The effects of X-ray on the radii of Red Blood Cells

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Abstract
X-ray could be used in different useful purposes either diagnostic or therapeutic. However, the living tissues such as blood can be negatively affected with the ionizing radiation like X-ray when the body was exposed. The irregularly effect can involve the mechanical features of RBCs like shapes and radius which represent the main factors in functional performance of RBCs. Thus, any defect in these characters could lead to lose the RBC roles. Our study aimed to find the morphological changes of RBCs when they exposed to X-ray by measuring the radii using He-Ne laser technique. Three blood groups; O, AB and A, were exposed to different X-ray doses; 60, 80, 100 and 120 Kilo Electron Volt (KeV) for 1 minutes and the radius was checked after applying low power laser beams which was previously used for accurate measurement to the RBC radius compared to biological measuring techniques. The results showed that the radii of fringes (r) of irradiated RBCs for the tested groups were smaller than that of non-radiated RBCs for all X-ray doses. The radii of fringes (r) directly and significantly increase with increase the distance between the slide and screen (D). Radii of the tested RBC (d) groups were significantly altered compared to control groups which could relate to impairment of sodium and potassium pump mechanism which can lead to loss the RBC membrane permeability. Thus, RBCs uptake or loss the materials and liquids resulting in decreasing or increasing their sizes.

Keywords: Red blood cells radius, RBC membrane, X-ray, irradiation

1. Introduction
X-ray; form of ionizing radiation, can be beneficially used in many medical applications including diagnostic and therapeutic methods (1). However, the living cells are relatively sensitive to rays depending on the dose and time of exposure (2). The main effect of radiation
on living tissues belongs to the changes of cellular chemical properties. These changes may
relate to separation of hemolytic molecules and production of secondary electrons (3) that
leads to generation of free radical roots and hydrogen peroxide in the body fluids (1). These
effects can directly increase with increasing the radiation dose (4).
Although, radiation can destroy the living cells, it can positively use in our life if it is properly
applied (5). The blood is highly sensitive to X-ray even in low doses (1,6). Different factors
could play an important role in radiation effects such as the exposure time and living
organism age. It was seen that there are several hematological changes during chronic
exposure to radiation (5). However, Talab et al. (7) found that the white blood cells count was
not changed during the chronic exposure and the effect were limited in elderly people only.
While Ikamaise \textit{et al.} (5) showed that the first exposure to ionizing radiation for few days can
lead to decrease the white blood cell count.

Another study was focused on the influence of low dose ionizing radiation on some blood
components in an animal model which examine the maximum RBC count after exposing to a
dose of 0.5Gy irradiation per day (8). The authors found that Infrared (IR) exposure results in
significant dose-dependent reduction of blood cell counts that can be regarded as a risk to the
health of individuals during irradiation exposure.

Red blood cells (RBCs) functions are mainly depended on the mechanical properties of RBCs
which mainly relate to their shapes, radius and flexibility features. Therefore, any defect in
these properties can lead to severe impairment of RBC functions (9). Exposing to irradiation
can damage the RBC membrane (10).

Based on this background, our study aimed to investigate the effect of relatively high doses of
X-ray (i.e., 60, 80, 100 and 120 KeV) on radii of different human blood groups RBCs by
measuring the radius using He-Ne laser.

2. \textbf{Materials and methods}
The blood samples were collected according to Al Se (11). Briefly, ten ml of fresh venous
blood of three groups; O, AB and A were collected from different healthy adults in
microbiology laboratory, Maternity and Children hospital in Al-Muthanna province, 300
kilometers south of Baghdad/Iraq. Each sample was divided into two tubes containing EDTA
to prevent blood clotting. The blood samples were spin down for 2 minutes at 2000 xg to
remove the plasma, white blood cells and platelets. One of tube from each sample can use as a
control.
Another tube was exposed to X-ray source of different doses (i.e., 60, 80, 100 and 120 KeV) for one minute per each dose using ECORAY - HF 525 Analogue X-Ray System which is available in this hospital. One drop of each blood sample was narrowly spread in a glass slide. Laser beam from He-Ne laser wavelength of 632.8 ηm was applied on each slide from different distances with constant space between front end of laser source and the slides (8 cm). The position of the source and the slide was adjusted to show ring fringes that appear on a screen behind the slide. The radius \((r)\) between the edge of ring (first dark ring fringe) and its center was recorded for different distances \((D)\) between the slides and the screen (10, 20, 30, 40 and 50 cm). The experiment was repeated thrice with different blood drops. The following equation was used to find RBC radius \((d)\):

\[
\frac{r}{D} = \frac{1.22 \lambda}{d} \quad \text{............... (1)}.
\]

Where:
- \(r\): radius of the fringe.
- \(D\): distance between the slide and the screen.
- \(\lambda\): laser wavelength \((= 632.8 \ \eta \text{m})\).
- \(d\): radius of blood cells.

The recordings of radius \((r)\) was plotted against the distance \((D)\) and the obtained figure was used to calculate the slope value \((r/D)\) which then used in equation (1) to find radius of RBCs \((d)\).

2.1 Statistical analysis

All values of counting were arranged and tabulated in Microsoft Excel program. All the results were analyzed as mean ± S.E.M using GraphPad Prism version 7 (Two way ANOVA (Tukey's multiple comparisons test) and One sample t test). P-value <0.05 was considered as significant.

3. Results:

The blood samples of O, AB and A groups were collected from different healthy adults in microbiology laboratory, Maternity and Children hospital in Al-Muthanna province and then exposed to X-ray source (used in radiology department of the hospital) of different doses for one minute only. Different people can be exposed to this kind of irradiation either patients or workers of the radiology department. However, they do not expose to high doses of the ray and the blood was not directly exposed to the radiation. While this true, the side effects of the
ray exposing still need more precautions as the blood cell features can be changed as we will see later.

By using He-Ne laser source, the radius of fringes (r) was recorded for different distances between the glass slides and the display screen after applying the source on the blood drops stacked on glass slides (figure 1, panel A). Laser was previously used for measuring the RBC radius and the results were relatively accurate compared to biological measuring techniques (12). It was also used to find out the RBC deformity (13). However, the high power of laser source may influence on the RBC radii. Therefore, the idea is to use only low power thus cannot biologically interfere with the RBC features (14).

![Diagram of experiments and fringes view](image)

**Figure 1: Illustration of experiments and fringes view**

In panel A, the laser source was fixed on a special holder and faced toward the glass slides containing the irradiated and control sticky blood drops. The distance between the glass and the source was 8cm. the source place was adjusted to get the rings and fringes (Panel B as explained by arrow) on display screen in the other side. The fringes radii were recorded for different distances between the slides and the screen.
Different ring fringes appeared on the screen (figure 1, panel B). The radii of fringes (r) of irradiated RBCs for the tested groups were smaller than that of non-radiated RBCs (figure 2). The data was analyzed using Two way ANOVA (Tukey's multiple comparisons test). Significant values=P<0.05. These differences between r and D regarding group O have significant effect in all tested D distances (figure 2, panel A) while the significant difference in other two groups was restricted in 10cm only (figure 2, panel B and C). The radii of fringes (r) directly and significantly increase with increase of the distance between the slide and screen (D) (figure 2).

**Figure 2:** the relation between the X-ray dose and radii of the fringes in different blood groups. The recorded fringes radii of blood group O (A), A (B) and AB (C) for different distances between the slides and the screen were botted against the X-ray doses using Graph Prism 7. The data was analyzed
using Two way ANOVA (Tukey's multiple comparisons test), Significant values=P<0.05. Data are the means ± SEM from three independent experiments carried out.

Radii of the tested RBC groups were significantly different compared to control groups (without radiation) (figure 3). RBC radii of O and AB groups were relatively elevated than that the control samples when 60 KeV and 80 KeV were applied. Then the curve is soon fall in other X-ray doses (100 KeV and 120 KeV). However, the radii of A group RBCs increased during applying 60 KeV, 100 KeV and 120 KeV doses, while they decreased after exposing to 80 KeV.

![Figure 3: the relation between X-ray doses and the radius of RBC.](image)

**Figure 3:** The relation between X-ray doses and the radius of RBC.

By applying the equation (1), the RBC radii of blood groups O, A and AB were calculated and blotted against the doses of X-ray using Graph Prism 7. The data was analyzed using One sample t test, Significant values=P<0.05. Data are the means ± SEM from three independent experiments carried out.

**Discussion**

X-ray is much more using in medical applications than other radiations. Therefore, our blood circulation can be directly affected by X-ray. The novelty of this project relates mainly to the impact of X-ray on the membrane of RBCs which consequently alter the red cells dimensions thereafter impairing their functions. In the microbiology laboratory of the maternity and children hospital in Al-Muthanna, blood samples of O, AB and A groups of healthy adults were obtained, and exposed for just a minute to radiation sources (used in the hospital radiology department). Patients and radiology staff can be exposed to this form of irradiation. They do not, however, expose themselves to high doses of radiation and the blood has not been directly exposed. Nevertheless, as the blood cell characteristics may change as we see
later, further precautions will still be necessary to get more explanation of the side effects of ray exposure.

Through applying the He-Ne laser source to the blood drops stacked on glass slides, it is reported that the fringes radius for different distances between the glass slide and the display screen were recorded (Figure 1, panel A). Laser was previously used for measuring the RBC radius and the results were relatively accurate compared to biological measuring techniques as it was used to find out the RBC deformity (13). One the effective tools to measure the changes in RBCs during photodynamic treatment of human red cells is Laser-assisted optical rotational cell analyser which provides an early indicator of RBCs impairment (15). The idea of use laser diffraction in measuring RBCs dimensions was found before as Johnson (16) mentioned that Ektacytometers (a machine used laser in its counting applications) can be commercially used in the measurement of RBCs membrane dimensions. This machine was basically designed by Bessis’s group at the Institut de Pathologie Cellulaire in 1975 (17). However, the high power of laser source may influence on the RBC radii. Therefore, the idea is to use only low power laser thus cannot biologically interfere with the RBC features (14).

The ring fringes appeared on the screen (figure 1, panel B) as a result of the diffraction the laser beams from the red blood cells (12). The radii of fringes (r) of irradiated RBCs for the tested groups were smaller than that of non-radiated RBCs (figure 2). The radii of fringes (r) directly and significantly increase with increase of the distance between the slide and screen (D) (figure 2) which could relate to increase the diffraction of laser beams from the red blood cells. These results agree with Al-Taii (12) who used Gamma rays, Beta particles and Alpha particles for RBC radiation.

Radii of the tested RBC groups were significantly different compared to control groups (without radiation) (figure 3). These changes can relate to the effect of X-rays on the RBC membrane characteristics. The main effect of X-ray exposing is haemolysis (18). However, this damage mainly depends on the dose of X-ray (19). Haemolysis of RBC due to irradiation is an osmotic effect which occurs due to impairment of sodium and potassium pump mechanism because of sulphhydryl groups diminution in the RBC membrane and the movement across the membrane can be impaired thereof (20). In addition, proteins included in RBC membrane can aggregate (21) and the membrane can be echinocyte after exposing to X-ray (22). Moreover, the damage could involve lipoproteins of the membrane which form the main unit of membrane structure (23) and thus, RBCs uptake or loss the materials and
liquids resulting in decreasing or increasing their sizes which could be responsible for the instability of the RBC radius. However, Tungjai et al. (24) found there is no effect of low rate X-ray on red blood cell fragility or cell wall stability.

The membrane of RBCs can be altered due to the effect of X-ray at 450 Gy which can lead to aggregate of the membrane’s proteins due to destruction of disulphide bonds (25). In addition, work is being conducted to determine the impact of radiation from electromagnetic fields on size and shape of human blood red blood cells due to the impact of Global Mobile Communications System (GSM) (26). In this study, blood groups of A and B the increase in size is not significant, 2.42% to 3.55%. However, AB and O blood groups showed significant changes in the size. These results could be quite important for the X-ray workers as they expose to X-ray doses for long times. Wherefore, it is recommended to continue testing their RBCs dimensions which could be a perfect indicator of the X-ray badly impact.

4. Conclusion
The RBC radii can be determined by using the laser source as a calculating toll while some experiments have used staining slides under the light microscope. The tested RBC groups showed considerably various radii compared to the control groups (without radiation). These modifications could relate to impact of X-rays on the targeted RBCs. The increases in radiation doses can lead to the lack of RBC membrane permeability thus resulting in failure of the function of sodium and potassium pump. Therefore, RBCs contents their materials and liquids were lost or the outer composition can cross into the RBCs without control which reduce or raise their sizes. RBC O group radii were more influenced than other examined RBC groups when they exposed to X-ray irradiation.

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