A Study on Size and Shape of Erythrocytes of Cancer Patients using Laser Diffraction Technique

C. RAMAKRISHNA RAO, KALEEM AHMED JALEELI¹, B. S. BELLOMBB¹ & ADEEL AHMAD¹
Department of Radiation Physics, M N J Institute of Oncology & Regional cancer Centre, Hyderabad -500 004, India.
¹Biophysics Unit, Department of Physics, Nizam College (Autonomous), Osmania University, Hyderabad -500 001, India.

Received 3 November 2008 Accepted 20 April 2009

Abstract - The subtle changes in the physiology of erythrocytes at the cellular level are 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, can not 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 suffering from different types of cancer. The laser diffraction method is very rapid and simple for assessing the average size of the cells. This could also be used with advantage as a diagnostic tool for assessing the variation in the size of human RBC.

Key words - Laser diffraction technique, Erythrocytes, electrical properties, diffraction ring.

1. Introduction
Cancer is a disease which arises from the abnormal and uncontrolled division of cells, known as cancer cells. They invade and destroy the surrounding tissues. Cancer cells are different from normal cells. They do not remain confined to one part of the body. They penetrate and infiltrate into the adjoining tissues and dislocate their functions. Some of the cancer cells get detached from the main site of origin and travel by blood and lymph channels. The blood is a heterogeneous fluid and, therefore, complex in nature. The size and shape are of clinical importance not only to characterize different cells but also in differentiating abnormal from normal blood cells. For example, the size of red blood cells differs from one individual to another but are distributed about a mean and hence the average size has to be determined. Similarly, there can be a change in the shape of the cells as is noticed in the case of sickle cell anemia [2]. The size and shape of blood cells are determined usually by microscope. But this method besides being tedious can not be
2. Materials and Methods

Fresh samples of normal human blood of 5 ml was collected from Lions club of Hyderabad. Blood was collected from the patients suffering from cancer cervix, breast, rectum, cheek, oesophagus, ovary, lung, thyroid, tongue, pituitary adenoma, osteosarcoma, multiple myeloma, bladder, Hodgkins disease etc from MNJ Institute of Oncology & RCC, Hyderabad. To avoid coagulation EDTA was added to the collected blood samples and stored in a nonconducting 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.

The technique of laser diffraction is based on Babinet principle [3 - 5], that gives Fraunhofer diffraction pattern on the retina of the observer. The 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 retina of the observer had its appropriate diameter on eriometer. This method is always used to find out the size of the spherical particles. Since this method is tedious and with a parallax error, a new sophisticated method using laser diffraction technique has been developed for the determination of size of erythrocytes. This arrangement consists of specimen slide, laser and screen for the determination of size of human erythrocytes. The sample was prepared by smearing a drop of fresh 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 sample 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" \( D \).

The angle of diffraction is given by

\[
\tan \theta = \frac{r}{D}
\]

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 wavelength of the laser light.

From the Rayleigh's criterion the size or diameter(\( d \)) of the cell can be written as

\[
d = \frac{1.22 \lambda}{\tan \theta} = \frac{1.22 \lambda D}{r}, \text{ where } \lambda = 6328 \text{ Å}^\circ
\]
3. Results and Discussion
The laser diffraction method is very rapid and simple technique. 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 pattern is a function of the variations in the size of the particles. Similarly the sharpness of the minima depends upon the consistency in cellular size and shape. All mammalian blood cells are of uniform 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 picture of diffraction of light by small particles. 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.

The mean diameter of RBC of normal human obtained by this method is 7.12 μ and where as for cancer cells is 9.35 μ. In the case of cancer, the size obtained is drastically increased due to changes taken place on the RBC cell membrane, because of high metabolic activity in the case of cancer and this leads to the changes in the cell morphology and size.

The study reveals the trend of diameter with respect to cancerous human RBC.

Table 1 - Size (d) of normal and cancerous erythrocytes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Type</th>
<th>Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal</td>
<td>7.12</td>
</tr>
<tr>
<td>2.</td>
<td>Cervix Cancer</td>
<td>10.5</td>
</tr>
<tr>
<td>4.</td>
<td>Penis Cancer</td>
<td>10.03</td>
</tr>
<tr>
<td>5.</td>
<td>Rectum Cancer</td>
<td>8.77</td>
</tr>
<tr>
<td>6.</td>
<td>Cheek Cancer</td>
<td>9.38</td>
</tr>
<tr>
<td>7.</td>
<td>Oesophagus Cancer</td>
<td>8.59</td>
</tr>
<tr>
<td>8.</td>
<td>Ovary Cancer</td>
<td>8.97</td>
</tr>
<tr>
<td>9.</td>
<td>Lung Cancer</td>
<td>9.68</td>
</tr>
<tr>
<td>10.</td>
<td>Thyroid Cancer</td>
<td>8.97</td>
</tr>
<tr>
<td>11.</td>
<td>Tongue Cancer</td>
<td>9.30</td>
</tr>
</tbody>
</table>

ISSN 0974 - 8970
Acknowledgement
Authors are grateful to the Principal, Nizam College, Osmania University, Hyderabad for providing laboratory facility and Director, MNJ Institute of Oncology & Regional cancer centre, Hyderabad for providing blood samples of cancer patients.

References