The Effect Of Laser Radiation And Viscosity Of Red Blood Cells On Erythrocytes Sedimentation Rate (ESR)

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(Received 22 / 6 / 2009 , Accepted 25 / 10 / 2009)

1:Abstract:

The purpose of this study is to investigate the *in vitro* effect of the hematocrit (packed cell volume) (HCT) , the viscosity of red blood cells and the He-Ne laser radiation on Erythrocyte Sedimentation Rate (ESR) . He-Ne laser wave length of 632.8ηm was used for irradiation with 5mm diameter beam spot on blood samples, with power density of about 30mw/cm^2 .The irradiation times were 10,20,30,40,50,60mint; so the doses of irradiation were 18, 36, 54, 72, 90, 108 J/cm^2 .The samples of blood were obtained from 18 volunteers ,and each sample was divided into two samples for irradiation and control . The ESR was measured after laser irradiation and was compared with un-irradiation control .the results of this study show the increase of ESR is related inversely with (HCT), the sedimentation rate decreases with the increasing of viscosity and the laser radiation reduce the erythrocyte sedimentation rate (ESR) of blood samples.

2: Key words: ESR, Red blood cells, viscosity of blood, rouleaux formation, westergreen method, laser effect

3:Introduction:

The erythrocyte sedimentation rate (ESR- some times called sed rate or sedimentation rate) is a type of blood test. It is a nonspecific screening test for various inflammatory diseases. it is a simple and inexpensive laboratory test for assessing inflammatory or acute response [1]. ESR is a measurement of the rate with which the red blood cells (RBC,s) settle in normal saline or plasma over a specified period [2]. There are many methods for determining the ESR: Westergen method, Wintrobes method ,Landu method[3]. Currently the Westergen method is recommended by the international committee for standardization in hematology [4].

The test measures the rate of gravitational setting in one hour of anticoagulant red blood cells (RBCs) from a fixed point in a calibrated tube of defined length and diameter held in an upright position [6,7] .Red cells carry a negative surface charge that impedes red cell aggregation. In the presence of a symmetric high molecular weight proteins especially those with a positive charge, the tendency for red cells to repel each other is reduced and red cell aggregation is promoted [4,7] **.**Red aggregate cells sediment more rapidly in the presence of inflammation; There is a cytokine mediated increase in proteins in the plasma as a part of the acute phase response and consequently an increase in the ESR [4] .The ESR is a type of blood test commonly used to screen for presence of inflammation. It is referred to as the erythrocyte sedimentation rate because it measures the rate at which red blood cells (erythrocytes) separate from the liquid part of blood (Plasma) and fall to the bottom of a test tube forming sedimentation because the ESR is directly related to certain blood proteins and those blood proteins are associated with various diseases,. An ESR test result is frequently abnormal (positive) long before other biochemical or physical signs indicate the presence of disease. The Erythrocyte Sedimentation (ES) occurs in three phases. First the cells tend to aggregate and form rouleaux formation and only fall slightly Fig. (1).

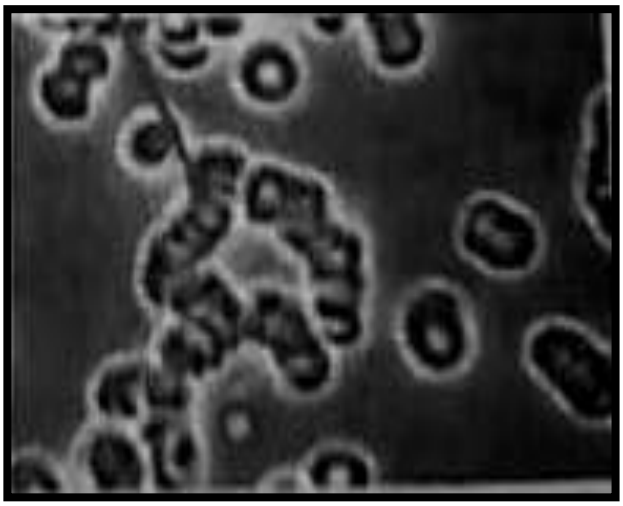


Fig 1: rouleaux formation [8]

In the second phase, of the fall is increased and as the cells pack this speed is decreased .During the third phase,[9,10]. Rouleaux formation is controlled by the concentration of fibrinogen and the amount of globulins present [11] .The steps of rouleaux formation seem to occur in the three stages ; first stage : contact by collision ;, second stage: Increase the contact area by RBC sliding on each other, and third stage Face –to-face adhesion [12].Blood is a non-Newtonian fluid, in which viscosity is independent of time and shear rate; It is a multiphase system such that viscosity depends on the viscosity of each of the individual phases. the viscosity of blood depends on the viscosity of plasma and concentration of red cells, aggregation of red blood cells, and also on the fluidity and deformability of blood cells. The viscosity of the red cells depends on both viscosity of the interior and the viscoelasity of the membrane while the plasma consist of a single phase [13] .

The ESR is affected by two major factors; biological factors and non biological factors. Biological factors are include the erythrocyte factor, the plasma ,the viscosity factor, the age factor and the race factor, and none biological factors include technical factors, physical factors such as the effect of laser radiation and some of the mechanical factors.

Laser radiation light cover a wide range of wavelengths, from about 150 to 1200 ηm , which includes the visible

range. Many type of lasers (gas, liquid, and solid) are available, they all operate on the principle from which they get their name ,e.g.ruby ,He-Ne , argon ,and dye laser. He-Ne laser is the first gas laser that was constructed on 1961. it is basic element is a glass tube filled with low-pressure mixture of two gases, helium and neon. When laser light strikes a tissue surface, it can be reflected, scattered, absorbed or transmitted [14].

4:Methods:

4-1: ESR measurements with different hematocrit:

Ten ml of fresh venous blood from the antecubital vein of healthy human adult was mixed with EDTA(Ethylene Damien Tetra Acetic Acid) in a tube (1.3mg/ml of blood) as anticoagulant. The anticoagulant blood sample was immediately centrifuged for 10min at 2000 r/min and at room temperature of 22-27˚C. The plasma, the Buffy coat and the upper most layers of packed red blood cells were discarded. The resulted packed RBCs were washed three times by resuspending them with suspending solution (normal saline). Recentrifugation and re-aspiration of the supernatant and the upper most layer of RBCs. The washed packed RBCs were diluted with normal saline at a rate 40% (4 parts of RBCs to 6 parts of NaCl silution). The westergren pipes were filled at (0) mark, allow to stand in vertical position. The erythrocyte sedimentation was recorded every 20 min for 120 min. At the end of one hour, the distance from the meniscus to the top of the column of erythrocyte is recorded as the ESR values. The mentioned procedures were done again with the different red blood cells concentrations(Hct)(30%, 20% and 10%) (3, 2, and 1 parts of RBCs to (7,8 and 9 parts of normal saline .

4-2: Viscosity measurements:

The washed packed RBCs were diluted with normal saline at 40%, 30%, 20% and 10%),(4,3, 2, and 1 parts of RBCs parts of red blood cells to 6, 7,8 and 9 parts respectively of normal saline for viscometer measurements. A U-shape capillary viscometer with a capillary diameter of 0.9mm, 17cm length and 2ml size were used for the measurement of RBC.s viscosity ; the U-shape tube viscometer consist of two limbs the left hand limb is essentially a pipit with two defining marks X and Y. The RBC suspension or distilled water was drawn up by suction into the bulb in the left hand limb and the time taken by the RBC suspension or distilled water to flow between the marks X and Y is accurately measured by a stop watch . the viscometer is thoroughly cleaned by distilled water after each measurement. All the measurements were carried out at a temperature of 37^0C . The relative and actual viscosity were calculated by using the equations .

The actual viscosity= [(flow time of the suspension(sec) / flow time of the distilled water(sec)] x [the viscosity of the distilled water (which is 0.6915Cp)] .

4-3: ESR measurement with laser radiation:

The washed packed RBCs were diluted with normal saline at 40 % (4 parts of red blood cells to 6 parts of normal saline),and each sample diluted blood was divided into two samples.The first sample was kept without irradiation and served as control, and the second sample was irradiated by laser radiation of 18 J/cm2. The ESR was carried out as described before. The ESR ratio with laser radiation and red blood cells $\mathbf{ESR_{(L)}}$ can be calculated by the following below equation:

$\mathbf{ESR_{(L)}=[ESR_{\text{with laser radiation}} / ESR_{\text{without laser radiation (control)}}]}$

The mentioned techniques were done again with the laser radiation doses at (36, 54, 72, 90 and 180 J/cm2

5: Results and discussion:

Statistical analysis: The. Statistical analyses were performed using students (t –est), taking $p< 0.05$ as the lowest limit of significance.

5-1: ESR measurements with different hematocrit:

The present study shows that the ESR is decreased with the increasing of the red blood cells concentration as in figures (2,3,4,5,6,7) and table (1). The results which indicate that ESR is related inversely with hematocrit (HCT), The possible explanation of this relationship is that the decreasing of concentration of red blood cells means the decreasing of heamatocrit (HCT) value which simply means decrease of the number of red blood cells per unit volume of the suspension , these reasons lead to decrease of viscosity of the red blood cells ,and according to the Einstein – Stokes equation $\mathbf{R^2=9\nu\eta / 2(\sigma 2-\sigma 1)}$, the velocity of the sedimentation decreased with the increased of viscosity , these leads to the decrease of the ESR ,Fig (8,9) and table (2) below.

Table 1: The relation between the sedimentation time and the different HCT values.

Hematocrit / ESR (mm/hr) / Time(hr)	50%	40%	%30	20%	10%
0.5	6	9	14	19	22
1	14	18	22	30	37
1.5	21	26	34	40	53
2	27	34	41	50	69
P	<0.05	<0.05	<0.01	<0.05	<0.01

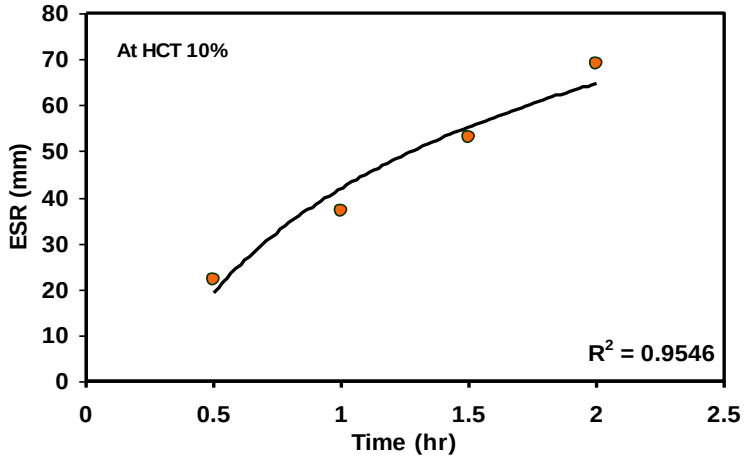


Fig 2: the relation between the ESR (mm) and time (hr) at HCT 10%

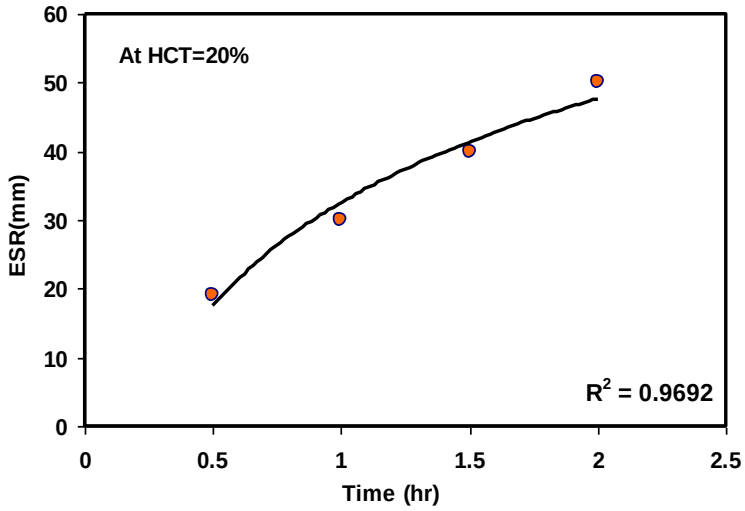


Fig 3: the relation between the ESR(mm)and time (hr) at HCT 20%

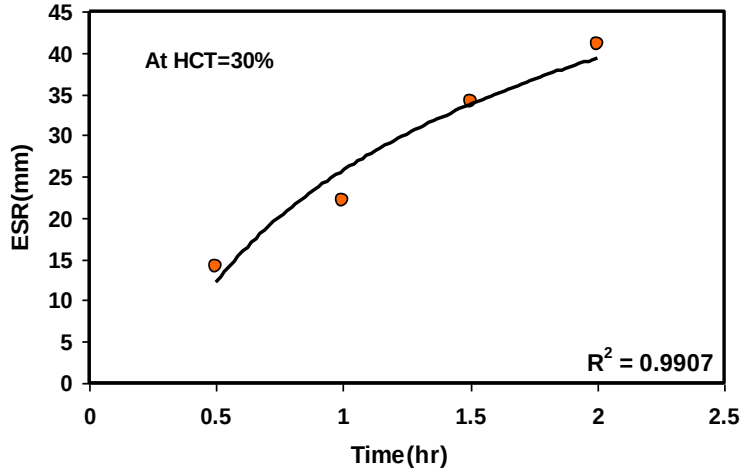


Fig 4: the relation between the ESR(mm)and time (hr) at HCT 30%

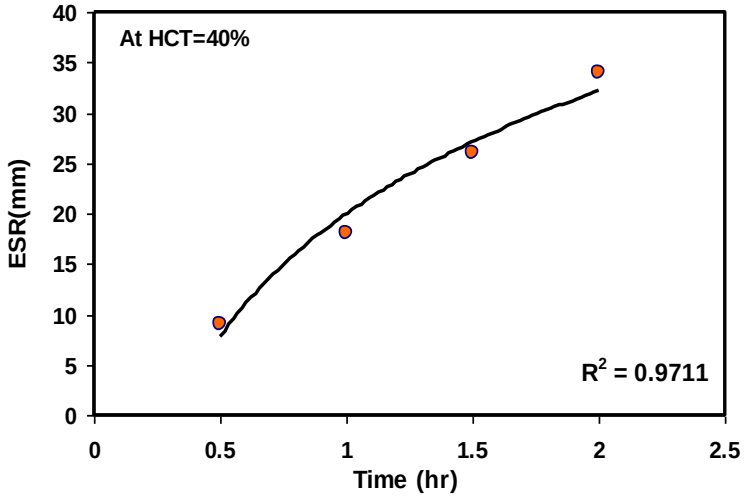


Fig 5: the relation between the ESR(mm)and time (hr) at HCT 40%

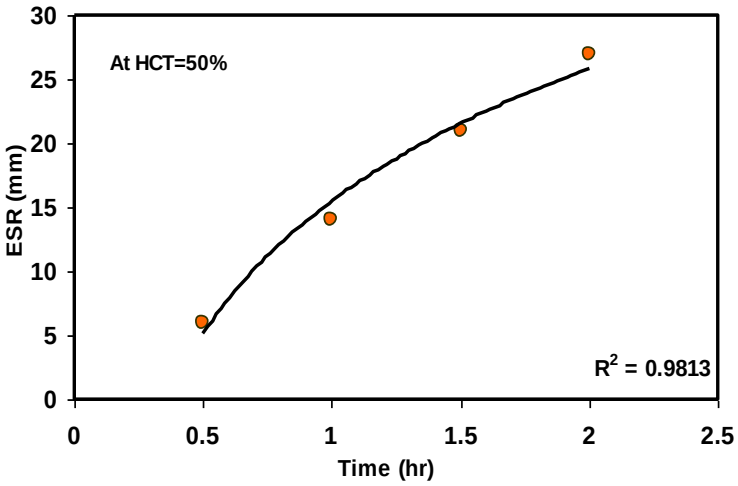


Fig 6: the relation between the ESR (mm) and time (hr) at HCT 50%

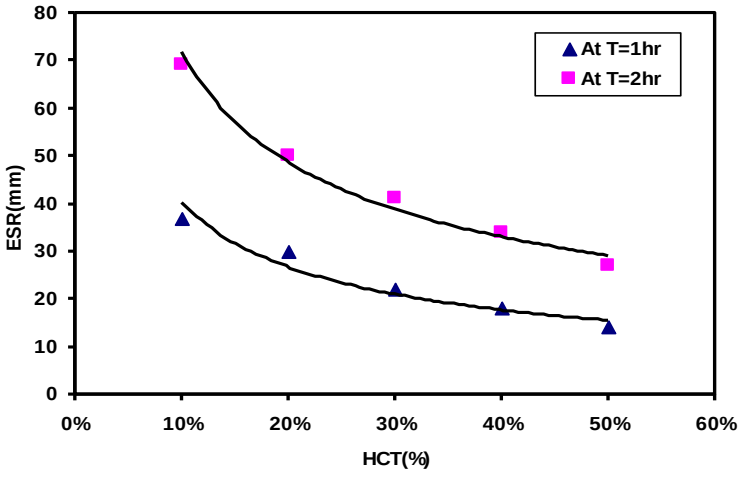


Fig 7 : ESR (mm) and different HCT(%)

Table 2 the relation between viscosity and HCT

Hematocrit	00%	10%	20%	30%	40%	50%
Viscosity	0.69	0.931	1.25	1.697	2.29	3.092
P<0.05						

Table 3 the relation between viscosity and ESR (mm)

ESR(mm/hr)	37	30	22	18	14
Viscosity	0.93	2.25	1.69	2.29	3.092
P<0. 05					

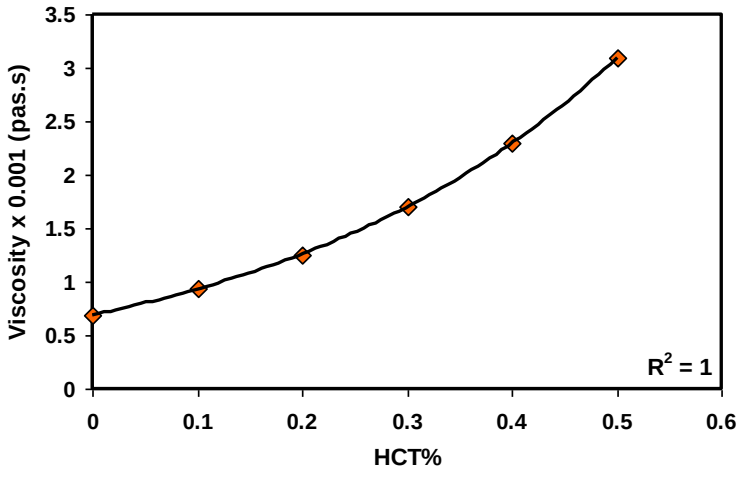


Fig 8: The relation between viscosity and HCT(%)

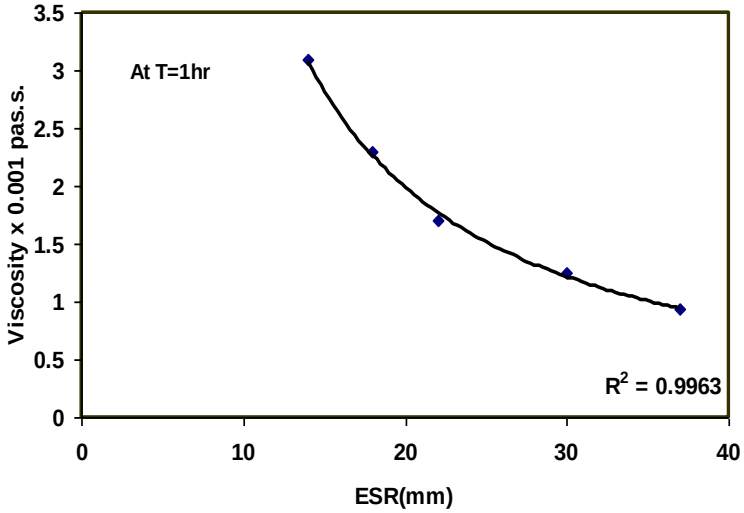


Fig 9: the relation between viscosity and ESR (mm)

The radius of sedimenting sphere (R) can be calculated from the Einstein – Stokes equation $\mathbf{R^2=9\nu\eta / 2(\sigma 2-\sigma 1)}$,where η is the plasma viscosity , σ1 is the plasma density , and σ2 is the red blood cell density ,and ν is the measured rate of sedimentation. In Fig(10) below the radius of the sedimenting sphere (which is directly proportional to the plasma protein concentration , the radius is increased by the either decreasing the HCT or plasma protein concentration .

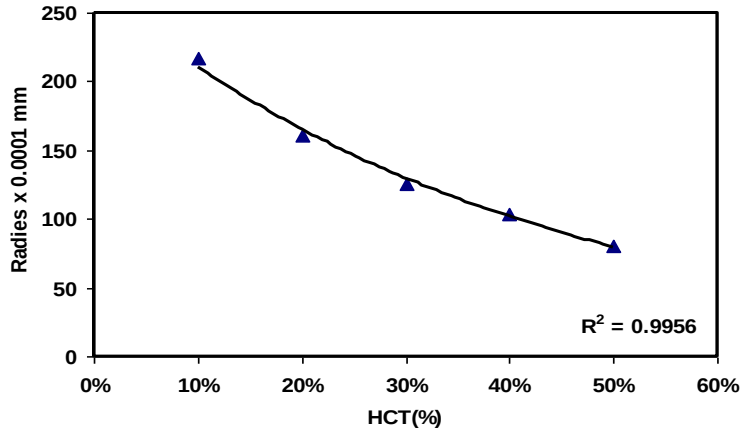


Fig 10: the relation between radius and HCT%

thus , a change in HCT masks the effect of changing plasma protein concentration and composition.

From the fig.10 and the relationship between the HCT and the ESR can be found the relation shape between the ESR and the radius . in fig (11) and table (5) show ESR increase with the increasing in radius of sedimenting sphere

Table 4: the relation between radius and HCT%

Hematocrit(HCT)	10%	20%	30%	40%	50%
Radius(mm)	217.1	161.1	126.1	103.3	80.5
P<0.05					

Table 5: the relation between radius and ESR(mm/hr)

ESR(mm/hr)	37	30	22	18	14
Radius(mm)	217.1	161.1	126.1	103.3	80.5
P<0. 05					

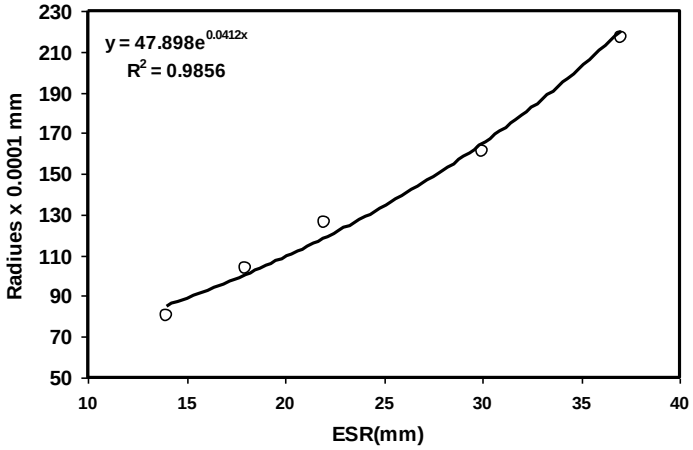
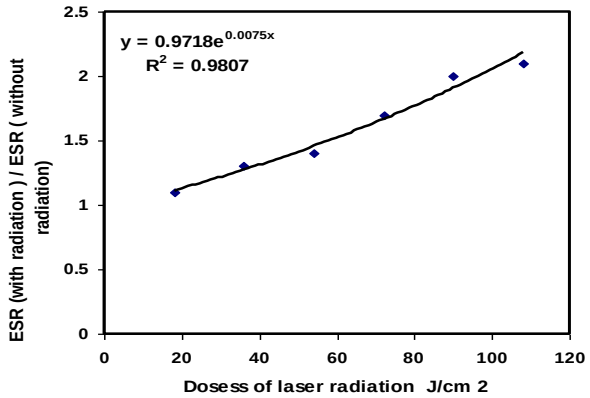


Fig 11: the relation between radius and ESR (mm)

5-2: ESR measurement with laser radiation:

Figure(12) shows the effect of laser radiation of doses (19.97,29.96,39.95,49.94 and 59.93 gray) on ESR for 50%,HCT constant red blood cells concentrations, ESR**(G.C)** levels increase with increasing the laser radiation doses for the used range of doses. (In absence of plasma) the red blood cells became important chief factor of determining the erythrocyte sedimentation rate (ESR), to explain the increasing in ESR $_{(L.)}$ levels with increasing the dose laser radiation produce alteration in molecules by :Intermolecular or intramolecular cross-linking is an effect caused by the creation of secondary bond between two molecules, (intermolecular cross-linking) or between two points of the same molecule , which were not linked before irradiation (intramolecular cross-linking) Cross-linking lead to increase the weight of molecule and decrease viscosity of the red blood cells these leads to the increase of the ESR [15].



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تأثير أشعة الليزر ولزوجة كريات الدم الحمراء على معدل ترسيب كريات الدم

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(تاريخ الاستلام:٢٢ / ٦ / ٢٠٠٩ ، تاريخ القبول:٢٥ / ١٠ / ٢٠٠٩)

الملخص:

أن الغرض من هذه الدر اسه هو لمعرفة تأثير كل من حجم الخلايا المتجمعة (HCT) ولزوجة كريات الدم الحمراء وإشعاع ليزر الهليوم نيون على معدل ترسيب كريات الدم الحمراء. استخدم ليزر الهليوم نيون بطول موجي 632.8ηm وكثافة قدره بحدود $30Mw/cm^2$ وفتحة تشعيع بقطر 5mm لتشعيع عينات الدم ، أن أزمان التشعيع كانت 10,20,30,40,50,60mint فكانت جرع التشعيع $18,36,54,72,90,108\ J/cm^2$. عينات الدم أخذت من ١٨ متبرعا، وكل عينة دم قسمت إلى قسمين شععت أحداها وتركت الثانية كمقياس للمقارنة. معدل ترسيب كريات الدم تم قياسه بعد الإشعاع وقورن مع النموذج القياسي . النتائج بينت أن الزيادة في معدل الترسيب يتناسب عكسيا مع حجم الخلايا المتجمعة(HCT)، وان معدل الترسيب يزداد بزيادة اللزوجة، و وجد أن أشعاع الليزر يزيد من معدل زمن الترسب لعينات الدم.