and still a major cause of mortality today, has many manifestations affecting the bone, the central nervous system and many other organ systems, but it is primarily a pulmonary disease. It is still a common disease in developing countries and a global trend in the resistance to antituberculosis drugs was observed (Bukhary *et al.,* 2007 and Daves *et al.,* 2009). Hemorheological changes in response to therapy have also not been fully determined in PTB patients living in developing countries (Awodu *et al.,* 2007). During treatment improvements in some of the hematological values such as, rise in hemoglobin and hematocrit levels (Alrajhi *et al.,* 2006). Primary billiary tuberculosis is an extremely rare entity in Pediatric age group from reported cases; this rare entity has scarcely been found in Pediatric age group (Gupta *et al.,* 1985; Bergdahl *et al.,* 1972; Kumar *et al.,* 2000 and Garg *et al.,* 2001). Currently 1/3 of the world's population are infected with tuberculosis bacillus with 8 million new cases and 3 million deaths due to tuberculosis estimated annually (Kaufman, 2002; Mustafa *et al.,* 2003; Neil Schluger, 2001; Wigginton *et al.,* 2001; Gonazalez-Jaurrero *et al.,* 2001 and Van Crevel *et al.,* 2002). Tuberculosis is a chronic granulomatous infection caused by *Mycobacterium Tuberculosis*.
Tuberculosis can cause an increase in Erythrocyte Sedimentation Rate (ESR), anemia and lymphopenia.Studies has also documented an increase in platelet counts in pulmonary and pleural tuberculosis (Kartaloglu *et al.,* 1995 and Hui *et al.,* 1994). In this study, we aimed to investigate the other coagulation parameters based on our previous study (Kartaloglu *et al.,* 1995) in which we found increased peripheral platelet counts in patients with pulmonary tuberculosis. Pohl (1951) observed the translational motion of neural matter caused by polarization effect in a non uniform electric field and named it as dielctrophoresis.Gopal Krishna *et al.,* (1994) reported dielctrophoretic characterization of normal and diseased blood. The characteristics of hemoglobin make possible to detect changes in the electrical and magnetic properties of blood cells and its constituents that are responsible for human illness (Basharath *et*

al., 2007).The characteristics of hemoglobin have not drawn much attention of physicists. However, some reports are available on magnetophoresis (Owen, 1978 and Svobada, 1986). The characteristics of hemoglobin make possible to detect changes in the electrical and magnetic properties of blood cells and its constituents that are responsible for human illness (Basharath *et al.,* 2007).

The blood is being studied extensively by the physiologists, bio-chemists and bio-medical engineers, but it has not drawn much attention of physicists. A survey of literature reveals that much attention has been not given to study the changes in RBC physiology of the blood drawn from the patients suffering from Tuberculosis at membrane level.

MATERIALS AND METHODS

About 42 patients suffering from Pulmonary T. B. (all males) were selected to study osmotic fragility of blood .Samples of volume 5 ml were drawn in the anticoagulant - EDTA at Andhra Pradesh Chest Hospital, Hyderabad and brought to the laboratory in siliconised bottles and stored at 4˚C until use. The experimental investigations were completed within three hours after the collection. Plasma was separated from blood samples by centrifuging the blood at the rate of 1500rpm about 15 minutes and the blood samples were prepared by mixing equal amount of plasma and erythrocytes. By this process hematocrit of sample is maintained to be constant.

Experimental

The Osmotic Fragility of Erythrocytes of Tuberculosis Patients' Blood

Osmotic fragility is the ability of RBC's to resist rupture (lysis) in hypotonic saline. The % of lysis increases as the RBC's becomes older. Red cells are bound by a membrane, which allows water to pass through while restricting the solutes. This is called osmosis. Red cells shrink due to exosmosis. When the red cells are placed in solution that is more concentrated (eg. 2% of hypertonic) then the concentration of solute inside. On the other hand the red cells will absorb water (called end osmosis) when kept in a hypotonic solution like water.

Red blood cells suspended in hypotonic solution of sodium chloride take up water, swell and become spheroidal and more fragile and eventually burst. The degree of fragility is inversely proportional to the concentration of sodium chloride and directly proportional to the thickness of the erythrocytes.

Reagents

Stock buffer saline:

1. Sodium hydrogen phosphate (di hydrate): 0.243gm
2. Disodium hydrogen phosphate (di hydrate): 1.7115gm
3. Sodium chloride: 9gm

Dissolve in distilled water and make up to 100ml.

Working saline: Dilute the stock saline 1 in 10 with distilled water (1ml stock + 9ml distilled water)

Procedure

Prepare NaCl solution of different concentrations using 0.9% sodium chloride solution and diluting it with distilled water. To each of the tube containing 5ml of dilute sodium chloride solution, add 0.05ml of heparanised blood with the help of 0.2ml pipette, mix well and wait for 30 minutes (Figure 1) transfer the content into the centrifuge tubes and centrifuge. Take the absorbance (OD) at 540 nm against distilled water as blank. The tube containing 0.1% saline serves as 100% lysis. Calculate the percentage of lysis in each tube.

$$\text{OD of each tube} = \frac{\%\ \text{of lysis} \times 100}{\text{OD of 0.1\% tube}}$$

If there is no hemolysis the red cells will be found at the bottom of the tubes with a clear saline solution above. If some hemolysis occurs, the saline will be tinged red with hemoglobin. If hemolysis is complete, the fluid will be uniform in colour throughout and these will be no red cells visible at the bottom of the tube.

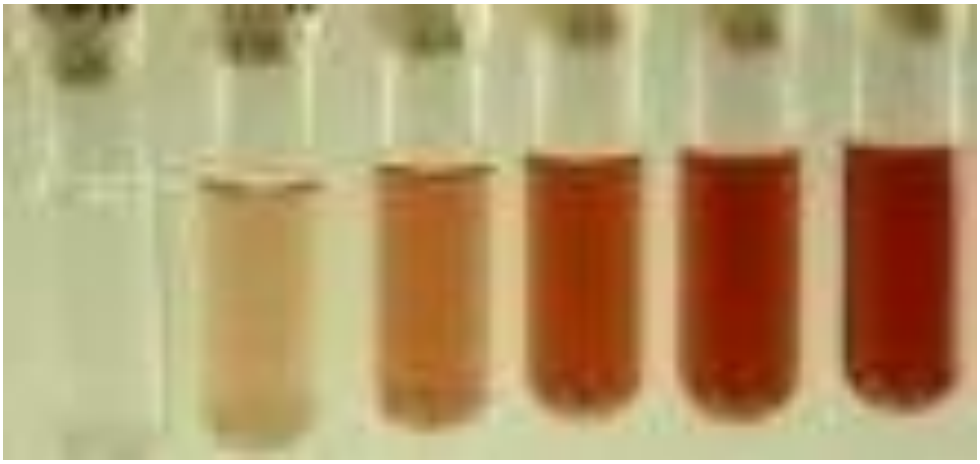

Figure 1: Experimental setup of osmotic fragility

The degree of hemolysis can be estimated by measuring the colour intensity of the supernant in a spectrophotometer, (Figure 2) at 540 nm and comparing it with 100%.

RESULTS AND DISCUSSION

The phenomenon of osmotic fragility or susceptibility to hemolysis of erythrocytes is made use in the present investigation. The number of cells undergoing hemolysis depends on the degree of hypotonicity of the extra cellular medium. The concentration of liberated hemoglobin in each test medicine is an index of the extent of osmotic hemolysis. The task is to examine the relationship between the extent of hemolysis and osmolarity of the medium in which the erythrocytes are suspended. The percent lysis of erythrocytes of tuberculosis patients' blood is significantly more than that of normal (Figure 6). It means erythrocytes of tuberculosis patients are more fragile than normal. The increase in percentage of lysis of RBC of TB blood is about 600, when compared to that of normal

Table 1: Data on percent lysis of healthy erythrocytes measured at 3.0% saline

Sample code	% lysis
N.S 1	15.9
N.S 2	17.9
N.S 3	16.9
N.S 4	16.7
N.S 5	3.9
N.S 6	7.5
N.S 7	7.7
N.S 8	21.0
N.S 9	8.7
N.S 10	7.8

Table 2: Data on percent lysis of erythrocytes measured at 3.0% saline for tuberculosis patients

Sample Code	% lysis
HB01	86.5
HB02	91.9
HB03	75.5
HB04	87.9
HB05	98.0
HB06	88.0
HB07	82.0
HB08	84.6
HB09	78.6
HB10	91.8
HB11	95.4
HB12	93.5
HB13	94.7
HB14	77.1
HB15	91.1
HB16	88.9
HB17	97.7
HB18	93.1
HB19	76.2
HB20	97.6
HB21	96.9
HB22	84.6
HB23	95.1
HB24	98.0
HB25	89.5
HB26	91.5
HB27	83.1
HB28	58.5
HB29	44.4
HB30	62.5
HB31	88.2
HB32	91.5
HB33	97.4
HB34	73.2
HB35	93.2
HB36	94.3
HB37	61.0
HB38	80.4
HB39	42.1
HB40	64.5
HB41	95.7
HB42	83.3

Table 1 gives the data on percent of lysis of erythrocytes measured at 3% saline for normal.
Table 2 shows the data on percent of lysis of erythrocytes measured at 3% saline for tuberculosis patients. A large variation is observed in percent lysis among tuberculosis patients, when compared with that of normal. Plots are drawn between different physical parameters.

Table 3 - Data on clinical investigation on blood and erythrocytes of tuberculosis patients

Sample Code	**E.S.R(mm) 1st hour**	**Hemoglobin Content (gm %)**	**R.B.C Count Millions/cu.mm**
Normal	0 – 5	14 – 16	4.2 – 6.5
HB01	40	11.5	3.7
HB02	40	11.5	3.7
HB03	60	12.0	4.0
HB04	50	10.5	4.0
HB05	40	12.0	4.0
HB06	40	11.6	3.9
HB07	120	10.5	3.4
HB08	70	11.8	3.8
HB09	15	12.0	4.0
HB10	50	11.0	3.5
HB11	60	12.0	4.0
HB12	65	11.5	3.7
HB13	50	11.5	3.7
HB14	100	11.0	3.5
HB15	80	12.0	4.0
HB16	125	11.5	3.7
HB17	90	10.5	3.4
HB18	70	11.5	3.7
HB19	35	12.0	4.0
HB20	80	11.5	3.7
HB21	40	12.0	4.5
HB22	90	11.0	3.3
HB23	20	12.0	3.5
HB24	30	14.2	5.1
HB25	65	11.5	3.7
HB26	60	11.0	2.0
HB27	50	10.5	3.5
HB28	40	10.5	4.0
HB29	40	13.0	4.0
HB30	90	10.0	3.0
HB31	25	5.50	3.0
HB32	35	6.50	3.0
HB33	65	10.0	3.3
HB34	40	13.0	4.0
HB35	60	10.5	3.0
HB36	50	10.3	3.0
HB37	70	15.5	2.2
HB38	50	8.60	2.5
HB39	50	11.1	4.1
HB40	120	9.10	2.8
HB41	120	9.10	3.6
HB42	60	5.30	2.4

I also studied some physical properties. Various plots are drawn between different physical parameters.
% of lysis = 95.182 - 2.02446 HB

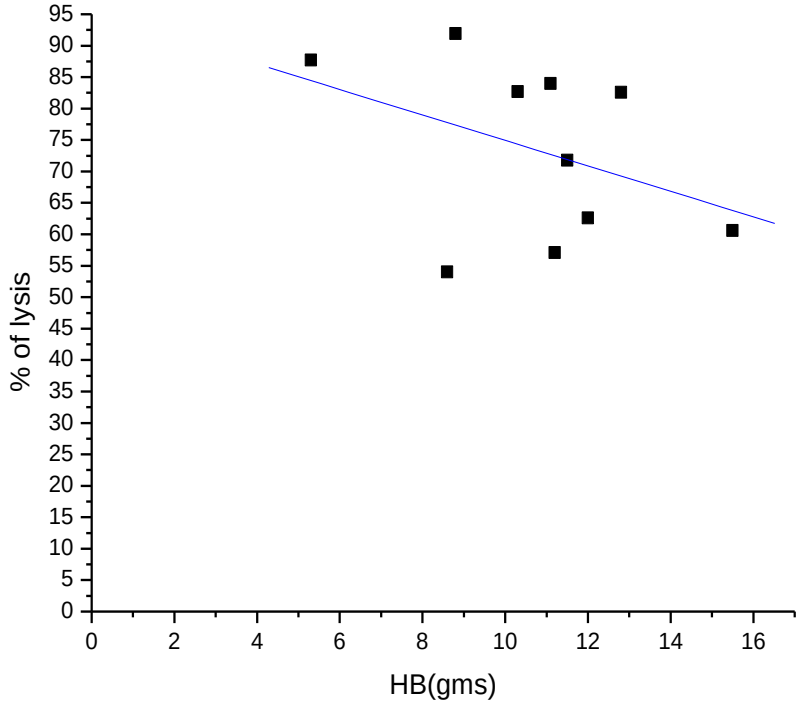

Figure 3: A plot between HB and % of lysis of blood of T.B patients

Figure 3 reveals a best fit plot between the percent of lysis on Y-axis and H.B on X-axis. It is interesting to note that percent of lysis decreases linearly as the H.B increases.

% of lysis = 91.52982 - 0.2453 ESR

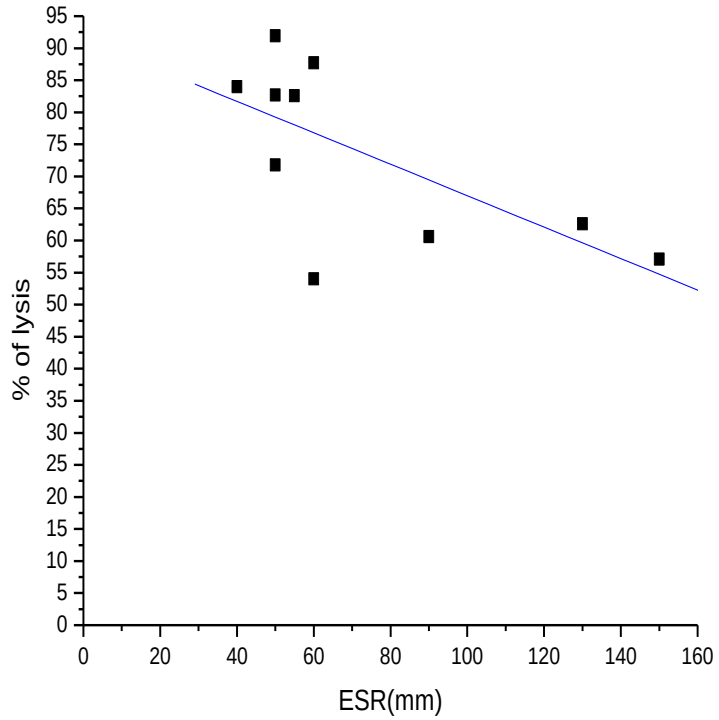

Figure 4: A plot between ESR and % of lysis of blood of T.B patients

Figure 4 reveal a graph plotted between percent of lysis on Y-axis and size of RBC on X-axis. A considerable increase in percent of lysis with the increase of size of RBC is evident from the graph.

% of lysis = 51.8678 + 2.11977 a

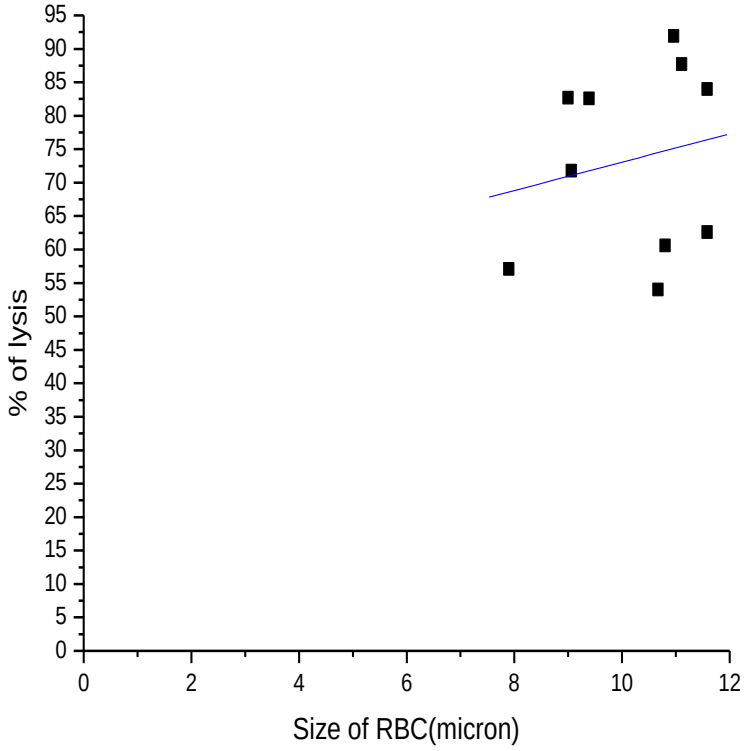

Figure 5: A plot between Size of RBC and % of lysis of blood of T.B patients

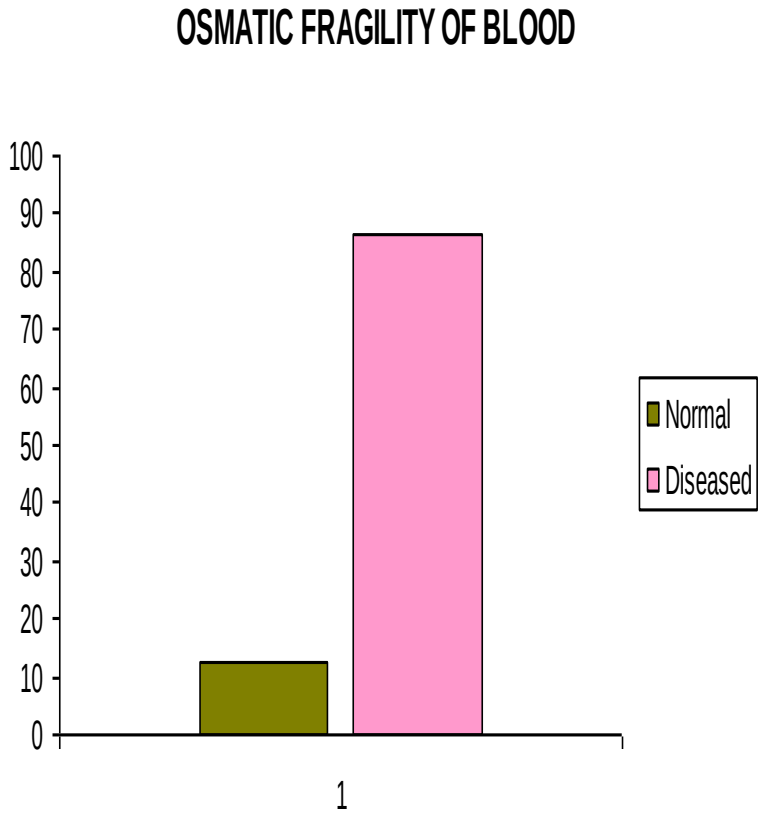

Figure 6:Osmotic fragility of diseased and normal blood

Figure 5 shows the graph plotted between percent of lysis on Y-axis and ESR on X-axis. The best fit graph shows the decrease in percent of lysis linearly with the increase of ESR.The below figure shows the variation of osmotic fragility of diseased blood with normal.

The ESR of tuberculosis patients' blood can be attributed to the following factors, concerned with whole blood and its constituents – plasma and RBC.

a. Increase in the
b. Size of RBC.
c. Decrease in the hemoglobin content.
d. Low concentration of fibrinogen.
e. Aggregation or formation of RBC's.
f. Interaction between the RBC and plasma proteins.

Here, in the present investigation, the increase in size of RBC and decrease in Hemoglobin and blood viscosity may contribute significantly to raise the ESR. The study on ESR suggests that ESR can be used as the first step of clinical examination so as to predict the cardiovascular and pulmonary disease in advance.

The study reveals a decrease in hemoglobin in tuberculosis patients.

Erythrocyte sedimentation rate (ESR) is an important index of hemorheology. It increases significantly in the case of tuberculosis patients.

ESR can be used to predict the cardio vascular and pulmonary diseases in advance.

DISCUSSION

Percent lysis of erythrocytes of tuberculosis patient's blood is significantly more than that of normal. It means erythrocytes of tuberculosis patients are more fragile than normal. The present study suggests that if biophysical parameters are correlated properly with clinical aspects, may be very much useful in medical discipline for the diagnosis of Tuberculosis

At diagnosis the mean hemoglobin concentrations were 11.46 g dL^{-1} for males. The value is significantly lower than the mean values for healthy male persons (Omar *et al.,* 1983; Akintunde *et al.,* 1995 and Hoffbrand *et al.,* 1993) g dL^{-1}, respectively.

In the present study, platelet count is found higher in male PTB patients as compared with the normal values. The findings are in agreement with the earlier reports (Omar *et al.,* 1983 and Akintunde *et al.,* 1995) but contradictory to some of the results indicating thrombocytopenia. It was suggested that in evaluating results of hematological values in PTB, lymphopaenia rather than lymphocytosis, should be considered (Akintunde *et al.,*1995).The RBC count is lower in tuberculosis patients (3.5Millions/cu.mm) than normal (4.2-6.5Millions/cu.mm).

Erythrocyte Sedimentation Rate (ESR), in untreated male PTB patients was found to be 62.3 mm h h^{-1}, respectively. The value is significantly higher than the standard normal values given (Hoffbrand *et al.,* 1993). The findings are fully supported by different reports specifying higher ESR values for PTB patients (Hoffbrand *et al.,* 1993; Stenius-Aarniala *et al.,* 1979; Chia *et al.,* 1979; Olaniyi *et al.,* 2003; Olaniyi *et al.,* 2003 and Akintunde *et al.,* 1995).

Conclusion

Based on the results of the present study, it was concluded that all such changes were associated to metabolic disorder accompanying chronic infections in PTB patients. The varied hematological abnormalities observed in PTB patients suggested that the differential diagnosis of tuberculosis should be entertained in patients with varied hematological disorders. Percent lysis of erythrocytes of tuberculosis patient's blood is significantly more than that of normal. It means erythrocytes of tuberculosis patients are more fragile than normal.

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