Influence of cholesterol on shape parameters of erythrocytes in hyperglycemic subjects

Kolesterolün hiperglisemik olgularda eritrositlerin şekil parametreleri üzerine etkisi

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Abstract

Objective: We determined the morphological changes of erythrocytes in blood samples of diabetic patients with varying levels of hyperglycemia with normo- and hyper-cholesterol concentrations and compared them with cells of healthy subjects.
Methods: The shape analysis was carried out by shape descriptors based on projected area, perimeter and form factor, as measured by the processing of erythrocyte images. Blood smears were collected from normal subjects and from glycemic subjects with normo- and hyper-cholesterol levels. After image processing techniques like edge enhancement, thresholding, filtering, contour extraction, and pattern analysis and recognition, the images were used for shape analysis.
Results: The shape parameters, which quantified the changes in erythrocytes in diabetic subjects with normal cholesterol level, showed significant deviation from the shape of normal cells. Cells of diabetic subjects with hyper-cholesterol level showed more deviation than cells with normal cholesterol.
Conclusion: These changes lead to hyper aggregation and to a decrease in deformability of erythrocytes and hence increase microcirculatory complications. *(Turk J Hematol 2009; 26: 77-81)*
Key words: Hyperglycemia, hypercholesterolemia, morphology

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Özet

Amaç: Değişik seviyelerde hiperglisemiye sahip normo- ve hiper-kolesterollü diyabetik hastalardan elde edilmiş kan örneklerinde eritrositlerin morfolojik değişikliklerini belirledik ve sonuçları sağlıklı bireylerin hücreleriyle karşılaştırdık.
Metodlar: Şekil analizi, eritrosit görüntülerinin işlenmesiyle ölçülen, ve tahmini alan, çevre ve form faktörünü baz alan şekil belirleyicileri ile yapıldı. Normal bireylerden ve normo- ve hiper-kolesterol seviyelerine sahip glisemik bireylerden kan yaymaları yapıldı. Görüntüler kenar güçlendirme, eşikleme, filtreleme, sınır çıkarma, yapı analizi ve tanıma gibi görüntü işleme tekniklerinden sonra şekil analizinde kullanıldı.
Bulgular: Normal kolesterol seviyesine sahip diyabetik bireylerin eritrositlerindeki değişiklikleri belirleyen şekil parametreleri, normal hücrelerin şeklinden anlamlı bir sapma gösterdi. Hiperkolesterol seviyesine sahip diyabetik hastaların hücreleri normal kolesterollü hücrelerden daha fazla sapma gösterdi.
Sonuç: Bu değişiklikler, agregasyonun artmasına ve eritrositlerin şekil değiştirebilme özelliğinde bir düşüşe yol açar ve böylece mikro dolaşım ile ilgili komplikasyonlar artar. *(Turk J Hematol 2009; 26: 77-81)*
Anahtar kelimeler: Hiperglisemi, hiperkolesterolemi, morfoloji

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Introduction

Hemorheologic properties of erythrocytes are altered by many factors, such as aggregation, deformability, viscosity and shape changes. The shape of the erythrocytes can be altered due to many diseases-diabetes mellitus (DM), malaria, and hypercholesterolemia, etc. DM is a metabolic disorder characterized by hyperglycemia (high blood sugar) and other signs, as distinct from a single disease or condition. Hypercholesterolemia is high-level cholesterol in the blood that can cause plaque to form blockages in arteries. Both hyperglycemia [1] and cholesterolemia [2,3] can alter the shape of the erythrocytes, which may lead to increase in aggregation and decrease in deformability. The hypercholesterolemia process induces changes in the erythrocyte membrane, primarily attributed to accumulation of cholesterol in the membrane [3,4]. These observations show that the gradual change in erythrocyte shape, as observed microscopically, may reflect the effect of cholesterol in the arterial vessels. Any deviation from the discoidal shape alters the deformability of these cells [4].

These alterations induce morphological changes in the erythrocytes, leading to formation of flat cells [5] or discocyte [6]. Hence, variation in morphometric parameters such as surface area and perimeter may show the extent of changes in the flow properties of these cells. Digital analysis of morphometric characteristics of erythrocytes show that such an analysis could be valuable in diagnosing coronary artery disease [7], determining the effect of cholesterol in capillary flow [8], monitoring erythrocyte aggregation [9], and detecting changes in erythrocytes due to blood storage [10].

The objective of the present work was to determine the influence of cholesterol in DM on shape changes such as surface area, perimeter and form factor of erythrocytes.

The shape of normal cells was compared with those of patients with diabetes with normal cholesterol (Group 1) and with hypercholesterol (Group 2) levels. To determine the influence of cholesterol in DM, the blood samples were collected such that samples had the same glucose range (210-260 mg/dl) in both groups. Samples of normal cholesterol (below 230 mg/dl) with the above glucose range and hyper cholesterol (above 280 mg/dl) in DM were collected. Area (A) and perimeter (P) was calculated in terms of pixels. For calculation of A, the contour of the cell was filled with pixels. By counting their number, A in terms of pixels was determined. Similarly, by counting the number of pixels, the P of the same cell was determined. Based on these, the form factor (FF) = P*P /4*pi*A was calculated. This parameter is the measure of compactness or roundness of the cell and its variation indicates the deviation of the shape in the image from that of a disc (FF for disc =1).

Materials and Methods

2.1 Erythrocyte shape analysis

Healthy subjects selected for the study had no clinical diseases and their plasma and serum bio-chemical levels were within normal range. Their systolic and diastolic blood pressures were within normal range and hematocrit within 36 to 45%. The ages of healthy and diabetic subjects ranged from 30-60 years and the duration of the disease for the diabetic subjects was more than five years. DM had no other associated diseases including micro- and macro- complications. The statistical analysis of the data was carried out by Student t-test.

2.1.1 Image acquisition

Figure 1 shows schematic of the smear imaging system. Blood smears of erythrocytes of normal subjects (n=15) and of diabetic patients with normal cholesterol (n=15) and hyper-cholesterol (n=15) levels were obtained and air-dried. These were viewed by Leitz Dialux 22 (Germany) transmission video microscopic system. A well-collimated beam of light from 12 V 100W tungsten halogen lamp was used for viewing purposes. The erythrocyte images were obtained by video microscopic system at a magnification of 40. These images were recorded on videotape by a VHS video tape recorder (National NV-370, Japan). The recorded data of the selected cells was digitized and stored in the computer for further analysis.

2.1.2. Edge enhancement

An edge is a boundary between two regions with relatively distinct gray level properties. The edges of the cell image show a sudden transition in the gray level intensity. These were enhanced by amplifying the gray level difference at these points and smoothing the areas of constant gray levels. The idea underlying the edge detection technique is the computation of a local derivative operator. To achieve the edge enhancement, the Sobel operator was used. The pixel intensity at any point is replaced by the computed value of the intensity gradient around the neighborhood of the pixel under consideration.

2.1.3Thresholding

The pixel gray levels of the image were compared with a selected threshold value and were classified based on whether the pixel value was greater than or less than the threshold value. If the pixel value is greater than the threshold value, a value of 1 is assigned to that pixel or 0 value otherwise. A binary image of the erythrocytes was obtained by this procedure.

2.1.4 Filtering

The binary image obtained by above procedure had an extended boundary, with a noisy pattern of isolated intensity over the edges. In order to avoid the patches, the image was filtered by a median filter.

2.1.5 Contour extraction

This was achieved by successive deletion of the outermost layer of the image until a connected unit pixel width framework or skeleton remained.

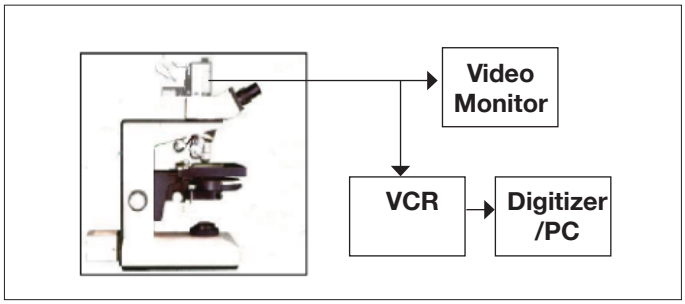

Figure 1. Schematic of smear imaging system

2.1.6. Pattern analysis and recognition

These images were used for shape analysis. The parameters computed for the present analysis were: perimeter (P), area (A), perimeter to area ratio (P/A) and form factor (FF). From the measured value of the pixels along the contour and number of pixels within the contour, P and A of the erythrocyte contour were determined. Based on these, P/A and FF (P*P/4 π A) were calculated. Figure 2 is a flow chart illustrating computation of the cell shape parameters.

2.2 Shape parameters

The thinned images of the erythrocytes obtained by the above procedures were used for erythrocyte shape analysis. The parameters computed for feature extraction in the present study were P, A, P/A, and FF. These are computed by the following procedures.

2.2.1 Perimeter (P)

The perimeter of the erythrocyte was obtained from the thinned image by counting the number of pixels on the contour.

2.2.2 Area (A)

The contour of the erythrocyte was then filled with pixels and the number of pixels in the filled region was counted. The area was also measured in terms of pixels.

2.2.3 Perimeter to area ratio

The perimeter to area ratio = P/A.

2.2.4 Form factor (FF)

FF = P ² /4 n A

This ratio is a measure of the compactness or rudeness. Disc has the minimum FF of value equal to one. The FF of a normal erythrocyte is found to be 1. This confirms the accuracy of the method, as the normal erythrocytes are disc-shaped. The other conformity test is that the radii computed by the P and A are comparable.

Results

The shapes of the erythrocytes as obtained by phase contrast microscope are shown in Figure 3. The normal shape of erythrocytes is affected by glucose and cholesterol levels and due to this process these cells acquire different shapes. The change was greater in glycemic samples with hyper-cholesterol (Group 2) than in glycemic samples with normo-cholesterol (Group 1) level. The processed contours also showed various membrane changes induced by the hyperglycemic process compared to normal cells. The shape descriptors of these cells, A, P, A/P and FF, as calculated from the contours of these cells, are given in Table 1. A significant increase in P shows that the membrane is irregular, which is also associated with the decrease in its A. The changes in shape parameters of glycemic samples with normo- compared with hyper-cholesterol level were significant. The FF showed a significant increase in glycemic subjects with hyper-cholesterol (Group 2) compared to normo-cholesterol levels (Group 1), indicating more deviation in the erythrocyte shape in diabetic subjects with hyper-cholesterol than those with normo-cholesterol compared to normal erythrocytes.

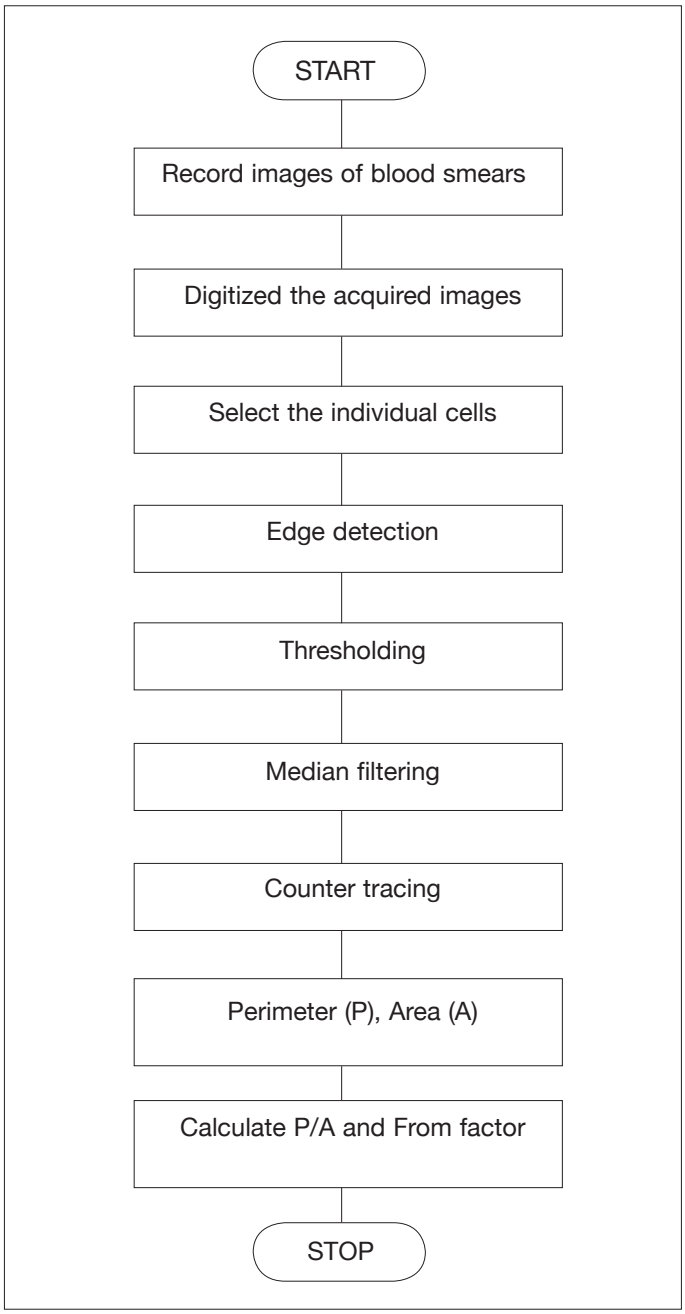

Figure 2. Flow chart for computation of cell shape parameters

Table 1. Morphological parameters of erythrocytes: perimeter (P), area (A), perimeter/area ratio (P/A) and form factor (FF) of normal cells and cells of DM patients with normo- (Group 1) and hyper-cholesterol (Group 2) levels

Erythrocyte (n=15)	Perimeter (P) (pixels)	Area (A) (pixels)	P/A	Form factor (FF)
Normal	135±14#	1472±132	0.09±0.01	0.98±0.07
Group 1	139±14*	1360±136*	0.10±0.01**	1.13±0.14***
Group 2	155±18*	1289±155*	0.12±0.09**	1.43±0.33**

#Mean±SD, *p<0.1, **p<0.05, ***p<0.025

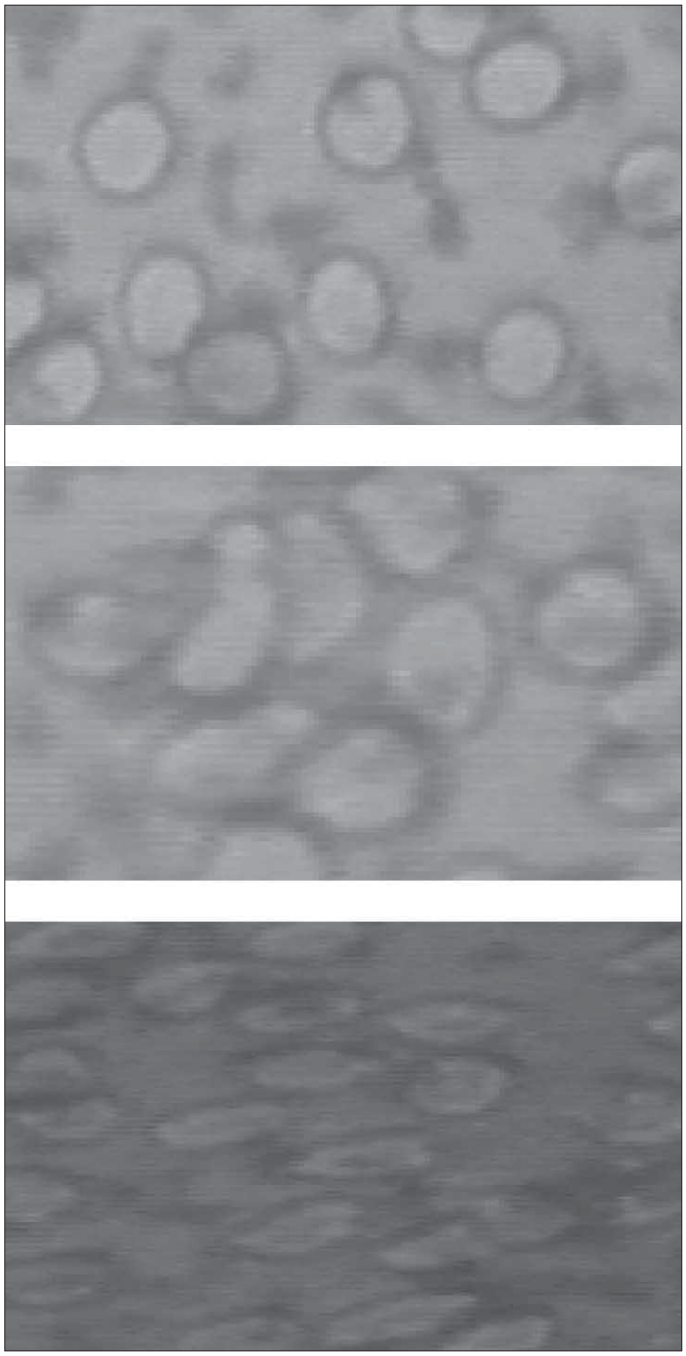

Figure 3. Images of erythrocytes as obtained by phase contrast microscope. Normal (a), DM with normo-cholesterol (b), and DM with hyper-cholesterol (c) level

Table 1 shows the shape parameters of normal cells and cells from diabetic subjects with normo- and hyper-cholesterol level. P/A and FF of the cells in diabetic subjects were large compared to normal cells. Due to the changes in the shape parameters, the shape of the erythrocytes deviated from its normal discoidal shape.

Discussion

Analysis of shape parameters of erythrocytes is very important in clinical application to determine disease severity. Erythrocytes in clinical conditions are associated with altered morphology, leading to abnormal rheological behavior, as observed in several hematological disorders [3,4,6,10-12]. Shape transformation affects the aggregation of erythrocytes directly either through deformability or altered shape. For a similar shape alteration in erythrocytes in the cardiovascular system, this process may affect their distribution in the micro vessels [11].

Shape parameters were quantified in this present work in DM subjects with normo- and hyper-cholesterol level and compared with the shape of normal cells, which are circular in shape. Any deviation from the circular shape is determined by the FF, whereas variation in P indicates irregularity in the contour of the erythrocytes [1,10]. The mechanism by which mature red blood cells change their shape under physiological and pathological conditions has been a subject of considerable interest [13]. The hypercholesterolemia process induces changes in the erythrocyte membrane [3].

The present results show that increase in glucose concentration and in glucose with cholesterol concentration produces an increase in P and decrease in A, indicating the development of membrane irregularity. This is further confirmed by the significant increase in P/A and the FF, which leads to decrease in deformability [14]. The aggregation of erythrocytes is also enhanced due to change in shape, which leads to microcirculation complications [15]. The deviation in erythrocyte shape and increase in filtration time may be partly responsible for the short lifespan of diabetic cells [16]. Such cells due to their altered properties may not be able to carry out tank-tread motion while flowing through microvessels [12].

In conclusion, the shape of the erythrocytes significantly changes with blood glucose levels compared to normal cells. The alteration is more significant in subjects having both increased glucose and cholesterol.

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