CHAPTER 1: INTRODUCTION

Human blood is a non-homogeneous system composed of deformable cells suspended in plasma. It contains 55% of plasma and 45% of cells. These cells are suspended in plasma as erythrocytes, leukocytes and thrombocytes. Leukocytes and thrombocytes form a small fraction. The erythrocytes are about 5 millions/cu.mm, White cells vary from 5000 to 8000/cu.mm and platelets from 250000 to 300000/cu.mm. The ratio of cells in normal blood is 600 erythrocytes for each leucocyte and 40 thrombocytes. The plasma is about 90% water by weight containing 7% plasma proteins, 1% inorganic and 1% other organic substances. Hence from rheological point of view, blood is considered as a suspension of erythrocytes in plasma. The averaged dimensions of erythrocytes are: 7.65µm, minimum thickness 1.44 µm, maximum thickness 2.84 µm, volume 97.91 µm³ and surface area 135 µm² (Y.C. Fung, 1993).

The properties of matter affecting deformation and flow are called rheological properties and science dealing with how the matter is deformed and flows when forces are applied to it is called rheology. The clinical hemorheological studies deal with pathological hemorheological abnormalities and aim to evaluate their links to diseases, diagnosis as well as to therapeutic approaches. The outline of hemorheology and its link to the function of living body has materialized in many different ways. The formed elements, erythrocytes, leukocytes and thrombocytes are subjected during blood flow in the
cardio-vascular system to mechanical loads which induce their deformation and clinical activation within the physiological limits as well as outside these limits under pathological conditions. Due to this, it leads to aggregation of erythrocytes and aggregation of platelets. Flow behavior of blood plasma or serum is affected by protein components. The contribution of proteins is related to their molecular weight and shape. Small globular molecules such as albumin are of less significance. On the other hand fibrinogen, a high molecular weight protein, has strong effect on plasma viscosity. Fibrinogen affects also the aggregability of erythrocytes, the interaction between WBCs and vascular wall and then adhesion which is the cause of disturbances in microcirculation (Lowe, 1988).

Blood is a non–Newtonian fluid. Hematocrit, plasma, viscosity, red cell aggregation and deformation determine the flow properties of the blood. The rheology of normal blood varies with shear stress and is mainly determined by plasma viscosity and hematocrit at high shear rates. In normal blood flow especially in arteries, the erythrocytes allow themselves to be carried along by the axial current and interactions are consequently reduced to a minimum. But due to some changes in rheological properties, the erythrocytes may lose their capacity to adapt to flow, leading to aggregation of cells.

Red cell aggregation plays a significant role in low shear flow. Blood viscosity is high at low shear rates, where red cell aggregates, and low at high shear rates where red cell participate in flow by elongation, orientation and deformation. Red cells flow as rotation of membrane around the cell interior under high shear rates. The plasma viscosity is determined by its dissolved
macro molecular components and its primary component is water. The contribution of individual protein fraction is related to the mass and shape of the molecules. Fibrinogen a large and elongated molecule has a strong effect on plasma viscosity. The normal range of plasma viscosity is between 1.10 and 1.35 m Pas at 37 degrees centigrade. Serum viscosity is lower than plasma viscosity (Stoltz et al, 1999).

Blood circulation is governed by microrheological properties of its various constituents, particularly the erythrocyte aggregation and deformability. Fahraeus (1929) showed that erythrocytes aggregation is in the form of rouleaux, which occurred physiologically. Aggregation is a result of interaction between erythrocyte and the plasma proteins in the suspending medium and is a reversible phenomenon. The adsorption of proteins between the membrane surfaces leads to formation of inter-cellular bridges. Once such a bridging occurs, the cell membranes ability to deform further aids in the formation of successive bridges resulting in maximum contact area. The cells adhere to one another in a face-to face manner to give maximal surface contact to form three-dimensional cellular structures.

A balance between attractive and repulsive forces acting among the cells determines the aggregation mechanism. The equation of these balancing forces is given by

\[ F_a = F_b - F_e - F_s - F_m \]
Where $F_a$ is the resulting aggregating force, $F_b$ the binding forces, $F_e$ the electrostatic repulsive forces of negatively charged cell surface, $F_s$ the shearing force that tends to disperse aggregate, and $F_m$ is the force to overcome the membrane elasticity. The binding forces have been extensively studied and it is well established that the large plasma proteins such as fibrinogen and macroglobulin determine $F_b$ and cause erythrocyte aggregation. The forces holding the aggregates together are rather weak in normal blood, allowing them to disperse in high shear conditions. However in pathological conditions, the adhering is associated with increased force, thus requiring high shear force to disaggregate the formed aggregates (Chien, 1975). In some extreme clinical cases the aggregation is so intense that masses of the cells clump together.

The aggregation of erythrocytes depends on factors like charge, deformability, shape, and plasma proteins. In flow condition it depends on relative motion of cells in plasma, which alters collision frequency of cells, increases aggregation or shear force leads to disaggregate the cells. The progressive increase in aggregation increases the viscosity and promotes increase in the size of aggregates and the rate of sedimentation. Increase in adhesive properties of red cell aggregates produce the phenomenon of intra vascular aggregation in most vessels. Any reduction in pressure enhances this effect, leading to occlusion of increasing number of micro vessels.

Red cell aggregation results from the bridging between adjacent cells by specific plasma proteins (fibrinogen and globulins) adsorbed on the cell surface. Aggregation could also be induced by macromolecules such as high molecular
weight dextrans. The cell-to-cell interaction in aggregation process is also influenced by the properties, which determine cellular deformability (i.e., cell surface area, cell volume, shape, internal viscosity and visco-elasticity). Thus aggregation process depends on the property of red cells and specific plasma proteins (fibrinogen and serum globulins) adsorbed on the cell surface. Chien (1978) has put forward the aggregation may facilitate blood flow and that the high viscosity observed at low shear rates is due to reversible red cell aggregation and low viscosity at high shear rates is connected to red cell deformability. The appearance of non-thixotropic and visco-elastic behavior can be observed in hysteresis, transient and oscillating flows. The specific properties are mainly connected with erythrocyte aggregation and disaggregating mechanisms and with red cell orientation and deformation phenomena. The role of sialic acid on the surface of membrane is in the form of electrostatic repulsive force, due to negative charge of its carboxyl group, in erythrocyte-erythrocyte interactions.

The erythrocyte hyper aggregation may lead to many consequences such as decrease in blood flow rate, decrease in volume concentration of erythrocytes in capillaries, change in erythrocyte deformability, increase in peripheral resistance and tendency to develop venous thrombosis.

Erythrocyte deformability is its ability to undergo shape transformations under different flow conditions and squeeze through vessels smaller than its own size owing to its larger surface to volume ratio, internal viscosity and flexible membrane properties (Stoltz et al., 1999). Erythrocytes are much more flexible and are easily deformed in flow not only in small vessels and also in large
vessels. During this process, the cells are completely adapted to flow by a continuous process of membrane rolling around the cytoplasm. The deformability of erythrocyte is due to absence of nucleus, low cytoplasmic viscosity, viscoelasticity of membrane (E.A. Evans et al, 1979) and high ratio of surface to volume, giving an excess area of about 40% (B. Bull et al, 1984). The deformation of blood cells in response to circulating forces related cell geometry such as shape, volume and surface area of membrane and intrinsic factors such as elastic and viscous properties of cell membrane, cytoplasm and active contractile system (M. Paulitschke et al, 1993). This is also the prime determinant of their survival within the circulation for 120 days since ‘stiff’ cells are sequestered by the spleen. The ability of cells to deform determines the blood flow dynamics both in macro- and microcirculation. In microcirculation even minor changes in erythrocyte deformability may exert a significant effect on blood flow resistance that aggravates the flow disturbances (Mchedlishvili et al, 1998).

Alterations in either erythrocyte aggregation or deformability pose a risk of sluggish flow, stagnation and other circulatory complications. These hemorhelological parameters could be altered in diabetes mellitus and in several diseases (McMillan, 1992). The reasons for such alterations are primarily due to the changes in erythrocytes themselves or plasma in which the cells are suspended. The importance of erythrocyte aggregation and deformability in human circulation is obvious and their role in micro-vascular pathology is significant in diabetes mellitus (Stoltz et al, 1999). Alterations in these
parameters will lead to cardio and cerebral vascular complications. Hence the analysis of these parameters is essential.

**Aggregation Measurement Techniques**

A simple and indirect quantification of erythrocyte aggregation is carried out by measuring the erythrocyte sedimentation rate (Chien, 1975). However it is a time consuming method, as the sedimentation is measured at physiological hematocrit, and it does not provide the precise details of the aggregation process. Viscometry on the other hand exploits the property of increase in the apparent viscosity of the blood due to the erythrocyte aggregation. Hence the measure of viscosity at low shear rates was used to quantify the aggregation. But such measurement could be erroneous due to the changes in the other factors contributing to the blood viscosity. A modified version of viscometer is the rheoscopic method wherein the erythrocyte aggregates are visualized while measuring the viscosity (Schmid-Schoenbein, 1975). In both these techniques the erythrocyte aggregation level is found fairly correlated to the viscosity at different shear rates and the methods yield quantitative measurement of rheological parameters in terms of viscosity, shear stress and shear rate.

Microscopic technique is based on direct observation of the aggregates under stasis conditions. Microphotographs of aggregates are analyzed for the number of aggregates per unit volume (Chien, 1975).

The laser aggregometry techniques employ continuous recording of back-scattered or transmitted light signal from the suspension of erythrocytes (Stoltz et al., 1999). Similarly in ultrasound technique, the backscattered signal is analyzed
to quantify erythrocyte aggregation (Swarnamani et al, 1989). By applying the pattern recognition procedures on the backscattered ultrasound Doppler signals the sizes of the aggregates are determined in another study. In our approach to laser aggregometry, the aggregation process is quantified by analyzing the laser-transmitted intensity through the erythrocyte suspension in a glass chamber as this technique provides the aggregation parameters under dynamic conditions (Singh and Kumaravel, 1994).

**Deformability Measurement Techniques**

The techniques for measurement of deformability are well established. They include, viscometry (Chien, 1975), rheoscopy, micropipette aspiration, and filtration (Stoltz et al., 1999) methods. In viscometry, correlating the viscosity of the suspension with various shear rates leads to precise measurement of parameters involved in the deformability. The problem with this technique is the interference of the aggregation at low shear rate. In rheoscopic method, deformation of cells at various shear stresses is directly visualized through the microscope. The change in length and width of a stable oriented cell due to elongation, quantifies the deformability. Micropipette technique involves the application of a specific negative pressure to allow the red cell to be aspirated through a micropipette of a known diameter. Deformation by uniaxial elongation technique is based on the adhesiveness of an erythrocyte to the surface and its elongation in one direction. In osmotic hemolytic technique, the cells are putting in hypotonic solution, so that osmotic pressure is balanced by membrane elasticity and tension. The filtration technique is very advantageous over the
others in terms of its simplicity. Here the erythrocyte suspension in physiological saline is allowed to flow through the torturous path of the micro pore filter under gravitation. The flow rate quantifies the deformability of erythrocyte (Nagaprasad and Singh, 1998). The optical hemorheometer used in this work is based on this method.

The aggregation and deformability of erythrocytes alters due to many factors and diseases like diabetes. The importance of erythrocyte aggregation and deformability in human circulation is obvious and their role in micro-vascular pathology is significant in Diabetes mellitus (Stoltz et al, 1999). Alterations in these parameters will leads to cardio and cerebro vascular complications. Hence the analysis of these parameters is essential in Diabetes mellitus.

The shape of the normal erythrocyte is discoidal. The major advantage of the biconcave shape is the favorable surface to volume relation for cell deformation and for exchange of metabolic product across the membrane. The shape of erythrocyte will be altered in diseased blood and it is very important in microcirculation as it affects the aggregation and deformability of the erythrocyte. The aggregates size and passage of cells through capillaries depends on shape and size of the erythrocyte. Therefore it is important to analyze the shape parameters in normal and diseases.

Many studies with different experimental techniques have been reported on hemorheological properties in Diabetes mellitus as well as hyper cholesterol subjects, but the study of hemorheological parameters in subjects having diabetes mellitus and hyper-cholesterol combination have not been reported
widely. So the novelty of the present work is to study the alterations of hemorheological parameters that deviated from physiological limits in diabetes and how much it is deviated further with presence of hyper cholesterol in diabetes mellitus, to know the disease severity which leads major risk factor for the life of human being. Considering the importance of such studies in diagnostic medicine, the objectives of the present work are

- **Measurement of Aggregation parameters of erythrocytes in control (a) diabetes mellitus (Type II) with normal cholesterol (b) and diabetes with hyper cholesterol subjects (c).**
- **Measurement of deformability and shape parameters of erythrocytes in the above subjects**
- **Analysis of influence of hyper cholesterol on hemorheological (aggregation and deformability) and shape parameters of erythrocytes in hyperglycemic subjects**