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Alteration in Erythrocyte Deformability in Diabetes Mellitus

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Abstract

Diabetes mellitus (DM) is a metabolic disorder, characterized by varying or persistent hyperglycemia, which induces several changes in the erythrocyte membrane and its cytoplasm, leading to alteration in the deformability. Techniques applied to measure this are based on filtration of erythrocyte suspension through a membrane and to obtain diffraction pattern under sheared conditions. Ektacytometry requiring less quantity of blood with disposable flow chamber used to measure the deformability of erythrocytes obtained from patients with diabetes and also associated with nephropathy and retinopathy. A decreasing trend of deformability in these patients is observed. The shape parameter form factor, as determined by image processing procedure, increases with the increased of blood glucose levels and shows a pattern similar to filtration time of erythrocyte suspensions through cellulose membranes. Further work is suggested to detect micro-level changes in cell membrane in diabetic patients

Key words: Diabetes mellitus, erythrocyte deformability, filtration, ektacytometry, shape descriptors.

Introduction

Blood flow through cardiovacular system is complex, primarily attributed to the flow properties of blood and viscoelastic properties of blood vessels. The varying shear rates at different locations of the cardiovascular system affect the flow properties of blood. The deviation from the homogeneous flow in large vessels to heterogeneous flow in microvessels is primarily attributed to the physiological flow conditions and rearrangement of the cellular components, which is basically dominated by erythrocytes. The multiprofile flow in arterioels is reduced to single profile of erythrocytes in capillaries, the vessels which are primarily responsible for exchange of gases and metabolic products. Any alteration in the blood composition can affect the flow of erythrocytes in the microvessels.[1]

Diabetes mellitus (DM) is a metabolic disorder characterized by varying or persistent hyperglycemia (elevated blood glucose), attributed to the decreased production of insulin or improper utilization of glucose.[2] Diabetes is the most common cause of polyneuropathy, with approximately 50% of diabetics affected within 25 years of diagnosis[3] and is responsible for over 50% of the non-traumatic amputations,[4] nephropathy[5,6] and blindness in adults.[7] Infants born of diabetic women are at increased risk of fetal malformation, prematurity, spontaneous abortion, macrosomia, and metabolic derangements.[8,9] Diabetics have a higher incidence and prevalence of large vessel disease[10,11] and occurrence of non-enzymatic glucosylation of hemoglobin.[12] The patients with Type 1 or Type 2 diabetes are affected by this series of changes.[13,14] Hyperglycemia can also result from stress response after stroke even in the absence of diabetes.[10]

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The blood flow resistance in the cardiovascular system is primarily associated with the rheological properties of blood and blood vessels. In recent years, it has been shown that the aggregation of erythrocytes affects the distribution of plasma and erythrocytes through capillaries.[15,16] Aggregation of erythrocytes is associated with the formation of chain-like structure under low flow conditions which disaggregate with the increase of shear stress.[1] This is an important mechanism associated with the re-arrangement of erythrocytes under various flow conditions and even for this the erythrocytes have to deform to provide required contact area to form intra-cellular bridges for aggregation process.[17] Flow of erythrocytes in capillaries is essential for exchange of metabolic products, which is associated with the deformation of the erythrocytes to adapt to flow conditions, as shown by parachute or slipper shape of the erythrocytes.[18] The aggregation and deformability of erythrocytes are important for blood flow in cardiovascular system. Aggregation of erythrocytes involves several parameters related to erythrocytes and suspending medium, thus making it difficult to link the observed changes to specific parameters. In contrast to this the deformability as a measured parameter is related primarily to defromation of erythrocytes.[1]

The measurement of erythrocytes deformability and its correlation with the disease process is an emerging area in Hemorheology. Some of the earlier developments have been described in the reviews by Chien[19] and Mchedlishvili.[20] During the recent years significant developments, in terms of research studies and technological developments have taken place. Through these some of the micro-level changes associated with erythrocytes membrane and its interior, which could affect their deformability, are identified. The objective of this work is not only to review critically these developments but also to discuss some of the techniques along with their results, which could be applicable in the future development of this field.

Determinants of Erythrocyte deformability

The erythrocytes during flow in microcirculation and micro-channels (in-vitro) deform significantly from their resting biconcave shape.[1,18] This is an essential feature of blood flow through microvessels and primarily attributed to the red cell geometry, rheological properties of intracellular fluid and microviscosity of erythrocyte membrane.[19] The availability of the excess surface area compared to as required to hold the erythrocyte volume allows the cells to take various shapes during circulation. The excess surface area are strongly dependent on the variability of the composition of intracellular fluid and membrane bilayer and skeletal proteins. The enzymes located in the intracellular fluid and membrane, and availability of the ATP further contributes in the regulation of the deformability.[21]

Diabetes mellitus, being a metabolic disorder, affects the functioning of the erythrocytes through interaction with the membrane and intracellular constituents. Some changes are associated with the impairment of glucose utilization process, whereas, others are induced by the impairment of mechanisms due to disease process, thus affecting the erythrocytes properties.[22] Some of these changes which directly or indirectly affect the functional characteristics of erythrocytes are given below:

Oxidative stress

Diabetic erythrocytes have higher malondialdehyde (MDA) (an indicator of lipid peroxidation),[23-24] and decreased glutathione (GSH) and membrane -SH group[25] compared with normal erythrocytes.[25,26] Oxidative stress and increased insulin production contribute to endoplasmic reticulum stress, protein misfolding, and induction of the unfolded protein response, leading to pathological protein.[27,28] The oxidative stress may further induce erythrocytes shape changes as observed under in vitro conditions by incubation of erythrocytes with H_2O_2

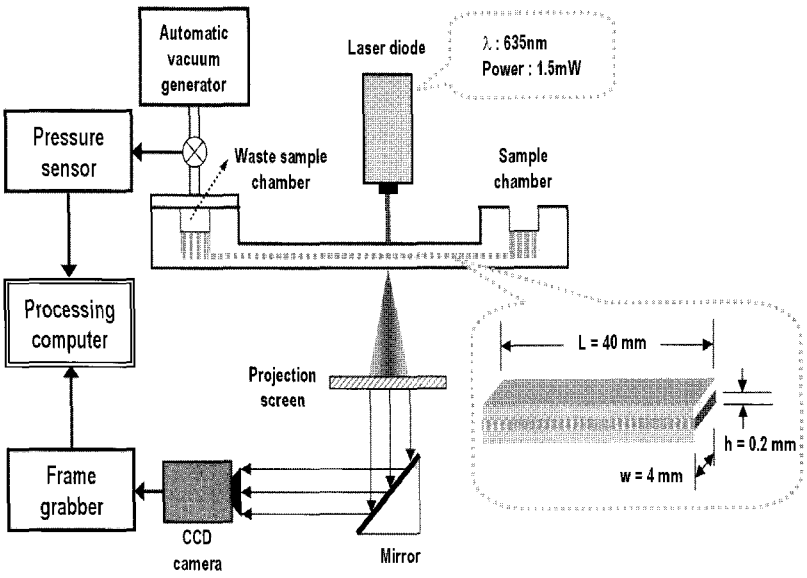

Figure 1. Schematic diagram of microfluidic ektacytometer

or ascorbate/Fe^{2+}, which transform discocytes to echinocytes due to involvement of membrane proteins.[27]

Lipids

The electronic spin resonance, using spin-labeled fatty acids, has shown structural changes at 0.6-0.8 nm from the membrane surface in the lipid bilayer of diabetic erythrocytes.[29] The membrane cholesterol is increased but there is four fold increases in phospholipids concentration in the membrane leading to a highly significant decrease in the ratio of cholesterol to phospholipids.[29-30]

Skeletal proteins

Spectrin and actin are the two main structural proteins that together form a sub-membraneous cytoskeletal meshwork that is responsible for the viscoelastic properties of the erythrocyte membrane.[31] The spectrin-actin network combined with protein 4.1, which provides erythrocyte membrane the ability to withstand the stresses of circulation, has its origins in various levels of structural organization.[31-33] The labeling of erythrocyte membranes with [3H]-borohydride, which labels glucose residues bound to proteins, revealed that several proteins are heavily glycosylated compared with nondiabetic membrane. In particular, the proteins beta-spectrin, ankyrin, and protein 4.2 are the most glycosylated and spectrin is oxidatively damaged.[34]

Enzymes and ionic balance

The alteration of activity of (Na^+/K^+) ATPase, which plays a central role in the regulation of intra- and extra-cellular homeostasis, is thought to be linked to several complications of diabetes mellitus.[35] In DM patients serum and intra-erythrocyte sodium and serum potassium levels are increased significantly in patients as compared to control subjects. The (Na^+/K^+)ATPase levels are significantly decreased which may cause disturbance of intracellular ionic balance and thus acceleration of cellular ageing.[36] Magnesium in the cell is largely associated with ATP, as the complex Mg-ATP. ATP is less stable when it is not complexed with magnesium, so the loss of magnesium makes the cell more susceptible to stress, leading to an increased uptake of Ca^{++}.[37-38] and diminished Ca^{2+}-ATPase activity in comparison to

healthy individuals. [39]

Measurement Techniques

The deformability of erythrocytes, depending on applied shear stress, is primarily responsible for their shape transformation.[19] Under constant shear rate conditions the magnitude of shape deformation is used as a parameter to measure erythrocyte deformability. Similarly the time taken by erythrocyte suspension under constant pressure to flow through a membrane is also used as a measure of erythrocyte deformability.[19] Based on this principle several techniques have been developed and used for measurement of erythrocyte defromablity. Functional principles of some of the prominent techniques are given below:

Filtration techniques

The filtration techniques are based on flow of erythrocyte suspension through a membrane of straight pores of diameter smaller than that of erythrocytes.[40] The measurements are carried out with suspensions of erythrocyte at different hematocrit under flow conditions. This procedure has recently been modified by measuring the flow of suspensions through cellulose membrane of curved slits of width around 20μm and length 400μm by a low pressure system. The measured filtration time of erythrocyte suspension through cellulose membrane is inversely proportional to the deformability of erythrocytes. [41-43] Filtration of erythrocyte suspension is a simple and comparatively economic procedure, especially by the initial filtration method, but requires erythrocyte suspension free of leucocytes and platelets, and filter membranes of uniform pore diameter.

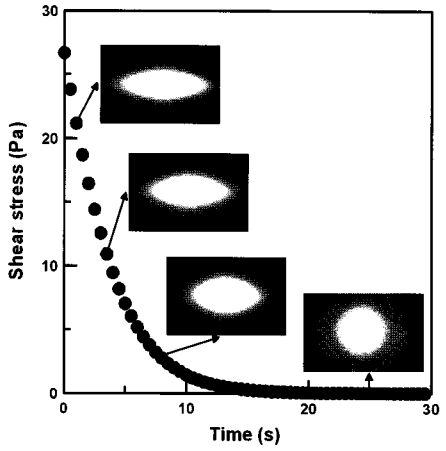

Figure 2. Variation of shear stress at various time intervals after the flow of erythrocyte suspension is initiated in the slit of ektacytometer. The diffraction pattern of erythrocytes corresponding to various shear stress are also given.

Ektacytometry

This technique is based on diffraction pattern of erythrocytes while flowing through a channel or being sheared between cone and plate geometry. During flow the erythrocytes are elongated and their diffraction pattern changes from circular to elliptic form. While maintaining the constant shear stress, the elongation index is directly related to the deformability of erythrocytes.[44]

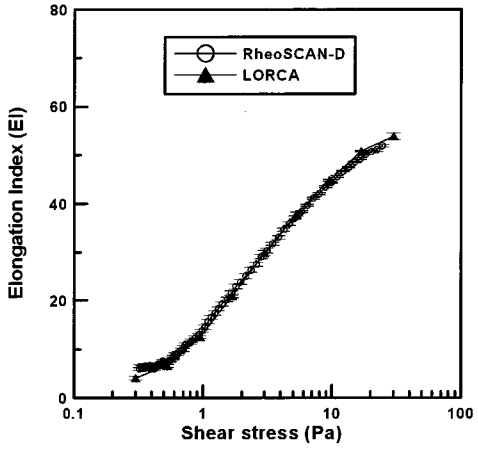

Figure 3. Comparison of elongation index at various shear stresses as measured by Rheoscan-D and LORCA ektacytometers

Based on this principle two instruments: Laser-assisted Optical Rotational Cell Analyser (LORCA: AMC, Amsterdam, The Netherlands)[45] and a Shear Stress Diffractometer (RHEODYN SSD, Myrenne, Germany) [46]are developed. From the diffraction pattern obtained during the shearing process the elongation index,a parameter related to deformability, is calculated.[47] These techniques are currently used in many laboratories but the sample size is large and involve frequent cleaning during deformability measurement of large number of samples.

Recently a new instrument, RheoScan-D (Sewon Meditech, Korea), functioning based on similar principle, is utilized for measurement of erythrocyte deformability (Fig. 1).[48] The shear induced deformation during flow of erythrocyte suspension (blood sample size 6.0μL) in a disposable rectangular channel under the application of negative pressure at its exit end, is measured. With the increase of shear stress at various time intervals the diffraction pattern changes from circular to an elliptical shape. This pattern is recorded by a camera and analyzed by a computer. Figure 2 shows an example of the flow of erythrocyte suspension in a channel and the corresponding diffraction pattern at different shear stresses. The elongation index is calculated by EI = (A-B)/(A+B), where A and B are semi-major and semi-minor axes of the elliptical erythrocyte.

By using the same blood sample, a comparative analysis of the elongation index of erythrocytes by the measurement with LORCA and Rheoscan at various shear stresses is carried out (Figure 3). No deviation is observed between the measured EI values by both the technique but some deviation above the range presently used for EI measurement is observed.

Morphological analysis of erythrocytes

During the disease process of diabetes the erythrocytes are subjected to morphological changes, which increase with the severity of the disease, as observed by the

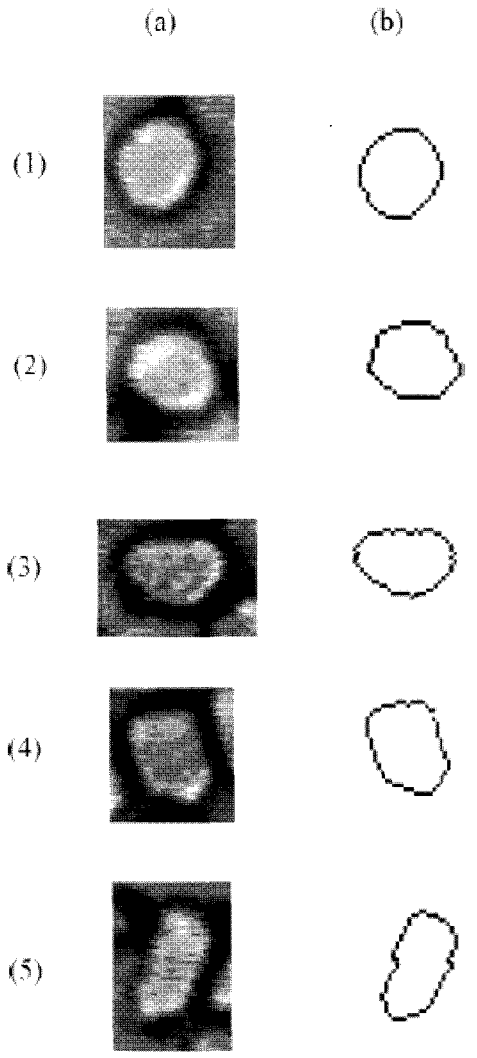

Figure. 4. The shapes (a) and contours (b) of erythrocytes of healthy subject (1) and diabetic patients at glucose concentrations158 mg/dl (2), 172 mg/dl (3), 210 mg/dl (4) and 304 mg/dl (5) ref.[49]

glucose level in the blood. Blood smears of erythrocytes of diabetic patients at different glucose levels are obtained and dried in the air. The images of the erythrocytes are obtained by video-microscopic system and after digitization these images are processed by edge enhancement, thresholding, filtering, and contour extraction procedures. Figure 4 shows the processed contours of erythrocytes obtained from patients with

blood glucose levels at various concentrations. By determining the number of pixels along the contour and after filling up the area with pixels the shape parameters perimeter (P) and area (A) are calculated, respectively. From these parameters the perimeter to area ratio and form factor ($P^2 / 4\pi A$) are obtained.[49]

Alterations in Erythrocyte Deformability

Diabetes mellitus, as mentioned above, produces a series of changes in the various constituents of the erythrocyte membrane and its interior. Each one of these constituents affects the functional characteristics of erythrocytes through impairment of its deformability.

Based on measurement of erythrocytes deformability by different methods several investigators have found that the deformability decreases with the increase of severity of the disease.[50-53] In diabetic patients, early impairment in red blood cell deformability appears in patients with normal renal function, and progressive impairment in red blood cell deformability is associated with renal function loss in all patients regardless of the presence or absence of diabetes.[51] The changes in the cytoskeleton decrease the deformability of erythrocytes.[34] The aging process of the erythrocytes may also affect the deformability as the erythrocyte deformation index is decreased with its aging process in a nonlinear fashion, with increasingly greater changes in the later part of the erythrocyte life span.[54] The ektacytometry procedure has been found to be sensitive to detect membrane bound specific changes in diabetes.[34]

The slit ektacytometry has been an effective technique not only to measure the deformability of erythrocytes but also to differentiate the influence of the severity of the disease. Figure 5 shows the variation in elongation index in diabetes and its associated complications. By filtration procedure the changes in erythrocyte deformability at various glucose levels were also measured. With the increase of glucose level in blood the filtration time of erythrocytes is reduced.[49] A comparison between filtration time and form factor (FF) is shown in table 1. The FF also shows an increasing pattern similar to that of filtration time. Significant to highly significant changes in erythrocyte perimeter, area and form factor have been observed with the progression of the disease which are directly related to the decrease in deformability as measured by filtration technique.[49]

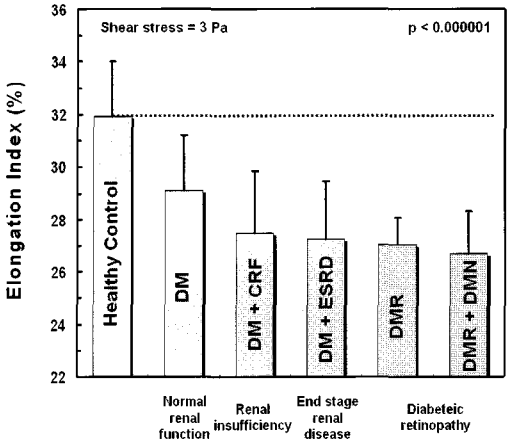

Figure. 5. Comparison of erythrocyte elongation index of healthy controls and diabetes (DM), and diabetes associated with chronic renal failure (DM + CRF), end stage renal disease (DM+ESRD), retinopathy (DMR) and retinopathy and nephropathy (DMR+DMN). The elongation index of erythrocytes in various diseases show significant variation compared to that of normal erythrocytes.

Table 1. Comparison between filtration time and form factor at various glucose levels (based on data in ref [48]).

S.No.	Glucose (mg%)	Filtration time (s)	Form factor
1	< 120	1.56 ± 0.15	0.98 ± 0.07
2	120 – 160	1.65 ± 0.12	1.13 ± 0.14
3	161 – 200	2.06 ± 0.15	1.16 ± 0.19
4	201 - 240	2.49 ± 0.29	1.21 ± 0.15
5	> 241	3.47 ± 0.39	1.31 ± 0.21

Conclusion and Future Perspectives

The role of erythrocytes in the microcirculation is very important in the transport of metabolic products. This require close to normal morphological and deformable characteristics of erythrocytes. But as discussed above the DM induces changes by impairing various mechanisms associated with its membrane and interior. The measurement of erythrocyte deformability is a good indicator for the assessment of these changes. Although through deformability, it is not possible to pinpoint exactly the mechanism in the erythrocyte which could have been impaired, still an overall assessment of the clinical status of blood flow is obtained. Erythrocyte deformability, if supported by biochemical parameters, then the extent of changes could directly be related to compositional changes in erythrocytes and the pharmacological intervention could be implemented.

During the last decade the technology has advanced significantly. The present emphasis is on in vivo assessment of diabetic conditions. In this regard the essential requirements are to check the glucose level and its effect on erythrocyte deformability. This article reviewed various procedures to measure erythrocyte deformability but out of these the present technique, which require minimum quantity of blood and disposable measuring chamber may be a good choice. The image processing procedure for detection of area and perimeter of cells not only provide information about shape changes in erythrocytes due to enhanced level of glucose in blood but also indirectly show the change in erythrocyte deformability. Thus by this procedure both parameters could be quantified. But this requires large computerized data base which could provide clinical information by interlinking various parameters.

There are several direct and indirect mechanisms associated with membrane for maintenance of normal deformability. Some of these mechanisms are impaired during diabetes. For identification of these further technological developments are required, which could be beneficial in pharmaceutical interventions.

Acknowledgement

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