Influence of diabetes on aggregation, deformability and shape parameters of erythrocyte

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1. Introduction

Diabetes mellitus is a clinical term denoting a group of metabolic disorders which impair glucose utilization leading to hyperglycemia. During the plasma proteins, mainly fibrinogen, is enhanced and negative surface charge of the membrane is reduced. The degree of glycation of membrane proteins modifies the hemorheological characteristics of erythrocytes A significant difference in the packing of lipids in the external leaflet compared to that of inner leaflet of the bilayer is produced. Since modification in phospholipids asymmetry influences the adhesion of erythrocytes to endothelial cells, this may also be involved in red cells aggregation by altering the deformation ability in the formation of rouleaux, particularly through a modification in membrane lipid fluidity.

These alterations induce morphological changes in the erythrocytes, leading to formation of discocyte. These changes in shape prove to be deleterious, leading to short life span of these erythrocytes in the circulatory system. In patients with these conditions i.e., hyperglycemia combined with hypercholesterolemia the erythrocyte deformability is significantly decreased. As the deleterious effect of glucose is concentration dependent, the objective of the present work is to determine the influence of increasing glucose levels in diabetic patients on aggregation and deformability and shape parameters of erythrocytes.

2. Materials and Methods

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2.1 Techniques

a. He - Ne laser light aggregometer

This technique is based on the attenuation of laser light after passing through the erythrocyte suspension of hematocrit 5% placed in a glass chamber. Depending on the size of the formed aggregates and erythrocytes during sedimentation, the transmitted intensity (TI) shows respective variations in terms of fluctuations superposed on mean intensity. By sequential recording of the TI signal the aggregation process in terms of various parameters is analyzed. Further details of this are given elsewhere[1].

b. Optical deformability meter

This instrument is based on change in intensity of light transmitted during the flow of erythrocyte suspension through cellulose membrane. The mean passage time through the membrane is inversely proportional to the deformability of erythrocytes. Further details of this technique are given elsewhere[1].

c. Erythrocyte shape analysis

Blood smears of erythrocytes of normal subjects and diabetic patients with different glucose levels were obtained and dried in the air. The images of the erythrocytes were obtained by video-microscopic system (Leitz Dilux 22, Germany) at a magnification of 40× and recorded on a cassette by a VHS video-cassette recorder. After digitization these images were processed by edge enhancement, thresholding, filtering and contour extraction and further processed to obtain the perimeter (P) and area (A) of erythrocyte. Based on these the perimeter to area ratio (P/A) and form factor ($P^2/4\pi A$) were calculated.

2.2 Sample preparation

Fresh blood samples, prior to intake of food, were collected in the morning from healthy subjects (n = 10) and diabetes patients The suspensions of 5% hematocrit were prepared in the plasma for aggregation and in physiological saline containing 0.5 g% albumin for deformability measurement.

2.3 Results and Discussion

Figure 1 shows the distinct variation in transmitted intensity (TI) during formation and sedimentation of aggregates in diabetes compared to that of normal samples. Based on analysis of the above variation in the TI, various parameters related to aggregation process were obtained which are shown below Table 1.

A significant variation in shape parameters in patients with varying levels of glucose compared to that of healthy subjects is also observed.

3. Conclusion

In conclusion the above mentioned parameters as observed in blood samples collected from diabetes patients may affect the blood flow and thus lead to the complications in cardiovascular system.

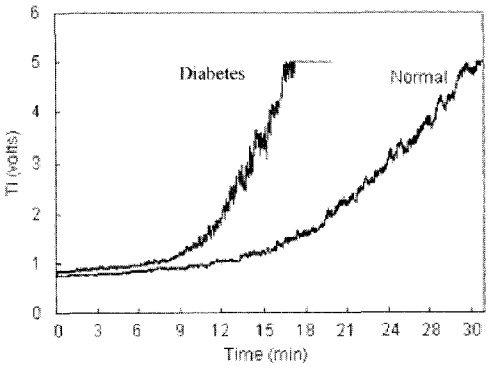

Fig. 1 The distinct variation in transmitted intensity (TI) during formation and sedimentation of aggregates

Reference

(1) M. Singh and M. Kumaravel, Clin. Hemorheol.15 (1995), 273 - 290.

Table 1

Aggregation parameters, process initiation time and process completion time, and passage time of erythrocytes of normal and diabetes patients of various groups

	Normal (<120 mg%)	Group 1 (120–160 mg%)	Group 2 (161–200 mg%)	Group 3 (201–240 mg%)	Group 4 (>241 mg%)
PIT (min)	$1.2 \pm 0.33^{\#}$	1.17 ± 0.28	1.2 ± 0.25	1.10 ± 0.20	$0.94 \pm 0.19^{*}$
PCT (min)	29.7 ± 2.56	$26.31 \pm 2.14^{*}$	$21.45 \pm 1.21^{**}$	$18.27 \pm 1.01^{**}$	$14.0 \pm 1.33^{**}$
Passage time (sec)	1.56 ± 0.15	$1.65 \pm 0.12^{*}$	$2.06 \pm 0.15^{**}$	$2.44 \pm 0.29^{**}$	$3.47 \pm 0.39^{**}$

$^{\#}$Mean $\pm$ SD, $^{*}p < 0.02$, $^{**}p < 0.0001$.