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Effect Of Ionizing Radiation Cesium Cs-137 On Isolated Lymphocytic DNA From Leukemia And Healthy Individuals Treated With Vitamin D.

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Abstract

Radiation is defined as the physical process where particles or electromagnetic waves pass through a medium or space. Ionizing radiation consists of either photon-radiation (gamma rays and x-rays) or fast moving sub-atomic particles (beta particles, neutrons, etc.). Gamma rays consist of electromagnetic energy in the form of photons emitted by radioactive nuclides such as caesium-137. Deficiency of Vitamin D is most common worldwide and associated with DNA breaking. This study was aimed to Vitamin D shared ionization radiation by attacking the DNA molecule and Vitamin D may potentiate the indirect effects of radiation.The design of the work was studied in vitro study is simply carried in the laboratory. Lymphocytes were obtained from chronic lymphocytic leukemia patients, known concentrations of lymphocyte cells prepared and different concentrations of synthetic vitamin D (1,25$(OH)_2$D3) prepared. Then the prepared solutions exposed to different time of radiation and different distance, monitored spectrophotometrically by 260/280nm ratio.The vitamin D effect hyperchromasia on lymphocytic DNA referring to increase in the absorbance of DNA solution, indicating separation of DNA strands, while ionization radiation effect hypochromasia on lymphocyte DNA referred to a decrease in the absorbance of DNA solution indicating DNA damage. The longer the exposure to ionizing radiation and the closer the radioactive source is to the sample, the greater the DNA damage.where it was found that vitamin D reduces the effect of radiation.

Keyword: DNA, vitamin D, ionizing radiation cesium Cs-1

Introduction

Ionizing radiation can be either naturally occurring background radiation or from a man-made source. As natural background radiation is ubiquitous, the exposure of human beings is inevitable. The sources of this background radiation include cosmic rays originating from outer space and radon, which is a naturally occurring radioactive gas present everywhere on Earth. Radon is classified as a known pulmonary carcinogen by the International Agency for Research on Cancer. Man-made radiation sources include X-ray equipment and nuclear reactors, which are used to generate nuclear energy. Radiation in the medical field is a growing source of man-made radiation. The use of medical procedures using ionizing radiation is increasing because of their value in diagnosing and treating various diseases (1).

DNA breaks have an essential role in cancer development. It depended on the electromagnetic wave frequency. Waves with Low-frequency, such as radio waves, are one end of the spectrum. X-rays/Gamma rays have a high frequency. Waves with high energy and high-frequency are called ionizing rays. The radiation composed from high energy can displace the orbit of the electron. Dislocation of the human tissues' electrons led to damage and DNA directly or indirectly. Dislocation of the electron and DNA break occurs by direct damage. The indirect damage occurs if the electron acts with the water, leading to hydroxyl compounds that damage the cell's DNA (2).

DNA damage occurs with several consequences. The single-strand DNA break is repaired by the cell without any sequelae. Breaking the double strand of DNA will produce abnormal linking between the strands, leading to adverse biological effects on tissue and cells level. DNA try to rejoin incorrectly, leading to cell death. It rejoins as asymmetrical translocation during division to produce cancer or abnormal division, leading to hereditary disorders. The radiosensitivity occurs for the cells leading to radiation exposure (3). Vit D is soluble in the fat, and the diet is a natural source; also, the seco-steroidal prohormone formed in the skin after exposure to (UVB, 290–320 nm) from the light of the sun. The steroid hormone precursor passes two-stage metabolism in the hepatic and renal tissue to produce the calcitriol (the active form), which binds with the vit. D receptor to make its function (4) (5). The role of vit D is to control Ca and P metabolism for bone remodelling. In the Past, studies found that vit D deficiency is associated with low sunlight exposure and leading to several skeletal diseases (6-9). Deficiency of Vit D is most common worldwide and associated with DNA breaking. The reports that study the relationship between DNA damage and vitamin D level in plasma are few. More reports found that vitamin D has a protective role against cancer. A high concentration of vit D decreased the prevalence of cancer such as breast cancer, ovary cancer, prostate cancer (10) (11). Vitamin D has many actions in the functions of the tissues, and it is a protective agent against radiation (12). The literature view is included minimal data about the role of Vit D with the damaged tissues due to the radiation. Administration of D3 (0.7 g) to the rat that is exposed to irradiation at (0.01 Gy daily for one month) will result in improving the activity of the glycolytic enzyme in bone marrow cells and erythroid cells (13)(14). found that the irradiated cell and treated with calcitriol are increased in growth. Vit. D is found in the radiosensitize cancer cells, such as breast cancer cells and lung tumor cells (15-18). Therefore, it is used as an adjuvant with radiotherapy for decreasing the side effects. In irradiated MCF-7, breast tumor cells (five times of 2 Gy for three days) are treated with an active form of vit D (100 nmol/L for 72 hours) stimuli autophagy and inhibit the proliferative after X-ray exposure (19). This effect was not observed in the human fibroblast cells and the BT474 breast tumor cell line (20). in non-small cell lung cancer cells, D3 is promoted of the response against irradiation by mediated VDR, AMPK pathways, and tumor protein p53. The bronchial cells and cardiomyocytes of the human did not develop radiosensitizer by vitamin D (21). The chronic exposure to irradiation, active form of D3 will cause changing of enzymes included vit. D metabolism (22). Administration of low vit. D level to people with radiological will be useful and have a protective role against harmful compounds such as free radicals. So, must take cure about deficiency of vit. D in patients during radiotherapy (23).

Materials and methods

The study consists of blood samples belonged to the patients with acute lymphocytic leukemia who attending the laboratories of the Baghdad Teaching Hospital, in Baghdad from December 2020 to February 2021. A venous blood sample (2ml) was collected at morning where 2 ml was placed in ethylene diamine tetra acetic acid (EDTA) tube. Samples of blood obtained from healthy subjects with match sex and age were also included in the present study for comparison. Extract DNA from lymphocytes using some materials, study was done by measurement of the absorbency DNA by spectrophotometer.

Effect of ionization radiation cesium Cs-137 on isolated lymphocytic DNA from leukemia and healthy individuals treated with vitamin D.

DNA lymphocytic (20µL) were treated with different concentration of vitamin D (1000IU, 500IU, 400IU, 200IU) and incubated for 10 minutes at room temperature, in addition to negative control group (lymphocytic DNA treated with vitamin d).The absorbance of samples were recorded by UV-spectrophotometer at 260nm and 280nm wavelength and then the ratio of λ260/λ280was calculated, then it exposed to different time of ionizing radiation cesium Cs-137 (1, 1.5, 3)hour with different distance (2-16 cm from the upper surface of test tube), The absorbance of samples were recorded by UV-spectrophotometer at 260nm and 280nm wavelength and then the ratio of λ260/λ280was calculated.

Results

Effect of radiation on isolated lymphocytic DNA from healthy individuals incubated with vitamin D

Effect of exposure distance

The samples of DNA solution were radiated for 180 minutes at a distance of 2, 4, 6, 8, and 16 cm from the source of radiation to the upper surface of the test tube opening. The DNA damage was higher at a short distance (2 cm) compared with a longer distance. The DNA damage at 2cm distance of the source of radiation was 11.3%, 3,3%, 4.7% and 14.2% when the DNA solution was treated with 1000IU, 500IU,400IU, and 200IU vitamin D, respectively (Table (1) [A],[B],[C], [D]. The higher DNA damage was observed when the DNA solution incubated with 200IU vitamin D (Table (1)[D]), while the lowest damage was observed with 500IU vitamin D (Table (3-3) B]). Figure (1) summarizes the effects of vitamin D at different concentrations and different radiation distances, which showed a non-linear relationship.

(Table (1)[A]): Effect of radiation exposure 3hour time at different distance (cm) on the DNA molecule solution treated with vitamin D at a concentration of 1000 IU.

Distance	dsDNA (µg/mL)		ssRNA (µg/mL)		ssDNA (µg/mL)		%
	Before radiation	After radiation	Before radiation	After radiation	Before radiation	After radiation	
2	3.55	3.15	2.84	2.52	2.343	~~2.079~~	11.3
4	2.65	2,55	2.12	2.04	1.749	1.683	3.8
8	3.3	3.3	2.64	2.64	2.178	2.178	0
16	4.4	4.4	3.52	3.52	2.904	2.904	0

(Table (1)[B]): Effect of radiation exposure 3hour time at different distance (cm) on the DNA molecule solution treated with vitamin D at a concentration of 500 IU.

Distance	dsDNA (µg/mL)		ssRNA (µg/mL)		ssDNA (µg/mL)		%
	Before radiation	After radiation	Before radiation	After radiation	Before radiation	After radiation	

2	8.05	7.75	6.44	6.2	5.313	~~5.115~~	3.3
4	7.05	6.9	5.64	5.52	4.653	4.554	2.2
8	7.4	7.35	5.92	5.88	4.884	4.851	1.7
16	8.55	8.5	6.84	6.8	5.643	5.61	0.6

(Table (1)[C]): Effect of radiation exposure3hour time at different distance (cm) on the DNA molecule solution treated with vitamin D at a concentration of 400 IU.

Distance	dsDNA (µg/mL)		ssRNA (µg/mL)		ssDNA (µg/mL)		%
	Before radiation	After radiation	Before radiation	After radiation	Before radiation	After radiation	
2	7.45	7.1	5.96	5.68	4.917	~~4.686~~	4.7
4	6.4	6.15	5.12	4.92	4.224	4.059	3.9
8	7.05	7.05	5.64	5.64	4.653	4.653	0
16	8.05	7.95	6.44	6.36	5.313	5.247	1.2

(Table (1)[D]): Effect of radiation exposure 3hour time at different distance (cm) on the DNA molecule solution treated with vitamin D at a concentration of 200 IU.

Distance	dsDNA (µg/mL)		ssRNA (µg/mL)		ssDNA (µg/mL)		%
	Before radiation	After radiation	Before radiation	After radiation	Before radiation	After radiation	
2	6	5.15	4.8	4.12	3.96	~~3.40~~	14.2
4	4.75	4.3	3.8	3.44	3.135	2.838	9.5
8	5.55	5.25	4.44	4.2	3.663	3.465	5.4

16	6.1	5.9	4.88	4.72	4.026	3.894	3.2

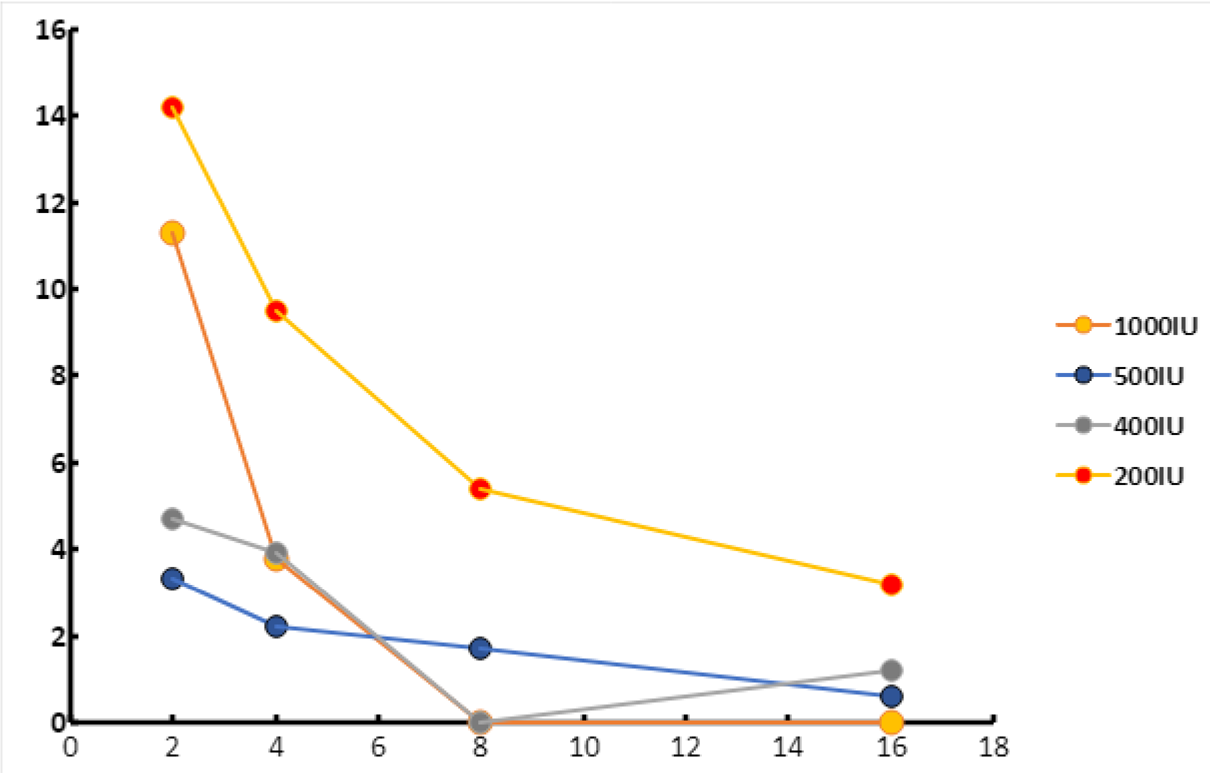

Figure (1): Effect of radiation exposure distance on the DNA solution treated with vitamin D at different concentration

Effect of radiation on the isolated lymphocytic DNA from leukemia incubated with vitamin D.

Effect of exposure distance:

The samples of DNA solution were radiated for 180 minutes at a distance of 2, 4, 6, 8, and 16 cm from the source of radiation to the upper surface of the test tube opening. The DNA damage was higher at a distance of (4 cm) compared with others. The DNA damage at 2cm distance of the source of radiation was 3.8%, 3.1%, 4.4% and 13.7% when the DNA solution was treated with 1000IU, 500IU,400IU, and 200IU vitamin D, respectively (Table (2) [A],[B],[C], [D]. The higher DNA damage was observed when the DNA solution incubated with 200IU vitamin D (Table (2)[D]), while the lowest damage was observed with 500IU vitamin D (Table (2)[B]).

(Table (2)[A]): Effect of radiation exposure 180 minutes at different distance (cm) on the DNA molecule solution (of patient with leukemia) treated with vitamin D at a concentration of 1000 IU.

Distance	dsDNA (µg/mL)		ssRNA (µg/mL)		ssDNA (µg/mL)		%
	Before radiation	After radiation	Before radiation	After radiation	Before radiation	After radiation	
2	2.63	2.53	2.1	2.02	1.73	~~1.67~~	3.8
4	2.8	2.675	2.24	2.14	1.848	1.766	4.5
8	2.75	2,7	2.2	2.16	1.815	1.782	1.8
16	3.05	3.05	2.44	2.44	2.013	2.013	0

(Table (2)[B]): Effect of radiation exposure 180 minutes at different distance (cm) on the DNA molecule solution (of patient with leukemia) treated with vitamin D at a concentration of 500 IU.

Distance	dsDNA (µg/mL)		ssRNA (µg/mL)		ssDNA (µg/mL)		%
	Before radiation	After radiation	Before radiation	After radiation	Before radiation	After radiation	
2	7.05	6.83	5.64	5.46	4.65	~~4.51~~	3.1
4	7.93	7.78	6.34	6.22	5.23	5.13	1.9
8	7.38	7.3	5.9	5.84	4.87	4.82	1
16	7.6	7.58	6.08	6.06	5.02	5.0	0.3

(Table (2)[C]): Effect of radiation exposure 180 minutes at different distance (cm) on the DNA molecule solution (of patient with leukemia) treated with vitamin D at a concentration of 400 IU.

Distance	dsDNA (µg/mL)		ssRNA (µg/mL)		ssDNA (µg/mL)		%
	Before	After	Before	After	Before	After	

	radiation	radiation	radiation	radiation	radiation	radiation	
2	6.25	5.98	5	4.78	4.13	~~3.94~~	4.4
4	7.28	6.9	5.82	5.52	4.80	4.55	5.2
8	6.93	6.75	5.54	5.4	4.57	4.46	2.5
16	7.075	7.025	5.66	5.62	4.67	4.64	0.7

(Table (2)[D]): Effect of radiation exposure 3hour time at different distance (cm) on the DNA molecule solution (of patient with leukemia) treated with vitamin D at a concentration of 200 IU.

Distance	dsDNA (µg/mL)		ssRNA (µg/mL)		ssDNA (µg/mL)		%
	Before radiation	After radiation	Before radiation	After radiation	Before radiation	After radiation	
2	4.55	3.93	3.63	3.14	3.0	~~2.59~~	13.7
4	5.9	5.48	4.72	4.38	3.89	3.61	7
8	5.48	5.13	4.38	4.1	3.61	3.38	6.4
16	5.75	5.43	4.6	4.34	3.80	3.58	5.7

Discussion

In present result vitamin D has been found protect DNA from radiation, findings support the potential benefits of combing a vitamin d analog with radiation to wipe out radiation resistant to protect DNA.as part of our study Effect of ionization radiation on DNA molecule extracted from lymphocytes healthy treated with vitamin D, Where The samples of DNA solution were radiated for 180 minutes at a distance of 2, 4, 6, 8, and 16 cm from the source of radiation to the upper surface of the test tube opening. The DNA damage was higher at a short distance (2 cm) compared with a longer distance. The DNA damage at 2cm distance of the source of radiation was 11.3%, 3,3%, 4.7% and 14.2% when the DNA solution was treated with 1000IU, 500IU,400IU, and 200IU vitamin D, respectively (Table (1) [A],[B],[C], [D]. The higher DNA damage was observed when the DNA solution incubated with 200IU vitamin D (Table (1)[D]), while the lowest damage was observed with 500IU vitamin D (Table (1) [B]), which showed a non-linear relationship. and other part of our study on effect ionization radiation on DNA lymphocyte with leukemia treated with vitamin, The samples of DNA solution were radiated for 180 minutes at a distance of 2, 4, 6, 8, and 16 cm from the source of radiation to the upper surface of the test tube opening. The DNA damage was higher at a distance of (2 cm) compared with others. The DNA damage at 2cm distance of the source of radiation was 3.8%, 3.1%, 4.4% and 13.7% when the DNA solution was treated with 1000IU, 500IU,400IU, and 200IU vitamin D, respectively (Table (2) [A],[B],[C], [D]. The higher DNA damage was observed when the DNA solution incubated with 200IU vitamin D (Table (2)[D]), while the lowest damage was observed with 500IU vitamin D (Table (3-8)[B]). This resulted obtained in this work showed that radiation exerts significant effect on DNA samples treated with vitamin d which is related to its different distance , exposure radiation at 180 minutes produces less damage upon DNA samples treated with vitamin D from without treated vitamin , on other side effect of radiation to damage DNA is increasing with increase radiation dose and decrease distance with reduction of the concentration vitamin D concentrations, the highest DNA damage was reached at distance 2 cm with 180 minute at concentration 200 IU, bearing in mind that a high concentration at 1000 concentration causes damage to DNA, A study by Mukai et al. indicated that vitamin D supplementation was a significant factor in prolonged metastasis-free survival after preoperative chemo radiation therapy for patients with pancreatic ductal adenocarcinoma (24). Radiation dermatitis occurs frequently during radiation therapy in cancer patients, and vitamin D ointment is helpful for its prevention (25). In a case report, vitamin D supplementation prior to surgery and radiotherapy in a patient with recurrent breast cancer altered certain biological cancer markers, such as estrogen receptor, human epidermal growth factor receptor, and nuclear protein Ki67 (26). Moreover, an increasing body of evidence suggests that gut epithelial vitamin D receptor signaling pathways play an essential role in maintaining the integrity of the intestinal mucosa. Vitamin D deficiency is associated with the severity of radiation-induced proctitis in cancer patients (27). Another study asserted that vitamin D has the potential to improve genetic inhibition and increase sensitivity to radiation, by acting as a switch between cytoprotective and cytotoxic autophagy (28)

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