Simultaneous influences of hematocrit in the erythrocyte medium on erythrocyte aggregation and sedimentation: a kinetic study by a laser scattering technique

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Date of acceptance 28 / 2/ 2010

Abstract

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, especially in the microcirculation. This study explained the kinetics of single cells rouleaux formation one- dimensional aggregate and three-dimensional aggregate, during simultaneous, and the effect of hematocrit on the process of aggregation and sedimentation. The present study was done on forty one healthy subjects. Laser light is passed through a well mixed sample of blood and the forward scattered light intensities recorded continuously. The samples were prepared with different hematocrit, (10%, 15%, 20%, and 25%). Increasing the hematocrit, (10%, 15%, 20%, and 25%) had significantly decreased the rate of rouleaux formation ($P< 0.005$) but increase in the rate of one and three dimensional aggregate formation. On the other hand the sedimentation rate is decreased significantly ($P<0.05$) with the increase in the PCV value. It was shown that changing the hematocrit have different effects on aggregation process and sedimentation.

Key word: RBC aggregation , ESR , rate Laser

Introduction:

Aggregation of red blood cells is the formation of reversible structures containing a number of these cells. While erythrocyte sedimentation rate monitors the tendency of unstirred red blood cells (RBCs) to form aggregate in plasma[1].The aggregation capacity of human red blood cells lies between that of the non-aggregated and the remarkably full sedimentation. The mechanism of the aggregation is an important parameter for understanding the rhulogical properties of blood [2].

Erythrocyte aggregation is normally reversible, whereas the formation of excessive, large and irreversible aggregates or clumps is the characteristic of diseased erythrocytes [3]. The rate and degree of erythrocyte aggregation depend on the physico-chemical properties of the suspending medium and erythrocytes and flow conditions[4][5].Changing the number of erythrocytes (changing the hematocrit) affect the shear stress of the suspending medium and its deformability and in turn these two factors affect mainly erythrocyte aggregation and its sedimentation that lead to variation in the scattered light intensity and so the rate and the time of each phase of the aggregation and sedimentation will be changed respectively[6].

Materials and methods:

The present study depends on a method that was modified from that of E. Muralidharan, in Biorheology, 1994, [7] and works on the same principles that is using

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laser light scattering. Fresh blood samples were obtained from the cubita vein of (41) healthy human subjects with heparin (0.03/5ml of blood). Samples were centrifuged at 3000 rpm for 10 min at room temperature. Plasma was separated from the red blood cells and divided into two parts. Control samples of erythrocytes were obtained by washing three times with isotonic phosphate buffered saline solution (50mM Sodium Phosphate, 3mM KCl, 90mM NaCl, 0.1g/dl D-glucose, PH 7.4).

The samples were prepared with PCV value of 10%, 15%, 20%, and 25% for RBCs volume of 100, 150, 200, and 250 µl suspended in plasma of 900, 850, 800, and 750 µl. The system of the measurement is shown in Fig.(1). A laser beam of a linear polarized He- Ne laser source of wave length (632.nm), generation power (1mW) and beam diameter (1mm) (Griffin Co.) was passed through erythrocyte suspension in a chamber (50×10×1)mm made of a microscopic glass plates. Blood column hieght was kept at 40mm. The forward scattered light intensity through the sample column was detected with a photocell (photodiode ampliphier).

The photocell was placed in front of the laser beam and it allowed the beam to pass directly through the crystal of the cell. The signals from the photocell passes 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.

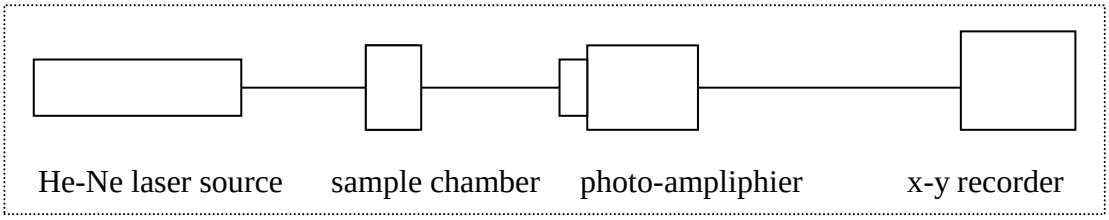

Fig. (1): The system layout

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.

Results:

Fig.(2) shows the pattern of rouleaux formation, one-dimensional aggregate and three- dimensional aggregate formation curve, of sample with 10%PCV suspended in plasma as it is recorded by laser assessted aggregometry used in this study.

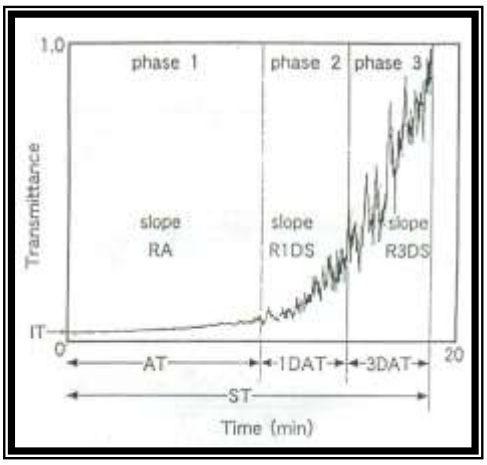

Fig. (2): Pattern of different stages of aggregation and sedimentation as recorded by laser scattering techniques.

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 is indicated by the appearance of fluctuations in the signal. These fluctuations are smaller in the beginning and become larger towards the end. The time at which the first sharp fluctuation appeares in the signal is termed AT (aggregation time). These fluctuations continue until the signal reaches the maximum without any variation. The time at which the signal reached the maximum is 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.

The study showed that the time needed for rouleaux formation is significantly increased ($P < 0.001$) with the increase in the PCV value.While the time of one-dimensional aggregate formation is significantly decreased ($P < 0.005$) with the increase in the PCV value (Fig.3) .

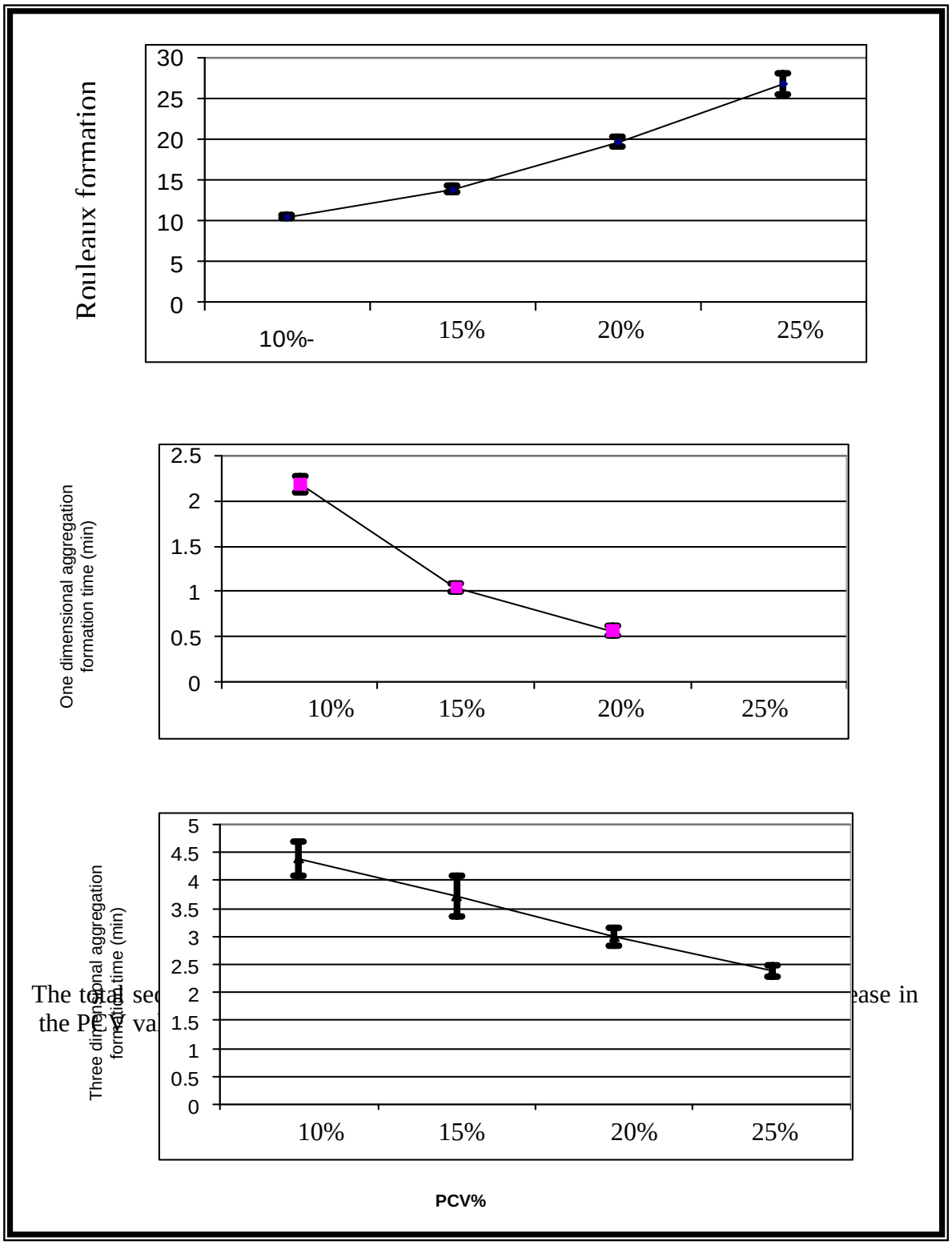

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Fig. (3): **Effect of PCV on erythrocyte aggregation stages time**

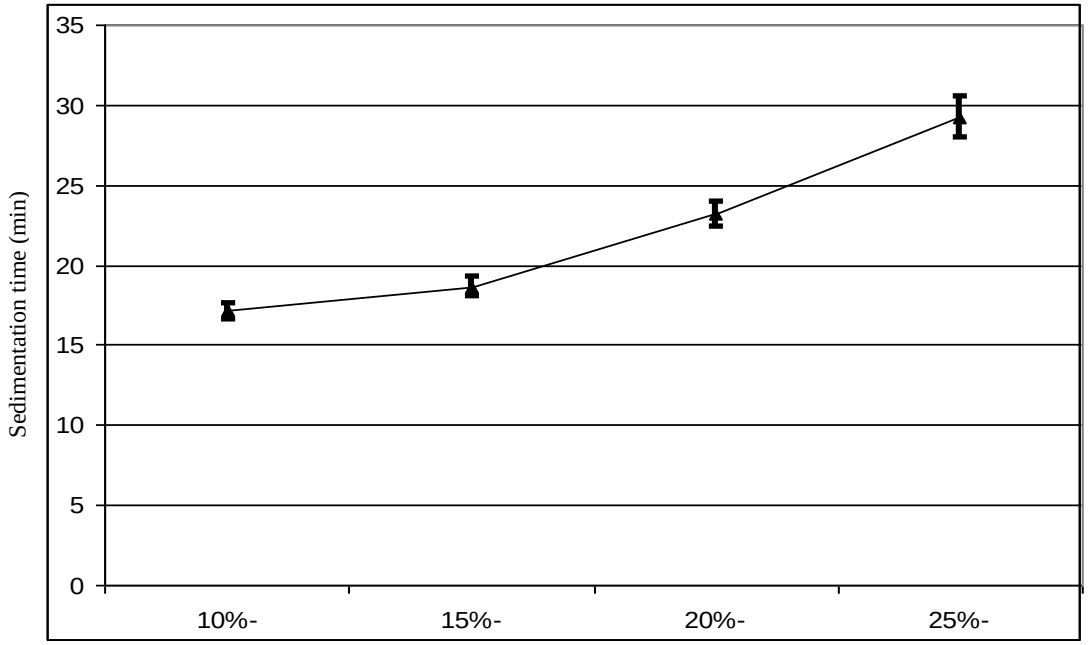

Fig. (4): Effect of PCV on erythrocyte sedimentation

Discussion

Erythrocyte aggregation has been widely studied and its importance is
well established in the reheological of microcirculation [5][6][8][9]. The mechanism of the aggregation and sedimentation is influenced by the alteration of the erythrocyte properties and the suspending medium, changing the deformability of the red blood cells and the distance between them as well as the viscosity of the suspending medium due to changing the hematocrit value. From this study we found that the time needed for rouleaux formation is significantly increased with increase in the hematocrit. This result is due to decreasing the degree of deformation and orientation which is suppressed as the hematocrit is increased and exceeds the macromolecules in the suspending medium of RBCs[10]. An adequate amount of macromolecules needed to link the erythrocytes together[11], so that increase of the hematocrit on the expense of macromolecules will not increase rouleaux formation.

Overcrowding of RBCs causes increase in the radius (a) of the aggregate of one and three dimensional that leads to increasing the transmission (decreasing the time) Fig.(3) and in turn means increasing the sedimentation of one and three dimensional aggregate formation and that is according to the following equation:

$$\nu = \frac{2a^2}{9\eta} g(\rho - \rho_o)$$

This equation is the determinant of sedimentation velocity (rate) include the radius of the particle (a), an acceleration due to gravity (g), density of the particle and the fluid (ρ and ρ_o) respectively as well as the viscosity of suspending medium (η).The present study showed that, in contrast to the aggregation time, the erythrocyte sedimentation time, decreases as the hematocrit of them is increased Fig. (4). This indicated an inverse relationship between the cell sedimentation rate and hematocrit[12].

The possible explanation of this inverse relationship is that increasing

the hematocrit lead to increase in the blood viscosity [13] which is influenced by sensitive to the PCV and according to the above equation the sedimentation rate decreased with increase in the viscosity. In addition, decreasing the sedimentation rate because the aggregated cells are packed at the bottom of the tube [14].

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ألتأثير الاني لتركيز كريات الدم الحمراء في معدل تجمع وترسب كريات الدم الحمراء باستخدام أشعة الليزر

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الخلاصة:

تجمع كريات الدم الحمراء ظاهرة فسيولوجية مهمة في الدورة الدموية .هذه الظاهرة تمثل الخصائص الاساسية للدم الطبيعي والتي تلعب دور مهم في الجهاز الوعائي القلبي, وخاصة في الاوعية الدموية الشعرية .

أجريت هذه الدراسة على 41 شخص طبيعي, تفسر هذه الدراسة تأثير زيادة تركيز كريات الدم الحمراء على مراحل التجمع, تكون اللفة والتجمع بمتجه واحد وبثلاث متجهات, وترسب كذيات الدم الحمراء.

طريقة اشعة الليزر النافذة استخدمت لهذه الدراسة. ويتم حساب شدة أشعة الليزگ النافذة بشكل مستمر خلال عملية التجمع والترسب.

زQاثې تركيز كريات الدم Чلحمراء (10%, 15%, 20%, 25%) يقلل بشكل xلحوظ وذا قيمة احصائية مردل تكون اللفة ولكن يزيد معدل تكون التجمع بمتجه واحد وبثلاث متجهات.

من ناحية اخرى زيادة تركيز كريات الدم الحمراء يسبب تقليل ملحوظ بترسيب كريات الدم الحمراء.

بينت الدراسة ان زيادة تركيز كريات الدم الحمراء له تأثيرات مختلفة على مراحل التجمع و الترسب لهذه الكريات.