Evaluation of the role of erythrocyte deformation on erythrocytes aggregation and sedimentation rate using He-Ne laser scattering

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Abstract

Background: The erythrocyte aggregation is an important physiological phenomenon in the circulation of blood. It is a basic characteristic of normal blood that plays a major role in the cardiovascular system, especialy in the microcirculation.

Objective: To evaluate the role of deformability of red blood cells on the aggregation and sedimentation of red blood cells.

Subjects & Method: The present study was carried out on thirty two healthy subjects. Laser scattering method was employed for this study. From scattered light intensity, profiles continuously obtained during aggregation and sedimentation of the aggregated erythrocytes. Different values of erythrocyte deformability were determined and evaluate their effects on each phase of the erythrocyte aggregation and sedimentation, rouleaux formation, one-dimensional aggregate and three- dimensional aggregate formation.

Results: Deformability values are expressed in term of rigidity index, the difference between medium and high rigidity index significantly decreased the rate of aggregation and the rate of three dimensional aggregate sedimentation.

Conclusion: Variation of the values of erythrocyte deformability from low to medium and from medium to high showed different effects on aggregation and sedimentation stages.

Keywords: Erythrocyte aggregation, sedimentation rate, deformability, laser light.

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Introduction

Erythrocytes aggregation and disaggregation are natural phenomena in the circulation of blood [(1)]. Aggregation of red blood cells is the formation of reversible structure containing a number of particles, while erythrocytes sedimentation monitors the tendency of red blood cells to form aggregates in plasma [(2)].

The formation of clumps of red blood cells under low or non-flow conditions, result from the attraction forces between the red blood cells. The cells adhere to each other in rouleaux aggregates. Slight mechanical force, such that occurs in the circulation, is enough to disperse these aggregates.

The process of aggregation affected by many physical and chemical factors. Chemical factors are concerned with modifications of either erythrocytes or suspending medium such as hematocrite, PH of the suspending medium, macromolecules and flow conditions [(3,4)].

Some investigations have pointed out the importance of cellular modifications to the erythrocyte aggregation, especially in relation to erythrocyte deformability, and erythrocyte filterability, (Ability of erythrocytes to change shape as they pass through narrow spaces, such as the microvasculare) [(5)].

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Figure 1: Erythrocyte deformability when passing through microcirculation

In large blood vessels, the resistance to blood flow depends to minor degree on blood viscosity but mostly upon the diameter of the vessels. This is due to the laminar blood flow and the deformability of erythrocytes under the high shear rate which reduce the viscosity of blood makes it ineffective. While the resistance to blood flow in the capillaries depends mainly on viscosity of the blood and erythrocyte aggregation under the low shear rate which increase blood viscosity [(6)].

The true capillaries are about 5 µm in diameter at the arterial end and 9 µm at the venous end, and since the red blood cells are flat disks of about 7 µm in diameter, thus when the sphincters are dilated, the diameter of the capillaries is just sufficient to permit erythrocyte to squeeze through in a "single file". Erythrocyte is a "bag" that can be deformed into almost any shape. This is because the normal cell has a great excess of cell membrane for the quantity of material inside, and due to the pliability of the cell membrane. Deformation does not stretch the membrane greatly & consequently does not rupture the cells as would be the case with old erythrocytes [(6,7,8)].

Materials and methods

The present study depend on a modified method of E. Muralidharan, in Biorheology, 1994,[(9)].Which work on the same principle of laser light scattering.

Five ml of fresh blood samples were drawn from the cubital vein of 32 healthy human subjects using heparin (0.03/5ml of blood), as anticoagulant, into a sterilized tube. 1 ml of the blood was used to measure the ESR (erythrocyte sedimentation rate) by Westergren method. Any record above 15 mm/hr for males and above 20 mm/hr for females) was excluded from the study (According to Bottlger, 1967) [(10)].

Four ml of the rest blood sample was put in centrifuge to separate erythrocytes from plasma and WBC coat

(3000 rpm for 10 minutes at 4°C). After removal of plasma & WBC coat, the erythrocytes were washed 3 times with iced cold normal saline (0.9% NaCl) and then re-centrifuge the sample again (3000 rpm for 10 minutes at 4 °C) to remove any particles that may be attached to the erythrocyte membrane.

Physiological measurements:

The measurement of erythrocyte deformability:

It was carried out by measuring the filtration time of the following two solutions:

The first solution 2 ml consisted of 5% of packed erythrocytes suspension prepared by mixing 100 µl of packed erythrocytes with 1900 µl of suspending medium.

(The packed erythrocytes were already washed three times with ice cold 0.9% NaCl as mentioned above).

The second solution (2 ml) was of cell free suspending medium.

The suspending medium was prepared from the following chemicals: (150 mM KCl, 0.5 mM Na_3EDTA, 10 mM Tris-HCl)

The pH of the solution was 7.4 at room temperature.

The filtration was through Whatman filter paper (number 1).

The deformability values were expressed in term of rigidity index (RI):

$$RI = \frac{\text{Filtration time of 2 ml 5\% packed erythrocytes suspension}}{\text{Filtration time of 2 ml of cell free suspending medium}}$$

This method was described by Al-Gailani and Al- Remadani (1998) [11].

The blood sample preparation:

To prepare the sample, 800 µl of dextran (mol. Wt.500.000) was added to phosphate buffered saline (P.B.S.) solution (50mM Sodium Phosphate, 3mM KCl, 90mM NaCl, 0.1g/dl D-glucose, PH 7.4), then 100 µl of bovine serum albumin of 0.5g/dl concentration was added to the tube, to prevent adhesion between RBCs and the wall of chamber, followed by addition of 100 µl of packed erythrocytes to get, after mixing, sample of 10% PCV. The experiments were carried out at room temperature.

The system of the measurement is shown in Figure 2; it is compose of a linear polarized He- Ne laser source, of wave length 632.nm, (Griffin Co.), and generation power 1mW. A beam of 1mm diameter was passed through erythrocyte suspension in a chamber of 50×10×1 mm; made of a microscopic glass plates, figure 3. Blood column hight was kept at 40mm. The forward scattered light intensity through the sample column was detected with a photocell (photodiode ampliphier) [9].

The photocell placed in front of the laser beam and allowed the beam to pass directly through the crystal of the cell. The signals from the photocell are passed through a light flexible cable to an amplifier (Grass 7P1F) for signal amplification. The sample chamber was mounted firmly on the holder so that the laser beam passed, exactly, through the center of the chamber.

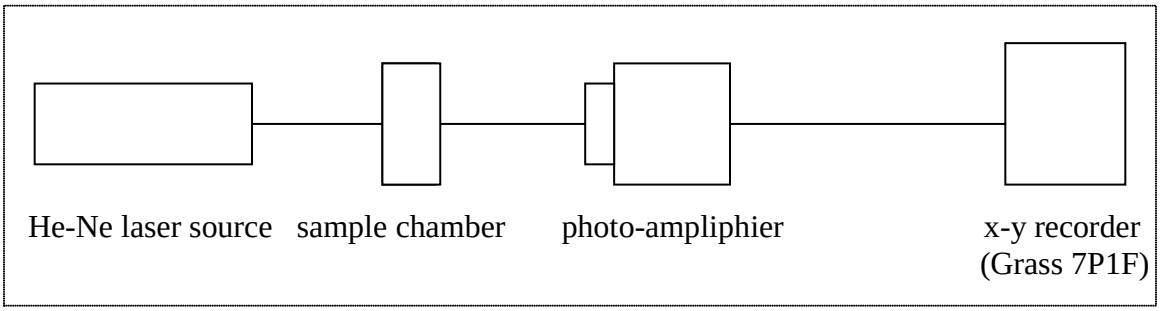

Figure.2: The system layout

The blood sample was gently introduced into the chamber by using a syring with long needle. Immediately after the sample was introduced, the forward- light signal was continuously recorded by the system.

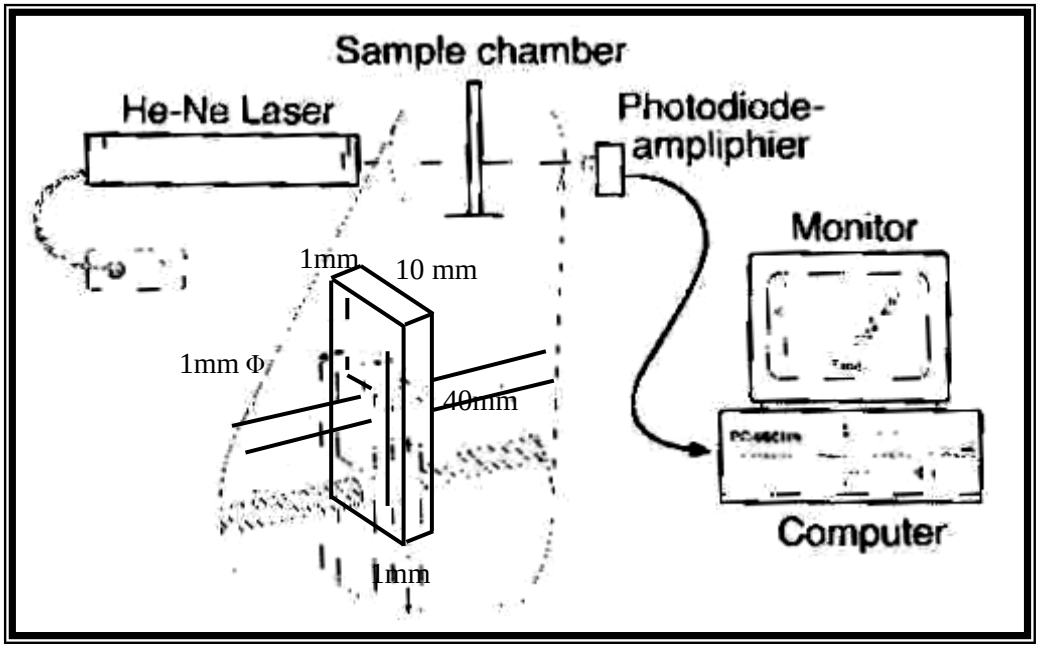

Figure. 3: Schematic diagram of an arrangement of light scattering experimental system (Muralidharan, 1994)

Results

Figure 4 shows the pattern of rouleaux formation, one-dimensional aggregate and three- dimensional aggregate formation curve, with sample of 10%PCV as it recorded by laser assessed aggregometry used in this study.

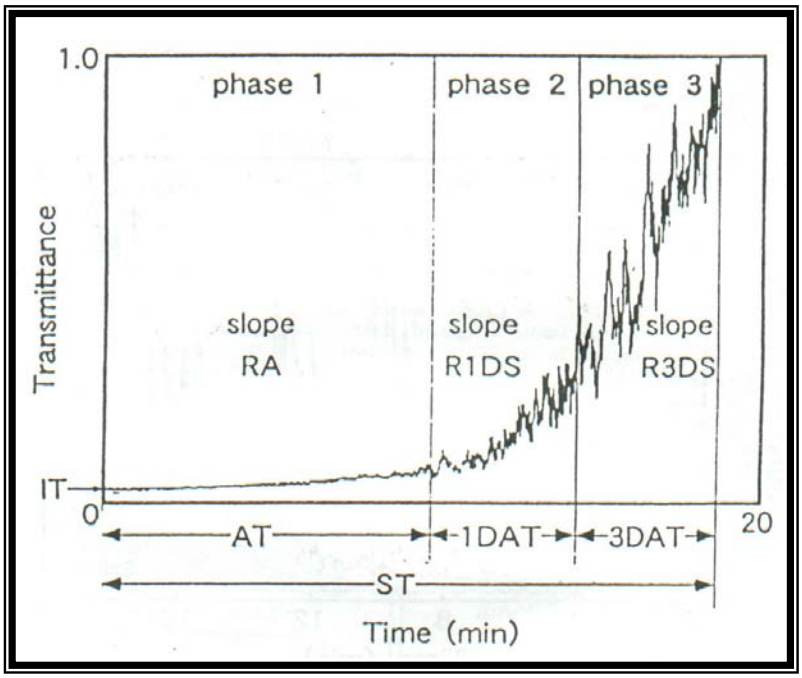

Figure 4: Pattern of different stages of aggregation and sedimentation as recorded by laser scattering techniques.

There was a slight increase in the signal due to the reorientation of single erythrocytes when the erythrocytes were monodispersed in the beginning of the aggregation process. The sedimentation of the aggregates formed was indicated by the appearance of fluctuations in the signal. These fluctuations were smaller in the beginning and became larger towards the end. The time at which the first sharp fluctuation appeared in the signal was termed AT (aggregation time). These fluctuations continued until the signal reached the maximum without any variation. The time at which the signal reached the maximum was termed ST (sedimentation time). The initial phase was due to the movement of single erythrocytes in the process of forming small aggregates. The rate of aggregation (RA) was obtained from the slope of this phase. The second phase was due to the sedimentation of small and one-dimensional aggregates. The duration of this phase was termed 1DAT (one-dimensional aggregation time).

The slope of this phase provided the rate of sedimentation of one-dimensional aggregates (R1DS). The third phase was due to the sedimentation of large and three- dimensional aggregates. The duration of this phase was termed 3DAT (three-dimensional aggregation time).

The rate of sedimentation of the three-dimensional aggregates (R3DS) was obtained from the slope of this phase. The light intensity fluctuation showed a clear visible in the signal between these phases.

Table 1: The values of ESR and deformability in this study

	ESR (mm / hr)	**Deformability (RI)***
Mean	**3.11**	**1.59**
SD (±)	**2.96**	**0.23**
Max.	**12**	**2**
Min	**1**	**1.25**

* Deformability expressed in term of rigidity index

The deformability of erythrocytes in this study was expressed in term of rigidity index, their values was ranged from 1.25 to 2 with a mean of 1.59±0.23.

The rigidity index results had been classified into 3 groups: low (1.25-1.49), medium (1.5-1.74) and high (1.75-2.0) as shown in table 2.

Table 2: Effect of low, medium and high erythrocyte deformability on aggregation and sedimentation parameters

		Low n=12	Medium n=11	High n=9
Time	AT	13.82±2.71	13.51±3.33	12.99±2.78
	1DAT	6.27±3.38	6.23±3.9	5.31±1.49*
	3DAT	2.03±1.06	2.76±1.3	2.47±0.76
	ST	23.02±4.55	22.49±6.86	20.77±3.27*
Rate	RA	0.037±0.008	0.039±0.007	0.045±0.015*
	R1DS	1.39±0.59	1.71±0.7	1.76±0.41
	R3DS	9.87±3.95	9.86±3.96	9.73±2.01*

* Significant difference between medium and high groups of erythrocyte deformability.

Table 2 shows a significant decrease ($P<0.01$) in the sedimentation time ST, one dimensional aggregation time 1DAT and in the rate of sedimentation of the three dimensional aggregates R3DS at high rigidity indexes of erythrocytes.

On the other hand there was a significant increase ($P<0.05$) in the rate of

aggregation RT at high rigidity indexes of erythrocytes.

Discussion

Some investigators have pointed out the importance of cellular modifications to the erythrocyte aggregation especially in relation to erythrocyte deformability. The contributions of the cellular alterations to the erythrocyte aggregation are smaller in magnitude, but the influence is significant [(5)].

The major determinants of the ability of deformation (deformability) of erythrocyte are enhanced by: [(12)]

a. Extracellular viscosity.
b. Membrane stiffness.
c. Cell geometry (surface area /volume ratio).

In the present study the deformability expressed in term of rigidity index (membrane stiffness) of erythrocyte.

When we compared the deformability results (RI) in this study with the aggregation stages and sedimentation, there was a significant decrease in the time needed for one dimensional aggregate formation (1DAT) and for sedimentation and significant increase in the rate of aggregation at high (RI) of erythrocyte, this is due to the presence of the macromolecules, which play the main role in the first stage of the aggregation process. The macromolecules act as a bridge and permit the RBCs to slide on each other to form rouleaux in the suspending medium [(13, 14)].

On the other hand, there was a significant decrease in the rate of the third stage (3DAS) at high (RI) of erythrocyte, this is due to decreased the deformability of erythrocytes, because the third stage of aggregation process depend on the shape and the deformability of erythrocytes and decreased the erythrocytes deformability made the size of the aggregate formed by erythrocytes of high (RI) are small, and the erythrocytes in this stage (three dimensional aggregates) are loosely packed [(15,16)].

This study showed different values of deformability have causes different effects on the three stages of aggregation and in turn on sedimentation.

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