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Development of cataract and corneal opacity in mice due to radon exposure

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This work investigates the radiation damage on the eye of albino mice exposed to effective radon doses ranging from 20.92 to 83.68 mSv. These doses were taken over 2–8 weeks using a radon chamber constructed by the National Institute for Standard (Egypt). The guidance on the quality assurance program for radon measurements was followed. Therefore, the estimated doses received by the laboratory animals meet the requirements of national standard. The refractive index(RI) and protein concentration were measured for soluble proteins of both corneas and lenses. In addition, the sodium dodecyle sulfate polyacrylamide gel electrophoresis (SDSPAGE) technique was used. The results show increasing of the RI of both cornea and lens proteins and decreasing in total protein concentration of exposed animals. These results were accompanied with changes in the SDSPAGE profile for both cornea and lens. Therefore, radon exposure produces substantial hazards to the cornea and lens of experimental animals and has a crucial role in the development of cataract and corneal opacity.

Keywords: radon; exposure; eye lens; cataract; cornea; corneal opacity

1. Introduction

Nowadays, it is well established that radon (^{222}Rn) is the only radioactive gas in the middle part of the long radioactive series of ^{238}U and represents 55% of the natural radiation sources (*1*). The main problem for the indoor workers, who are working in mines, is the inhalation of radon and its daughters. Most of the radon daughters attach themselves quickly to ambient aerosols. When the daughters are inhaled, they become lodged in the nasal passage, trachea and pulmonary tree or pulmonary parenchyma. Radon and about one-third of the deposited decay products are transported from the lung into the blood stream (*2*). Therefore, the blood itself and several other organs and tissues are exposed to α-particles from radon and its daughters.

The dose equivalent of both β-particles and γ-rays to the tissue are negligible due to their larger range and lower biological effectiveness as compared with α-particles. Miles and Cliff (*3*) could determine the doses of radon and its daughters that affect organs other than the lung. Radon inhalation constitutes internal exposure whereas external exposure comes mainly from the

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α-emitters in the surrounding environment. The hazards from internal exposure of radon exceed that from external one (comes from β-particles and γ-rays) by a factor ranging from few hundreds to more than thousand (*4*).

At low doses of radon exposure, a few data were obtained *in vivo* experiments especially in some organs other than the eyes. Therefore, this study was designed to investigate the effects of external and internal (inhalation) exposure of radon on the protein of the cornea and lens of albino mice.

2. Experimental work

Adult male Swiss albino mice, weighing 25–30 g and two months average age, were used for this study. The mice were selected from the animal house of Research Institute of Ophthalmology (RIO), Egypt, and received balanced diet.

2.1. *Radon exposure equipment*

The animals were exposed to known radon doses using the radon chamber constructed by the National Institute for Standard (NIS), Egypt, (*5*) for 6 h/day and five days a week. The constructed radon chamber has a cubic form with dimension $50 \times 50 \times 50\,cm^3$ ($0.125\,m^3$ volume). The walls of the chamber were made of 8 mm glass and equipped with pitchblende, which is a radium-bearing ore (used as a radon source). The volume of the chamber was designed to get an appreciable concentration of radon gas that emitted from the used source. In addition, a sufficient number of experimental animals can be exposed to radon inside the chamber. The chamber roof has a rectangular window of dimension $28 \times 28\,cm^2$. Above this window, an exposure shutter is fixed. The dimensions of this shutter are $30 \times 30 \times 26\,cm^3$ with two drawers (12 cm apart). Using this shutter, the equilibrium inside the chamber was reserved as well as possible during multi-exposure processes. For mice exposure to radon gas inside the chamber, the following steps must be followed:

1. After pulling the upper drawer, the mice cage was inserted, which was fastened by means of a string passing through a small hole in this drawer.
2. After withdrawal the upper drawer, the lower one was pulled to let the mice cage fall vertically inside the chamber, which then tightly closed again.

At the end of exposure, the above steps were repeated in the opposite manner.

2.2. *Measurements of radon doses*

The insertion of the mice cage in the chamber displaces the air inside it. The displacement leads to some leakage of radon during multi-exposure processes. This leakage was measured and evaluated by monitoring the radon concentration during animal exposure periods using an Alpha-Guard radon monitor (active technique) and CR-39 detector (passive technique).

The passive device was a diffusion cup (7 cm diameter and 5 cm height) fitted with a CR-39 detector at its bottom while the open end was covered with filter paper of 170 μm thickness. Moreover, an external (bare) CR-39 detector was fixed outside the cup. For getting good statistics, the diffusion cups with their external detectors were exposed to radon gas inside the chamber for different periods ranging from one to five days.

At the end of the exposure period, the detectors were collected and etched together under their optimum condition of 6.25 N NaOH solution at 70 °C for 6 h and stirring at 50 rev/min.

The samples were washed by distilled water, dipped for few seconds in 3% acetic acid solution, washed again in distilled water and allowed to dry in air. The track densities were counted using an optical microscope of 400× magnification power.

The radon concentration (C_o) was calculated by the following Equation (*6*):

$$C_o = \frac{D_o}{K_T t} \tag{1}$$

where D_o is the track density of the detector (inside the diffusion cup), t is the exposure time in days and K_T is the CR-39 detector response for radon measurements, which equals to 0.18 ± 0.02 track cm^{-2}/Bqm^{-3} d and is measured in the National Institute for Standard, Radiation Measurements Department, Egypt (*7*).

The track density ratio (D/D_o) between external (D) and filtered (D_o) detectors were used for calculating the equilibrium factor (F), where

$$F = \exp\left[a + b\left(\frac{D}{D_o}\right) + c\left(\frac{D}{D_o}\right)^3\right] \tag{2}$$

The free parameters a, b and c have the values of -5.902, 3.059 and -0.118, respectively (*8*).

The dose in term of working level month (WLM) can be calculated from

$$\mathrm{WLM} = \mathrm{WL}\frac{t}{170} = \frac{F \times C_o}{3700} \times \frac{t}{170} \tag{3}$$

where WL is the number of working level, t is the exposure time (hour) assuming that the exposure of 1 WL for 170 h produce 1 WLM.

Knowing the WLM makes it possible to calculate the effective dose in mSv, where (*9*)

$$\begin{aligned} 1\,\mathrm{WLM} &= 63\,\mathrm{m\,Sv} \\ &= 3.54\,\mathrm{mJ\,m^{-3}\,h} \end{aligned} \tag{4}$$

2.3. *Sample preparation*

The animals were classified into five groups each of 30 mice. Group I is a control group, and group II to group V inhaled radon for two, four, six and eight weeks, respectively.

The animals were killed and their eyes were excised. The cornea and lens were removed and homogenized in distilled water followed by centrifugation at 8000 rpm for 30 min. The sample of the supernatant was removed for the analyses of soluble protein.

The following measurements were carried out on the soluble protein of both cornea and lens:

- The refractive index (RI) using Abbe refractometer attached with temperature control unit at $37\,^{\circ}\mathrm{C} \pm 0.02$.
- Total protein using the method of Lowry et al. (*10*).
- Sodium dodecyle sulfate polyacrylamide gel electrophoresis using the method of Laemmli (*11*).

2.4. *Statistical evaluation*

The obtained results were statistically evaluated using the Student' t-test analysis. The probability level of $P < 0.05$ was considered as significant (*12*).

Table 1. Radon and its related parameters as measured by CR-39 inside the mice exposure chamber.

Time (days)	$D \times 10^3$ (Tcm^{-2})	$D_o \times 10^3$ (Tcm^{-2})	D/D_o	$C_o \times 10^3$ (Bqm^{-3})	Equilibrium Factor (F)	Working Level (WL)
1	24.93	11.65	2.14	64.72	0.60	10.48
2	46.60	20.62	2.26	57.28	0.70	10.90
3	62.48	29.47	2.12	54.57	0.58	8.58
4	84.56	37.75	2.24	52.43	0.69	9.73
5	93.66	45.03	2.08	50.03	0.55	7.41
Mean ± SD				55.80 ± 5.65	0.62 ± 0.06	9.42 ± 1.43

3. Results

The Alpha-Guard measurement of radon concentration during the exposure of mice showed some fluctuation around 62 ± 3 (4.8%) KBqm^{-3}. The day leakage (during the exposure) was recovered during the night but still some leakage occurred. After five days of consequence exposures, the overall leakage reached 15%.

The track density of the filtered CR-39 detector (D_o) was used to calculate the radon concentration using Equation (1). The mean radon concentration was equal to 55.80 ± 5.65 (10%) KBqm^{-3}. Using the ratio between external (D) and filtered (D_o) detector track densities, the equilibrium factor was equal to 0.62 ± 0.06 (9.6%) as calculated by Equation (2). Therefore, the number of WL was equal to 9.42 ± 1.43 (15%). Table 1 lists the details of these calculations.

The WLM was equal to 0.055 ± 0.008 per one exposure hour as calculated by using Equation (3) which is equivalent to 0.195 ± 0.028 mJm^{-3}. Therefore, the mice were exposed to radon dose equal to 1.65 ± 0.24 WLM/one week (based on six exposure hours per day for five days a week, *i.e.* 30 h/week), which is equivalent to an absorbed dose rate of 10.39 ± 1.51 mSv/week or 5.84 ± 0.85 mJm^{-3}h/week.

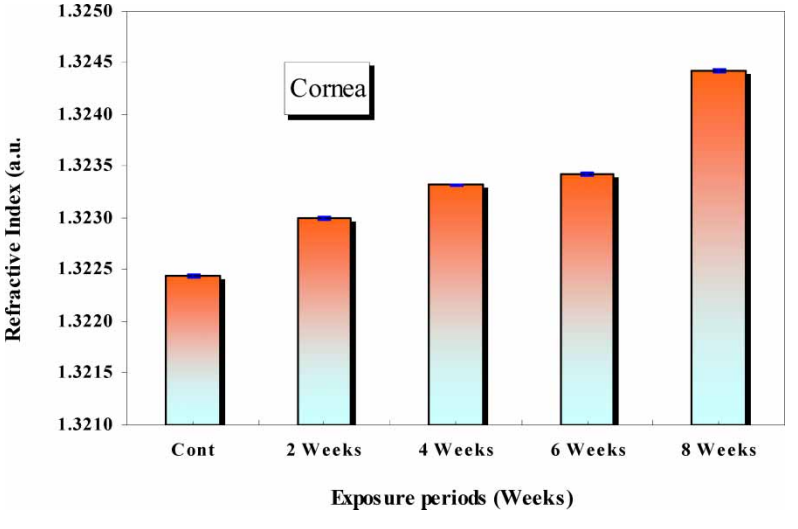

Figure 1. RI of the mice cornea protein after different periods of exposure to radon.

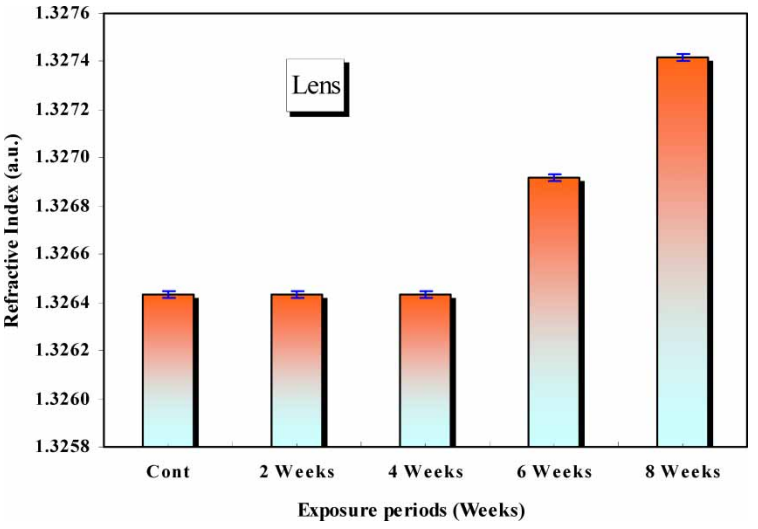

Figure 2. RI of the mice lens protein after different periods of exposure to radon.

3.1. *Refractive index measurements (RI)*

The RI of corneal proteins of mice exposed to radon α-particles from radon (Figure 1) showed a very high significant increase ($P < 0.001$) for all groups. In case of lens proteins of mice exposed to α-particles (Figure 2); there were non-significant changes in the RI for the groups exposed for two and four weeks. Moreover, it is clear from the figures that the groups, exposed to radon for six and eight weeks showed very high significant increases in the RI of lens protein ($P < 0.001$).

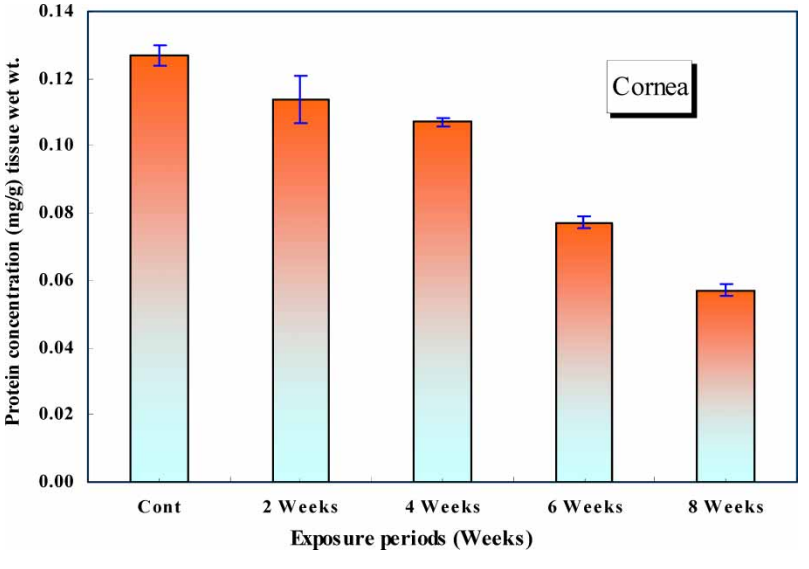

Figure 3. Protein concentration of the mice cornea after different periods of exposure to radon.

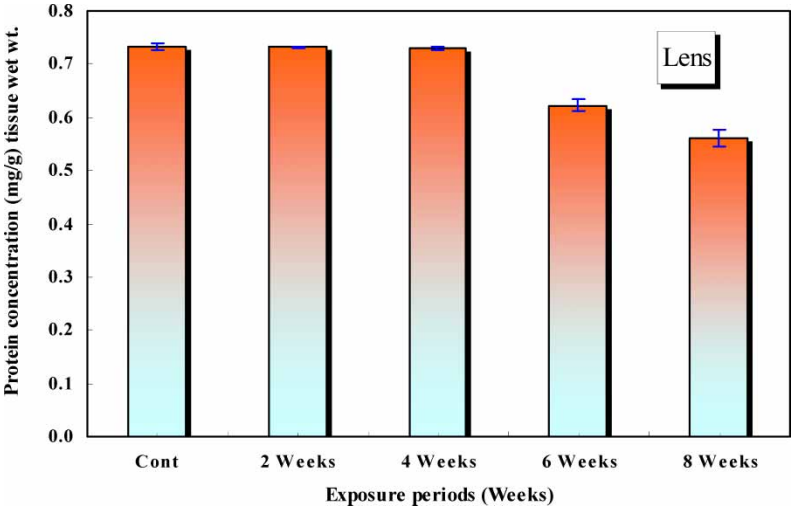

Figure 4. Protein concentration of the mice lens after different periods of exposure to radon.

3.2. *Total soluble protein*

The protein concentration of normal mice cornea was 0.127 ± 0.003 mg/g cornea wet weight. After exposure of mice to radon gas for two weeks, the total protein was significantly decreased to 0.114 ± 0.007 mg/g ($P < 0.05$). In addition, there were high significant decreases ($P < 0.001$) in cornea protein concentration, with respect to normal group, for mice exposed to radon gas for four, six and eight weeks (0.107 ± 0.001, $0.077 \pm 0.001$and 0.057 ± 0.002 mg/g, respectively), Figure 3.

Figure 4 shows the lens protein concentration for control (0.733 ± 0.006 mg/g lens wet weight) and mice exposed to radon gas for two, four, six and eight weeks. The data showed non-significant changes in lens protein concentrations after two and four weeks of radon exposure (0.732 ± 0.001 and 0.730 ± 0.003 mg/g wet weight, respectively). In contrast, there were high

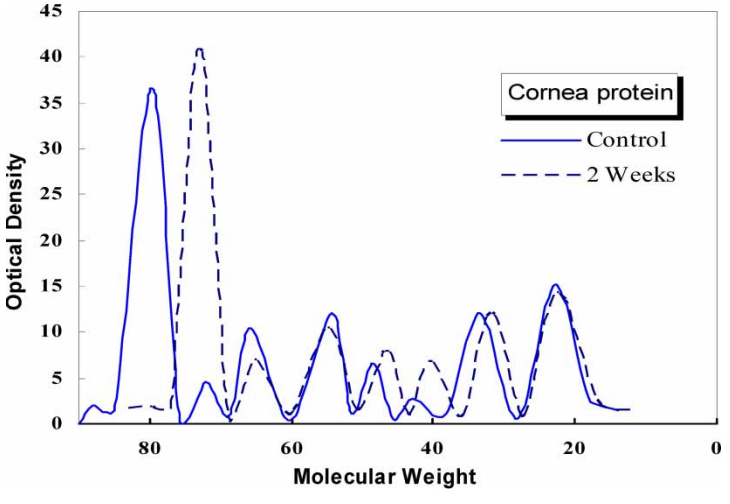

Figure 5. Electrophoretic pattern of mice cornea protein for control and two weeks exposure to radon.

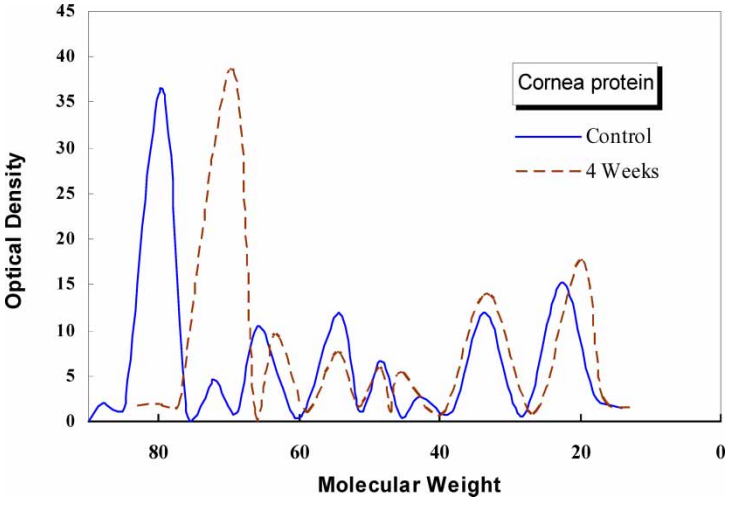

Figure 6. Electrophoretic pattern of mice cornea protein for control and four weeks exposure to radon.

significant decreases ($P < 0.001$) in protein concentrations after six and eight weeks of radon exposure (0.622 ± 0.011 and 0.560 ± 0.016 mg/g wet weight, respectively).

3.3. *Electrophoretic separation*

Figures 5–8 show the electrophoresis patterns of the corneal proteins for control mice and those exposed to radon for two, four, six and eight weeks. The electrophoresis pattern for control was characterized by the presence of several peaks (seven fractions) which varies in their intensities (heights) and broadenings (width).

After two and four weeks, there was slight shift of corneal proteins to the right indicating increase in protein mobility. After six and eight weeks of radon external exposure and inhalation, the protein mobility was significantly increased. Both heights and widths of the peaks were remarkably changed as compared with the control.

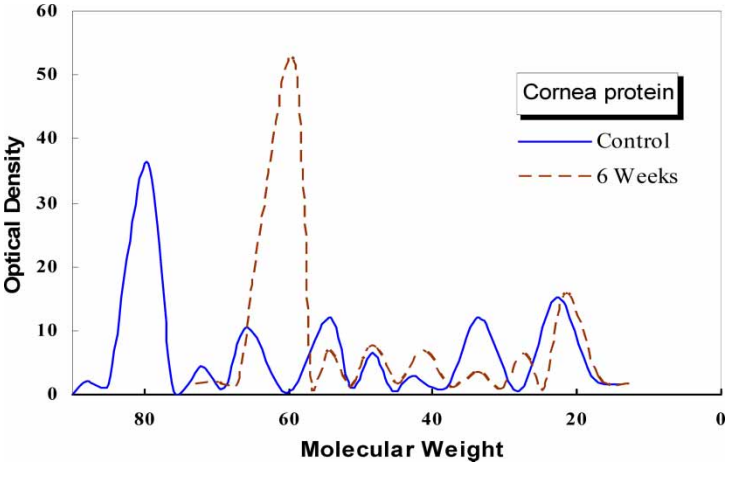

Figure 7. Electrophoretic pattern of mice cornea protein for control and six weeks exposure to radon.

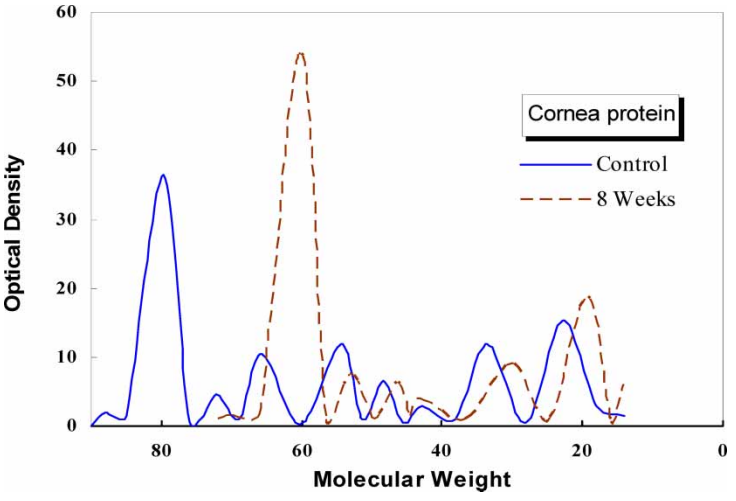

Figure 8. Electrophoretic pattern of mice cornea protein for control and eight weeks exposure to radon.

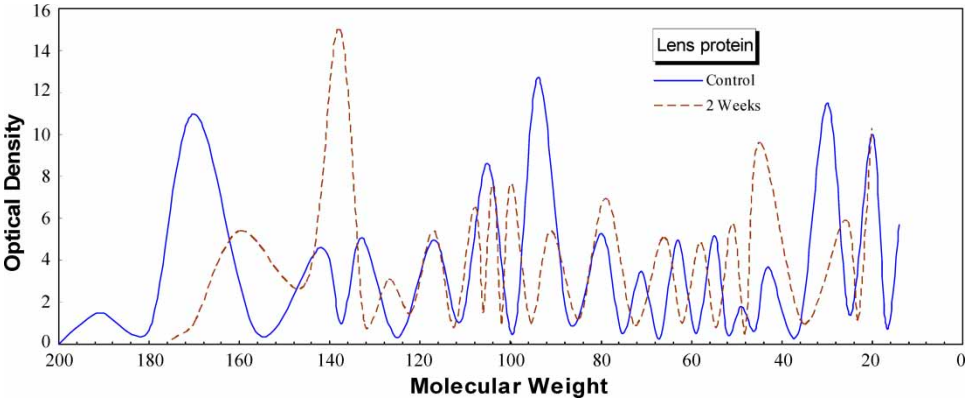

Figure 9. Electrophoretic pattern of mice lens protein for control and two weeks exposure to radon.

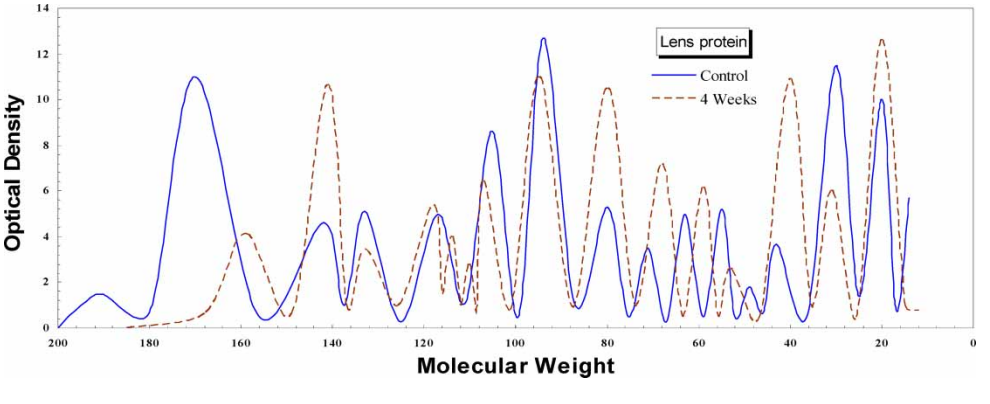

Figure 10. Electrophoretic pattern of mice lens protein for control and four weeks exposure to radon.

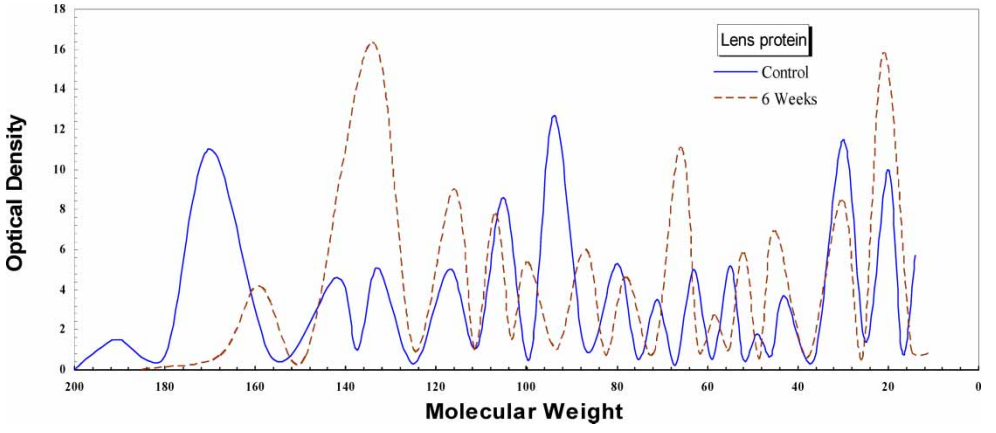

Figure 11. Electrophoretic pattern of mice lens protein for control and six weeks exposure to radon.

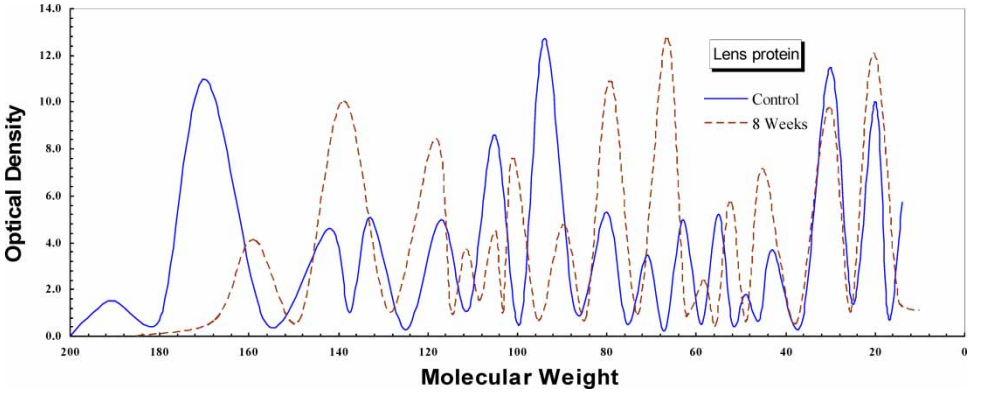

Figure 12. Electrophoretic pattern of mice lens protein for control and eight weeks exposure to radon.

Figures 9–12 illustrated the electrophoresis patterns of the soluble lens proteins for control and radon treated mice for two, four, six and eight weeks. The electrophoresis pattern for control was characterized by the presence of 15 fractions. After two and four weeks, the changes in electrophoresis pattern were significant in the low mobile group. After six and eight weeks of radon exposure, lens crystalline fractions were characterized by changes in the intensities of the peaks and increase in their mobilities. Both heights and widths of the peaks were remarkably changed as compared with the control. In addition, some protein fractions disappeared.

4. Discussion

The Alpha-Guard measurement of radon concentration showed that, the day leakage (during the exposure) was recovered during the night but still some leakage occurred. After five days of consequence exposures, the overall leakage reached 15%. For this reason, the chamber was sealed for two days without exposure to compensate for this leakage. After these two days, the concentration reached again its maximum. Therefore, the mice were exposed to well-known constant radon concentration at different weeks and the quality of radon measurements was assured.

The annual exposure and the annual effective dose are important parameters for the workers health. These estimated parameters are based on the chronic occupational exposure to radon gas in the work places, which is calculated by UNCEAR 2000. It was found that the average effective dose for the workers was almost 30 times higher than the world average dose (*13, 14*).

It was observed that there are high significant changes in the RI and protein concentrations of lens. Such case may be due to internal exposure produced from radionuclides reached the pigmented tissue of the eye through the blood stream following inhalation of α-emitting radionuclides. These results are compatible with the observation of Holtzman and Sha (*15*) who found greatest concentration of ^{226}Ra in the human choroids, sclera and iris for high body burden of ^{226}Ra. In addition, the obtained results agree with the work of Griffith et al. (*16*) who reported the appearance of cataract in eyes of a relatively young man of 47 years and attribute their results to radon inhalation.

Therefore, internal exposure by α-emitting radionuclides would be expected to be more effective than external penetrating radiations like γ- and β-rays, since α-particles have a greater biological effect than γ-rays as recognized by the quality factor used in calculating the absorbed dose for different types of radiation. The quality factor for α-particles is 20 is compared with 1 for γ-rays (*17*).

Internal radiation exposure of the eye may cause injury to the proliferating cells in the anterior epithelium of the lens, in which the radiosensitive cells were situated by free radical formation and oxidative effect. These injured cells and their breakdown products were accumulated at the posterior pole of the lens and lead to the obtained change in molecular weights of crystalline as detected in electrophoresis. The obtained changes in molecular weights of lens proteins are responsible for the local alteration in the RI and for the decrease in the concentration of the total soluble protein, which are characteristics of cataract.

As the iris and ciliary's body lie adjacent to the area of the lens epithelium, some of α-particles would reach the radiation sensitive cells in the germinative zone of the lens epithelium. Since the range of α-particles is about 35 μm in tissue (*18*), the distance between the iris and the sensitive cells will lie within the same range (*19*). Such local irradiation by α-particles is expected to be more effective than external penetrating radiation.

The external α-radiation is particularly harmless except when it passes through the cornea, because α-particles do not penetrate the outer layer of skin (*20*). The cornea of the eye is the most vulnerable external tissue to α-radiation, since the lens of the eye, which is at depth of 3.0 mm below the cornea, cannot transmit α-particles to the cornea.

The external α-particles from radon and plated out radon daughters caused significant changes in the RI and protein content of the cornea, which is a transparent tissue having high refractive power. These changes are attributed to the ionizing effect of α-particles which leads to disturbance in cells through changing their functions and decreasing the corneal transparency (*21*), which are characteristics of corneal opacity.

5. Conclusion

It could be concluded that, radon exposure leads to internal damage of the eye lens through inhalation and external damage through plated out radon daughters on the cornea. The percentage radon daughters reaching the lens through the blood stream is expected to be less than it is plated out on the cornea. For this reason, the effect on the cornea may occur faster than on the lens. The corneal damage appeared after two weeks following eye exposure to radon while the lens was affected after six weeks. Such changes indicated that the α-particles from radon and its daughters are particularly dangerous.

Eventually, in order to protect the mine workers from both the external and internal effects on the eye cornea and lens, it is recommended to have a good design of ventilation systems for these mines (internal protection) and advise workers to wear safety glasses and masks for external protection.

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