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Research Article

MORPHOLOGICAL PARAMETERS OF PATIENTS OF BLOOD SUFFERING FROM TUBERCULOSIS

***Manohar Reddy N.**
Department of Physics, S.V. College of Engineering, Tirupathi, Andhra Pradesh, India-517507
**Author for Correspondence*

ABSTRACT
The subtle changes in the physiology of erythrocytes at the cellular level on documented in the present laser diffraction study. Using this technique one can differentiate the morphologies of erythrocytes. The size and shape of blood cells are determined usually by microscope. This method, besides being tedious, cannot be extended to a large number of cells and samples. In view of this, a simple and quick method has been developed for determining the average size and shape of blood cells by employing laser diffraction technique. Blood samples were collected from normal healthy persons and patients from tuberculosis. The laser diffraction method is very rapid and simple for assessing the average size of the cells. This could also be used with advantages as a diagnostic tool for assessing the variation in the size of human RBC.

Keywords: *Laser Diffraction Technique, Tuberculosis, Erythrocytes, Diffraction Ring*

INTRODUCTION
India, a country with over 1.21 billion people, has the highest burden of tuberculosis (TB) in the world, accounting for 20% of the global incidence of TB, and an even higher share of global incidence of multi–drug resistant (MDR) TB (WHO Report, 2010). It is caused by a bacterium called mycobacterium tuberculosis, usually infects the lungs, but it can also enter the blood and infect almost any part of the body. This includes liver kidneys, stomach and gut, bones, skin, breasts, brain and spinal cord TB in these places is more common in children and people with weak immune systems, including people living with HIV (Murray *et al.*, 1990). Mycobacterium tuberculosis, the etiologic agent of tuberculosis is responsible for more deaths each year than any other single pathogen. The World Health Organization has established that there are about 10 million new cases of tuberculosis each year and that tuberculosis is responsible for at least 3 million deaths annual (Bloom and Murray, 1992; Kaufmann and Vanembden, 1993).
This disease is associated with considerable morbidity from pulmonary and extra pulmonary pathology. Pulmonary symptoms are progressive and include cough, hemoptysis, dyspnea and pleuritis. Extra pulmonary TB can involve the bones, joints, pericardium and lymphatic's and it can cause spinal cord compression from Pott's disease. Death is more common in older patients and infants, with estimated case fatality rates ranging from 0.3% in adolescents also experience significant morbidity from this disease. The social and economic impact of Tuberculosis (TB) which claims lives of more than 4, 00,000 people every year is devastating, especially as it affects the economically most productive age groups (T. B India, 2004). Furthermore, in virtually all countries, fewer female than male tuberculosis cases are notified (WHO Global TB Control, 2002). In India, it has been found that more men report with chest symptoms than women and the Revised National Tuberculosis Programme (RNTCP) advocating Directly Observed Treatment – Short Course (DOTS) detects nearly three times more male than female TB patients (Khatri and Frieden, 2000). Higher tuberculosis notification rates in men may partly reflect epidemiological differences, exposure to risk of infection and progression from infection to disease (Howson *et al.*, 1996). However, this may not be the only factor influencing this disparity. It has also been generally observed that women in developing countries. Confront more barriers than men in accessing health care services due to a variety of socio-cultural factor (Uplekar *et al.*, 2001; Hudelson, 1996). A complete medical evaluation for tuberculosis (TB) includes a medical history, a physical examination (symptoms), radio graphical examination (a chest X-ray), and microbiological examination of sputum, a tuberculin skin test and surgical biopsy. In this context, much attention has not been paid to examine the blood of TB patient either pathologically or biochemically for the diagnosis of Tuberculosis.

MATERIALS AND METHODS

Blood was collected from the patients (male) suffering from tuberculosis patients. To avoid coagulation EDTA was added to the collected blood samples and stored in a non conducting flask. Uniform smears of these samples were made on well cleaned slides. The time interval between the collection of samples and the slide making was well within an hour.

Experimental

The technique of laser diffraction is based on Babinet Principle (Calthrope, 1952; Charles, 1934), that gives Fraunhofer computational method of finding the average size of the diffracting particles is based on the measurement of the angle of diffraction, which involves the adjustment of the distance of the sample from the central hole such that the diffraction ring on the eriometer. This method is always used to find out the size of the spherical particles.

But this method is tedious and introduces parallax error. In view of this, a more sophisticated method using laser diffraction technique has been developed for the determination of size of human erythrocytes. The sample was prepared by smearing a drop of blood uniformly (thin film) on microscopic slide and then introduced in between the laser and the screen with the smeared surface facing the screen. A He-Ne laser of power 2 mW was employed for the diffraction purpose.

This laser when passes through the blood samples gives the well defined diffraction pattern on the screen. The radius r of the first order diffraction minima was measured for different samples for a known "sample to screen distance" D. A plot between radius of the diffraction ring r and D is drawn, the slope of which gives the tangent of the angle of diffraction.

The angle of diffraction (θ) is given by $\text{Tan } \theta = r/D$. where r = radius of the first circle; D = distance between slide and the screen. The mean diameter (d) of the blood cells was calculated using the equation taking into account the wave length of the laser light. From the Rayleigh's criterion the size or diameter (d) of the cell can be written as $d = 1.22\lambda / \text{Tan } \theta = 1.22\lambda \ D/r$,

Where $\lambda = 6328$ A°

A well defined diffraction pattern is readily obtained on the screen, the radii and widths of which could be easily measured if the pattern formed on a graph paper. The screen can also be calibrated (radii / axial lengths versus particle size) for a given distance, D so that the average size of the diffracting cells can be obtained directly without involving any calculations.

The size of the diffraction patterns is inversely proportional to the particle size. The width of the diffraction ring is a fraction of the variations in the size of the particles. Similarly the sharpness of the minim depends upon the consistency in cellular size and shape and hence the diffraction pattern produced by these cells is very sharp and clear.

However, laser diffraction method is very rapid and simple for assessing the average size of the cells and at a glance.

The method not only simple but also elegant, readily handled and can be easily demonstrated to a large gathering at a time especially to moderately sophisticated health science students which would provide them to look at physical optics as a set of relevant phenomena. This could also be used with advantage as a diagnostic tool for assessing if there is more than normal variation in the size distribution of human RBC from the width of the diffraction ring.

RESULTS AND DISCUSSION

The mean diameter of RBC of normal human obtained by this method is 7.12 μm and where as for tuberculosis patients' cells is 10.6 μm. In the case of tuberculosis, the size obtained is drastically increased due to changes taken place on the RBC cell membrane, because of high metabolic activity and this leads to the changes in the cell morphology and size. The study reveals the trend of diameter with respect to tuberculosis human RBC.

Table 1: Data on size of erythrocytes of T. B. patients' blood

Sample code	D (cm)	r (cm)	d (µm)
Normal			7.12
HB01	7.0	0.6	9.00
HB02	7.1	0.5	10.96
HB03	7.0	0.5	10.80
HB04	8.3	0.6	10.67
HB05	6.0	0.4	11.58
HB06	7.0	0.6	9.06
HB07	7.2	0.5	11.11
HB08	7.3	0.6	9.39
HB09	7.5	0.5	11.58
HB10	7.2	0.5	11.12
HB11	6.9	0.5	11.50
HB12	8.1	0.6	10.56
HB13	6.9	0.5	11.22
HB14	7.4	0.6	9.55
HB15	7.2	0.5	11.20

The large size red cells macrocytes usually have mature cytoplasm with adequate hemoglobin and are differentiated from reticulocytes, which are also large but immature. The excessive variation in size, called anisocytosis is seen with most anemias, prominent in sideroblastic anemia. The study reveals about increase in size of RBC of TB patients when compared with that of healthy persons (Figure 1). The increase in size of red cells of TB patients may perhaps be due to the hyper tonicity of the medium and an influx of water occurs, the cells swell, the integrity of their membranes is disrupted. The increase in size of RBC of TB blood is 36%. 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.

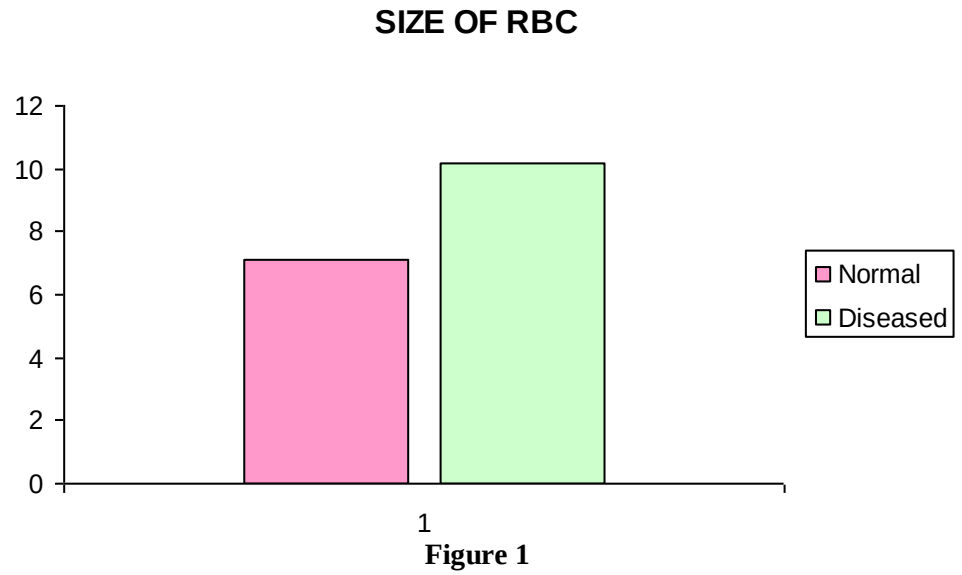

Figure 1

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