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# Evaluation of the effect of the helium-neon laser (632.8 nm) on erythrocyte sedimentation rate (ESR) and packed cell volume (PCV)

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**Abstract**
Erythrocyte aggregation is considering an essential physiological phenomenon in blood circulation, the interaction of lasers with biomaterials like blood is a crucial topic of study. It is a fundamental property of healthy blood that is crucial to the cardiovascular system. Evaluation of the vital processes of blood microscopically and in laboratory by examining the packed cell volume and erythrocyte sedimentation rate upon exposure to laser radiation for two different times and recording the results after all period of time and comparing them with the control group. In this study, the effect of radiation on packed cell volume and erythrocyte sedimentation rate was observed. In terms of viscosity speed and deposition for different periods of time, it was observed that when packed cell volume are exposed to radiation for a certain period of time (10 minutes), their values will decrease as a result of the amount of radiation generated against them, and this decrease increases when the time period increases (15 minutes) .While the erythrocyte sedimentation rate is inversely proportional to PCV, there is an increase in the erythrocyte sedimentation rate when exposed to radiation. While the microscopic examination has shown that various deformability values have varying impacts on the degree of aggregation when the radiation duration increases over long periods, which therefore has an impact on deposition. Therefore, knowing the type of radiation and concentration are very important to know these effects. Preferred from researchers to do more research on the effect of radiation on various blood functions.

## Introduction

Light amplification by stimulated emission of radiation, or LASER, is the abbreviation for the term. The laser was invented in 1960, and micromanipulations began using it in 1962 [1]. Researchers in the medical sector have discovered innovative uses for lasers, such as the potential to use them as an optical trap in rotating cells. Over the final three decades of the 20th century, it has been discovered that lasers have practical uses in the domains of operation, ophthalmology, automated physics, telecommunications, and other related sectors [2]. Through the use of photic-dynamic treatment, in which a laser with a 630 nm wavelength of waves is utilized to trigger a chemical process that results in the production of a poisonous

substance to kill tumors, laser therapy has advanced to the point where it is used to treat serious diseases like cancer. Additionally, lasers may be used to sterilize blood in blood banks, clearing it of germs and viruses [3].

In photochemical interactions, light causes changes in the tissue's chemistry. Photochemical interactions are the foundation of both biostimulation and photodynamic treatment [4]. It has been observed that the absorption spectra of whole blood, erythrocytes, and plasma may be used to analyze the photochemical processes that are brought on when blood is exposed to laser light in vivo [5]. While pulsed lasers have made a greater contribution to laser surgery, diode lasers and free electron lasers have been crucial instruments for cutting-edge medical research. To have any impact on a live biological system using a low power laser, the photons must be absorbed by electronic absorption bands that correspond to certain molecular chromospheres or photoacceptors [6]. In biological research & clinical trials, several sophisticated photonics approaches & laser stimulation techniques are successfully applied [7]. The hemoglobin absorption spectrum is consistent with this wavelength effect, indicating that hemoglobin may be one of the action targets when exposed to laser light [8].

Aggregation and disaggregation of erythrocytes occur naturally during blood circulation. Red blood cell aggregation is the reflex of development of blood cycle that works to produce and remove the blood cell to playing different functions [9]. The forces of attraction between the red blood cells cause clumps to develop when there is little or no blood flow. The cells form rouleaux aggregates (Figure 1) that hold onto one another. These aggregates may be dispersed with only a little mechanical force, such what happens during circulation. The aggregation process is influenced by a variety of physical and chemical variables. Chemical variables, Hematocrit (HCT), the PH of the suspention medium, large molecules, and the flow rate are examples of variables that relate to changes in either the RBCs or the suspension medium. [10]. According to some studies, erythrocyte aggregation is significantly influenced by cellular modifications, particularly those that affect the capacity of RBCs to alter morphology when they pass through small places like the microvasculare, their deformability, and their filterability. [11].

One form of blood examination is the Erythrocyte Sedimentation Rate (ESR). This test is a general screening tool for several inflammatory disorders. It is a quick and low-cost laboratory test for determining an acute or inflammatory reaction [12]. ESR measures the RBCs' rate of settling in millimeters per hour. Anticoagulated blood is allowed to stand for an hour within a unique, vertical tube (Westergren pipette). Over time, RBCs will sediment to the bottom, leaving clear plasma on top.via rouleaux formation, RBCs sand RBCs stack or form a column when they accumulate one on top of the other. Due to the high concentration of acute phase reactant proteins such globulins and fibrinogen that are produced during inflammatory processes, the creation of rouleaux occurs more quickly. There are several ways to decide. Currently, the International Committee for Standardization in Hematology recommends using Westergen Pipette technique [13].

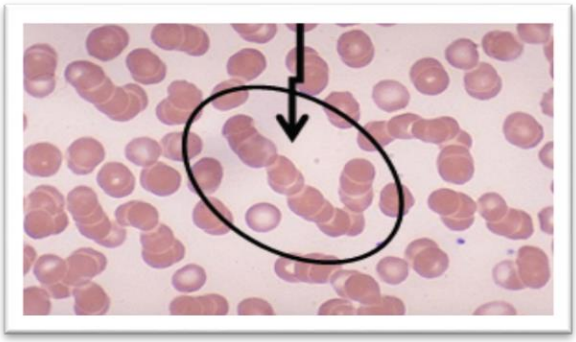

**Figure 1:** Rouleaux formation [14]

Therefore, laser is a device that generates light with certain characteristics [15], such as one wavelength and one color, and thus amplifies the light through the stimulated transmission of radiation, which have a many of several different types Figure 2.

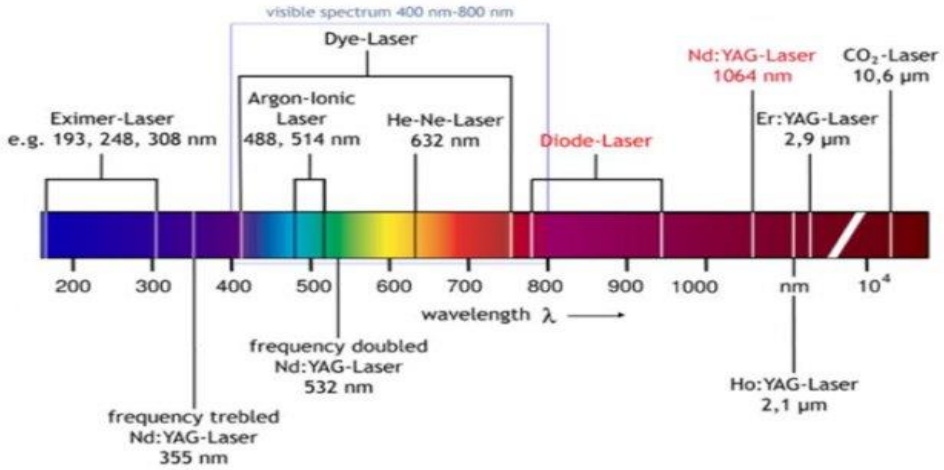


**Figure 2:** Types of laser according wavelength [16]

The blood has many functions, including those related to the transfer of oxygen from the lungs to the rest of the organs of the body and the excretion of carbon dioxide and vice versa outside the body in addition to the transfer of information between the organs and germs in the body as well as the transfer of food to the body [17].

Blood consist of many cells (Red, White, Platelet) like, Neutrophils, Eosinophils, Basophils, Monocyte and Lymphocyte [18] that have very important function in body human like keep anticoagulant, help immunity to remove any infection, know allergic disease and keeping the presenting between the normal range.

Packed cell volume may increase because of conditions that accompany dehydration, such as drinking alcohol, exposure to burns, diarrhea or may cause a decrease in packed cell volume due to anemia, pregnancy, or malignant diseases [19]. While erythrocyte sedimentation rate higher than normal result to cases of advanced age, pregnancy, and inflammation (rheumatic and rheumatoid joints, in addition to bacterial and immunological infections. erythrocyte sedimentation rate percentage may be less than normal as a result of taking various medications, heart failure diseases, or hereditary causes [20].

## Methods and materials

### Sample collection

1- The samples were taken between the hours of (9am - 11am)

2- They were collected from healthy people between (20-45) years (Male 12, Female 3) in College of Dentistry / Mustansiriyah University

3- The number of samples taken for the study was about 15 samples.

4- The left hand was chosen to draw blood from it after sterilizing the area with alcohol and placing the tourniquet directly 4 inches above venipuncture above the joint area (vein). Then the blood was withdrawn through the needle and added to an anticoagulant tube and another tube to conduct the examination.

5- The study period was from November to January 2023.

### Helium Neon Laser system

At an approximate power consumption of (0.5 mW), the Helium Neon Laser (Hamburg, Germany) was utilized in this work. It produces radiation in a beam that is collimated with an outer diameter of (4 mm), a light wavelength of (632.8 nm), and an energy ratio of (3.98 $mW/cm^2$) that was determined using this formula Figure (3).

Power Density ($mW/cm^2$) = output power (mW) / area ($cm^2$)

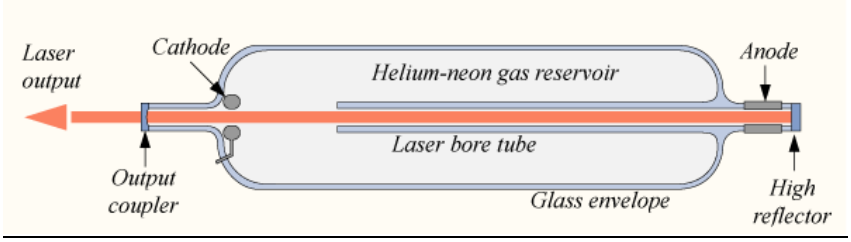


**Figure 3:** Helium Neon Laser (632.8 nm) (15)

### Microscopic examination

### Preparation of blood smears

One centimeter away from the slide's frosted area, two to five ml are positioned. The spreader is a second glass slide. Before being brought back into the drop, the spreader's edge is positioned at an angle of 30–45 [21, 22]. The spreader is then swiftly and softly moved forward, leaving a tiny layer of blood on the slide. Once prepared, the microscope slides need to be completely air-dried for a minimum of thirty minutes, excluding any "active" procedures such as stirring or blasting. This will avoid the overspread of monocytes and neutrophils. Blood smears that have been air-dried need should be colored or fixed as quickly as practicable because, if not, the blood film's plasma would produce a grey or blue color backdrop [23]. The blood was separated into two parts, the first was examined directly on a microscope, and the other was exposed to radiation for two different periods (10 and 15) minutes, after which it was examined on a microscope and the readings were recorded.

### Staining procedure

The slides were stored on two glass rods that were momentarily fastened to a tray. To hide the smear, Leishman stain was first added to the slide using a dropper. After then, the smear might be fixed for a minute. Following that, Distilled Water (DW) was added to the stain and stirred while the fluid was given a light air blow. Staining might take place for ten minutes. The slides were then rinsed with tap water to give them a pink color [24].

**Measurement Method to Packed Cell Volume (PCV)**

Antecubital vein is used to take venous blood, which is then put in anticoagulant (EDTA). Tourniquet stasis should be avoided since it might cause venous hematocrit values to increase. Carefully mixed blood is used, ideally on the rotating machine. Additionally, blood from the veins drawn by a capillary pierce and collected uses a capillary tube that has been heparin. Once well combined, blood is poured into the unmarked end of a simple capillary tube and allowed to quickly fill to about 3/4 of its total length. A horizontal tube is possible tipped to hasten fill. After being taken out of the blood, the tube is cleaned of extra blood, closed from one side, and replaced. Clay is then used to plug the unmarked end, which is subsequently inserted into centrifuge as seen with clay end resting in rubber. Each determination should be made in triplicate or duplicate to ensure accuracy. Centrifuge at a predetermined force for 5 minutes. This separates the RBCs in down while plasma in up, leaving a strip of median region composed of white blood cells and platelets at the interface.

The percentage of red cells in entire venous blood is known as the hematocrit. This may be accomplished using a constant bore capillary tube by measuring the range ratio of measurement unit. The start read capillary is configured with a clay interface set to zero. Then, move the etched line or frequency scale set to 100% and place next to the middle layer of plasma. Scroll down for where the red cell-white cell interface connects with the % spiral line. The hematocrit number is this percentage. The buffy coat layer should not be included in this figure. The volume of packed white cells should be noticed and documented if it is more than 2 percent. The blood was divided into two parts, the first is considered a control and measured directly, and the other was exposed to the laser for (10 and 15) minutes and measured again and compared them with control (Figure 7).

**Measurement Method for Erythrocyte Sedimentation Rate (ESR)**

To prevent erythrocyte shrinkage, blood was collected with the appropriate anticoagulant proportionate to blood volume, such as anticoagulant EDTA (0.5 mg/ml of blood). Together with refilling the tube, thoroughly combine the blood and anticoagulant. With the use of a completing the needle Pasteur pipette, we carefully fill the tube with blood while preventing air from accumulating in the lumen. We mend a specimen's meniscus to the zero line at tube's topmost. The tube was placed in an upright position, as indicated in, and set on a rack that will keep it there. By noting the amount of erythrocytes in the tube, the decline in erythrocytes was interpreted after an hour had passed. On the identical tube edge to the line's side, erythrocyte sedimentation rate is measured. The ESR is calculated as the decrease in cell number expressed in millimeters per hour of time, reading from the top down. The blood was divided into two parts, the first is considered a control and measured after 1 hour and the other was exposed to

the laser for two times (10 and 15) minutes, measured again after 1 hour and compared them with control Figure (5).

## Results

The normal range of Erythrocytes Sedimentation Rate (ESR):

Adult men 0-17 mm/ hr

Adult women 0- 25 mm/ hr

When conducting the test, it was noticed that there is an increase in the result when exposing the blood to radiation for a period of 10 minutes, and it increases with increasing time, Table (1) Figure (4).

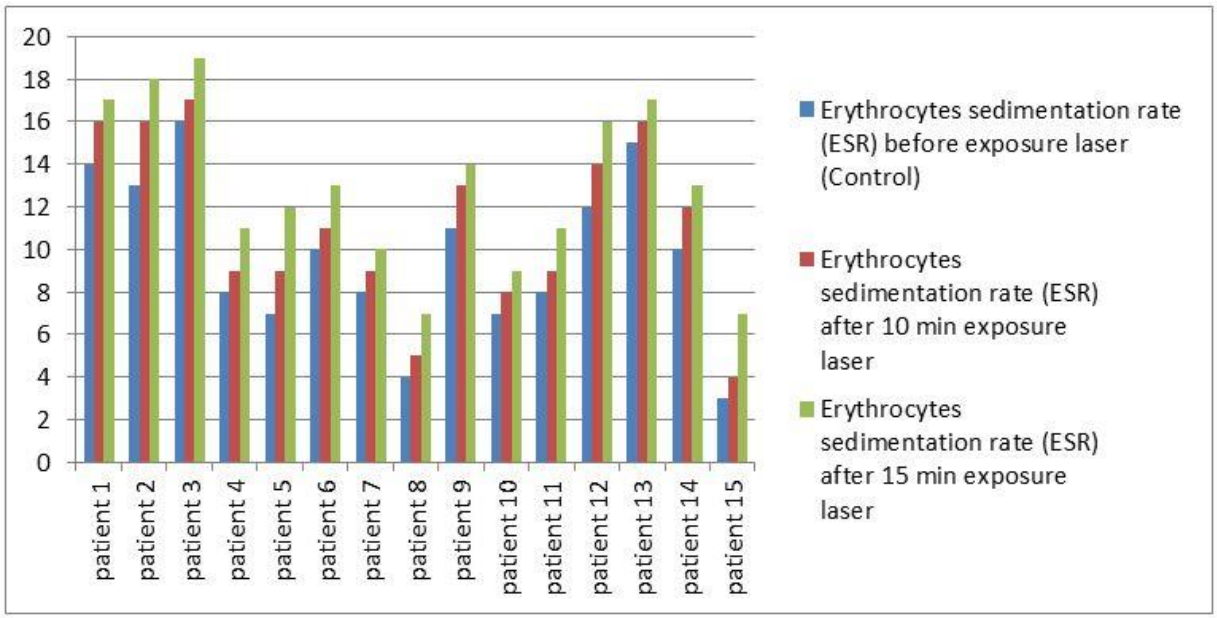


**Figure 4:** Showed effect the laser radiation on Erythrocytes Sedimentation Rate (ESR)

**Figure 5:** Showed separation of erythrocyte sedimentation rate after radiation

**Table 1:** Showed effect the laser radiation on Erythrocytes Sedimentation Rate (ESR)

| N | Gender | Erythrocytes sedimentation rate (ESR) (mm/ hr) / before exposure laser (Control) | Erythrocytes sedimentation rate (ESR) (mm/ hr) / after 10 min exposure laser | Erythrocytes sedimentation rate (ESR) (mm/ hr) / after 15 min exposure laser |
|---|---|---|---|---|
| 1 | M | 14 | 16 | 17 |
| 2 | M | 13 | 16 | 18 |
| 3 | M | 16 | 17 | 19 |
| 4 | M | 8 | 9 | 11 |
| 5 | M | 7 | 9 | 12 |
| 6 | M | 10 | 11 | 13 |
| 7 | M | 8 | 9 | 10 |
| 8 | F | 4 | 5 | 7 |
| 9 | M | 11 | 13 | 14 |
| 10 | F | 7 | 8 | 9 |
| 11 | M | 8 | 9 | 11 |
| 12 | M | 12 | 14 | 16 |
| 13 | M | 15 | 16 | 17 |
| 14 | M | 10 | 12 | 13 |
| 15 | F | 3 | 4 | 7 |

The normal range of packed cell volume (PCV):

Male: 40 - 54%

Female: 36 - 48%

When conducting the test, it was observed that when PCV are exposed to radiation for a certain period of time (10) minutes, their values will decrease as a result of the amount of radiation generated against them, and this decrease increases when the time period increases (15) minutes. That indicates of the light from the radiation has lessened red blood cell attachment Table (2) Figure (6).

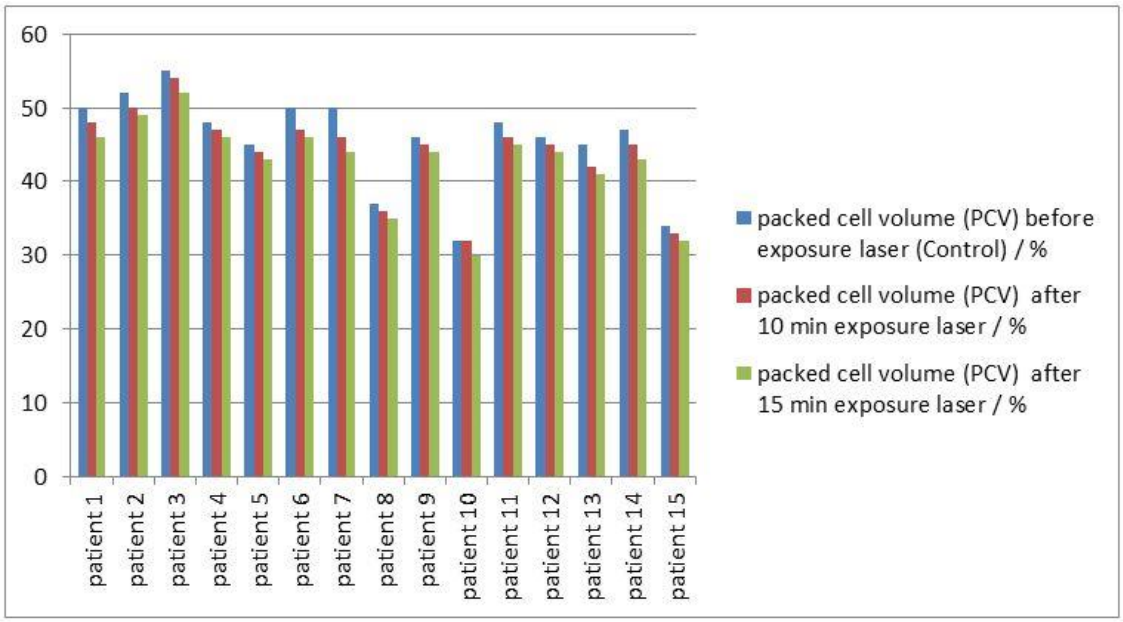


**Figure 6:** Showed effect the laser radiation on packed cell volume (PCV)

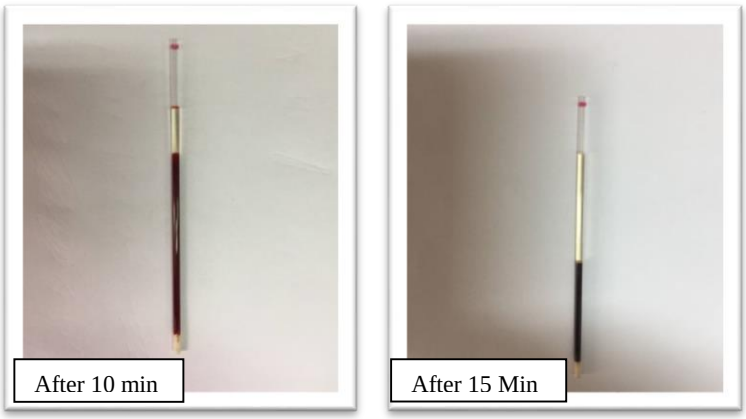


**Figure 7:** Showed separation of packed cell volume after radiation.

**Table 2:** showed effect the laser radiation on packed cell volume (PCV)

| N | gender | packed cell volume (PCV) before exposure laser (Control) / % | packed cell volume(PCV) after 10 min exposure laser / % | packed cell volume (PCV) after 15 min exposure laser / % |
|---|---|---|---|---|
| 1 | M | 50 | 48 | 46 |

| | | | | |
|---|---|---|---|---|
| 2 | M | 52 | 50 | 49 |
| 3 | M | 55 | 54 | 52 |
| 4 | M | 48 | 47 | 46 |
| 5 | M | 45 | 44 | 43 |
| 6 | M | 50 | 47 | 46 |
| 7 | M | 50 | 46 | 44 |
| 8 | F | 37 | 36 | 35 |
| 9 | M | 46 | 45 | 44 |
| 10 | F | 32 | 32 | 30 |
| 11 | M | 48 | 46 | 45 |
| 12 | M | 46 | 45 | 44 |
| 13 | M | 45 | 42 | 41 |
| 14 | M | 47 | 45 | 43 |
| 15 | F | 34 | 33 | 32 |

**Discussion:**

Some researchers have emphasized the significance physiological adjustments to RBCs aggregate, particularly in light of red blood cells deformability [25]. Major factors to capacity of deformation of red blood cells are boosted through extracellular viscidity. However, the contribute of physiology changes to red blood cells aggregate are lower scale. Membrane stiffness and surface area to volume ratio in cells [26]. In this study, the use of radiation on packed cell volume and erythrocyte sedimentation rate was observed In terms of viscosity speed and deposition for different periods of time , there is a relative difference between males or females in the level of results in erythrocyte sedimentation rate Figure (4) While the erythrocyte sedimentation rate is inverse with PCV, there is an increase in the erythrocyte sedimentation rate when exposed to radiation , as the test arrangement red blood cells for them in the same way and duration Table (1), while there is a relative difference between males and females in the analysis of packed cell volume, the reason may be attributed to the amount of proteins ( globulins ,fibrinogens) present in males figure (6), It may be more than females as a result of eating foods that contain proteins or fats, smoking or other factors, it was observed that when packed cell volume (PCV) are exposed to radiation for a period of time (10) minutes, their values will decrease as a result of the amount of radiation generated against them, and this decrease increases when the time period increases (15) minutes Table (2). That indicates of the light from the radiation has lessened red blood cell attachment. this might be caused by either a change in chemical composition caused by a less powerful bond created if an atom of hydrogen in a particular molecule draws attention to a particle of nitrogen, fluorine, or oxygen in another molecule, a phenomenon known as hydrogen bonding, or a mechanical modification because of changes in the gaps or goes that exist on areas and keep areas by means of interconnection [27-28]. the microscopic examination shown that various deformability values have varying impacts on the degree of aggregation when the radiation duration increases long periods, which therefore has an impact on deposition [29-30]. Helium neon laser with different wavelengths has many functions, including what enters therapeutic cases such as wound healing and blood disorders, as it is used in the medical field as a blood coagulant [31-32]. It is

considered one of the best and safest types of lasers for humans, because it contains a safe and appropriate wavelength, which is recommended for use among students in laboratories.

## Conclusion

The blood is affected when exposed to large amounts of radiation or any external conditions, which lead to the emergence of problems that may negatively affect the various functions of the blood. Therefore, knowing the type of radiation and its concentrations is important to know these effects. Radiation is involved in improving and speeding up the treatment of many issues in the industrial, agricultural and medical aspects. Radiation facilitates the work of doctors during surgical interventions because of its high safety when performing the operation and the exploitation of the time factor to reduce the time of performing operations. Hope the researchers to do more research on the effect of radiation on various blood functions.

## Acknowledgments

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# تقييم تأثير ليزر الهليوم نيون (632.8 نانوميتر) على معدل ترسيب كرات الدم الحمراء وحجم الخلية المعبأة

**كوثر حسين مسير، حيدر يوسف فالح***
فرع العلوم الأساسية، كلية طب الاسنان، الجامعة المستنصرية

**معلومات البحث:**
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تاريخ التعديل :2023/08/11
تأريخ القبــول: 2023/08/06
تاريخ الـنشر: 2023/12/30
**الكلمات المفتاحية:**
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**معلومات المؤلف**
الايميل:

**الخلاصة:**
يعتبر تراكم كريات الدم الحمراء ظاهرة فسيولوجية أساسية في الدورة الدموية، ويعد تفاعل الليزر مع المواد الحيوية مثل الدم موضوعًا حاسمًا للدراسة. إنها خاصية أساسية للدم الصحي الذي يعد ضروريًا لنظام القلب والأوعية الدموية. تقييم العمليات الحيوية للدم مجهريا ومخبريا من خلال فحص حجم الخلايا المعبأة ومعدل ترسيب كرات الدم الحمراء عند التعرض لأشعة الليزر لمرتين مختلفتين وتسجيل النتائج بعد كل فترة زمنية ومقارنتها مع مجموعة السيطرة. في هذه الدراسة، لوحظ تأثير الإشعاع على حجم الخلايا المعبأة ومعدل ترسيب كرات الدم الحمراء. ومن حيث سرعة اللزوجة والترسيب لفترات زمنية مختلفة لوحظ أنه عند تعرض حجم الخلايا المعبأة للإشعاع لمدة زمنية معينة (10 دقائق) فإن قيمها تنخفض نتيجة لكمية الإشعاع المتولدة ضدها. ويزداد هذا الانخفاض عندما تزيد الفترة الزمنية (15 دقيقة). وبينما يتناسب معدل ترسيب كرات الدم الحمراء عكسيا مع PCV، هناك زيادة في معدل ترسيب كريات الدم الحمراء عند تعرضها للإشعاع. بينما أظهر الفحص المجهري أن قيم التشوه المختلفة لها تأثيرات متفاوتة على درجة التجميع عند زيادة مدة الإشعاع على فترات طويلة، مما يؤثر بالتالي على الترسيب. لذا فإن معرفة نوع الإشعاع وتركيزه مهم جداً لمعرفة هذه تأثيرات. ويفضل من الباحثين إجراء المزيد من الأبحاث حول تأثير الإشعاع على وظائف الدم المختلفة