

## Chromosomal Aberration Study of Human Lymphocytes exposed to Americium -241 in vitro..

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### الخلاصة:

أخذت عينة دم وريدي من متبرع ذكر ذو صحة جيدة. عينة الدم بعد ان زرعت على الوسط قسمت الى مجموعتين, الاولى عينة سيطرة والثانية عرضت للاشعاع لعنصر الامريشيوم للكشف على تأثيره على التشوهات الكروموسومية. المزارع التي عرضت للاشعاع الامريشيوم اظهرت ثنائية الجسيم المركزي (52,8%), الكسور (20%), الشدفة (18.5%) والكروموسوم الحلقي (6.8%). وأظهرت فروقات معنوية باحتمالية اقل من (0.5) بالمقارنة مع عينة السيطرة. ثنائية الجسيم المركزي اظهر نسبة عالية بالمقارنة مع التشوهات الكروموسومية الاخرى. متوسط معامل الانقسام والانحراف المعياري كان  $(2.212 \pm 2.236)$  للعينة المعرضة للاشعاع اعلى من عينة السيطرة التي كانت  $(1.565 \pm 1.581)$ . يستنتج من ذلك ان التعرض لجسيمات الفا تسبب تشوهات كروموسومية في خلايا الانسان .

### Abstract

This study was intended to decide the impact of  $\alpha$ -partcales for acceptance of chromosome deviation. The venous blood sample was drawn from 30 years old healthy male donor. The blood sample after cultured in RPMI was divided into two groups, the first was as control and the second was exposed to radiation from Am-241 to detect the effect of it on chromosomal aberration (CA). The cultures exposed to Am-241 have dicentric (52.8%), breaks (20%), fragments (18.5%) and (6.8%) ring chromosomes. There was significant differences ( $p \leq 0.5$ ) compared with control sample. Dicentric show high percentage of chromosomal aberration compared with other CA. The mean of mitotic index (MI) and stander deviation was  $(2.212 \pm 2.236)$  for radiation exposed sample higher than that of control was  $(1.565 \pm 1.581)$ . It has been concluded the exposure to low  $\alpha$ -partcales could caused chromosomal aberrations in human cells.

### Introduction

In the last years, the world has witnessed in the use of X-rays and electromagnetic radiation in many of the devices that are used daily by the population, such as mobile and a lot of medical equipment and devices as well as the microwave oven and Internet connection.

The writing information emphatically underpins the conceivable acceptance of transmissible genomic unsteadiness when presentation to radiation (1,3). Be that as it may, the likelihood of presentation bringing about unfavorable wellbeing impacts stayed doubtful (4). Moulde et al. (5) when a late audit presumed that the present proof for a causal relationship amongst disease and introduction to

radiofrequency electromagnetic radiation RFR is feeble and unconvincing. At the point when epidemiological proof is feeble, mostly because of short follow up periods, research center studies are vital and in vitro studies are a decent supplement if the outcomes are replicable and steady crosswise over various techniques for testing.

Hereditary flimsiness can be broke down by utilizing cytogenetic parameters such as chromosoma aberrations (CA), sister chromatid exchanges (SCE), micronuclei (MN) and mitotic index (MI) that suggested to be the biomarkers of genotoxic and cytotoxic impacts and broad size for esteem mutagenic impact of operators in a brief timeframe in various situations [6]. Chromosomal

abnormalities in lymphocytes have ended up being connected with growth chance in people (7,8) and are viewed as useful pointers of presentation; besides they can be utilized as a part of a more quick screening for unfriendly impacts..

Americium is made by the human, radioactive component. It is no normally happening or stable isotopes of americium element. Immaculate americium is a brilliant metal. There are two vital isotopes of americium, americium - 241 and americium- 243, Both isotopes have the same concoction conduct in the environment.(9-10 ) Am-241 and Am-243 emit alpha particles (some of the time alluded to as alpha radiation).

The dominating business utilization of Am-241 is in ionization-sort smoke finders.( A commonplace family unit smoke indicator include 0.9 microcuries ( $\mu\text{Ci}$ ; a  $\mu\text{Ci}$  is one millionth of a curie) may be 33,000 Bq of Am-241 ). Am-241 is likewise utilized for mechanical gaging applications and in medicinal demonstrative gadgets. It found applications research, yet there is no worked applications for Am-243.

Am-241 can be discharged to nature from atomic reactors, atomic blasts, and mischances, and in addition from assembling items containing americium, (for example, smoke identifiers). Since Am-241 has a long half-life, it is available on the earth for quite a while. You might be presented to Am-241 by breathing the air, drinking of water, even eating nourishment contained Am-241; notwithstanding, the amount of Am-241 in water, air, soil, and sustenance are for the most part low, and of little wellbeing result. Individuals who create or handle Am-241 in smoke locators or different gadgets might be presented to more elevated amounts. On account of an atomic mishap, presentation could happen by breathing or eating dust

containing  $\alpha$ -particles from Am-241. (11).

So the present study was completed to decide the impact of  $\alpha$ -particles for acceptance of chromosome distortions.

#### **Materials & procedure :**

The samples of blood were drawn from 30 years old healthy male donor into heparinized vacutainers. No drug was taken by donor for 4 weeks before taken sample from him . The sample was classified into two kind; the first without exposed to element of Am as control and the second for exposure to Americium radiation by expose culture tubes to Am-241 disc in incubation .

Am-241 (small disc shape) used in this research was a sources of  $\alpha$ -particle with average dose in the surface equal to  $0.35\mu\text{Sv/h}$  and radiation activity equal to  $102.8\mu\text{Ci}$  (according to IRSRA\* test near the time of doing the research).

Culture of whole blood were prepared . Then adding 0.5 ml blood to 4.5ml medium consisting of RPMI-1640 (Gibco BRL) containing 0.2 mM L-glutamine , 15% fetal Calf serum (Gibco BRL), one hundred IU/ml from penicillin and fifty  $\mu\text{g/ml}$  of streptomycin . Finally add five  $\mu\text{g/ml}$  of PHA (Gibco BRL) for to stimulate lymphocytes for division . Then tubes of culture were put in thirty seven  $^{\circ}\text{C}$  for forty eight hours (with Am-241 disc for second group). Then prior to harvesting about one hours, colcemid  $0.1\mu\text{g/ml}$  (Gibco BRL) was add to tubes of cultures. The tubes of culture were centrifugation to precipitate the lymphocyte and treated with a  $0.075\text{M}$  KCl, for ten min . Then centrifugation for tubes and taked the pellet to fixed in a 3:1 mixture of methanol:glacial acetic acid and then dropped on to cooled clean slides and air dried . Slides were stained with 5% Giemsa stain (sigma) . One hundred Cells with mitoses were analyzed for each sample and chromosomal aberrations (breaks,

dicentrics, fragment and rings) were counted. Mitotic index(MI) was calculated 1000 cells for each slide.(12)

Statistical analysis was done chi-square for chromosomal aberration. Mean and

standard deviation (SD) was done for MI. The significance ( $P < 0.01$ ) of the differences between exposed and control end-point Means was analyzed using Student's t-test.

## Results

**Table( 1 ) Distribution of chromosomal aberration caused by exposing to Am-241 radiation.**

Sample	Total cell	Cell with chromosomal aberration	Break	Dicentric	Fragments	Ring	Total abnormalites
exposure to Am241 radiation	750	41	14(20%)	37(52.8%)	13(18.5%)	6(8.5%)	70
control	800	11	2(6.8%)	14(48.2%)	0	2(6.8)	18
Total	1550	52	34	51	13	8	88

**Table( 2 ) shows mean mitotic index(MI) of exposure to Americium radiation and control.**

Sample	Total counted cells	Total number: dividing cells	meanof MI &SD
exposure to Am-241 radiation	<b>5650</b>	<b>125</b>	<b>2.212±2.236<sup>#</sup></b>
control	<b>5750</b>	<b>90</b>	<b>1.565±1.581</b>
Total	<b>11400</b>	<b>215</b>	<b>1.885±4.116</b>

<sup>#</sup> t-test:  $P < 0.01$

Different types of chromosomal aberrations were show ins lymphocyte cultures expose to Am-241 element. These aberrations included chromatid break, dicentric, fragment, and ring chromosome.

In this study 750 cells count, 41 were aberrations. The all chromosomal aberration of sample exposure to Americium radiation was 70; 20% of them breaks,52.8% dicentric chromosomes,18.5% fragments and 8.5% ring chromosomes. comparison with control sample was the total number of cell with chromosomal aberrations was 18 : include 6.8% breaks,48.2% dicentric,0% fragments and 6.8% ring chromosomes. There was significant differences ( $p \leq 0.01$ ) between exposed sample and control.

Dicentric show high percentage of chromosomal aberration compared with other CA.

The sample exposure to Americium radiation show a significant of MI when compared with control ( $P < 0.01$ ). MI rate ( $2.212 \pm 2.236$ ) of exposed sample was higher than that of control ( $1.565 \pm 1.581$ ) (Table 2).

## Discussion:

The essential center of this in vitro study was to assess if introduction to Am-241 radiation would induce chromosomal damage in human lymphocytes.

The comes about saw in this study are in close concurrence with portrayed beforehand, that show freely of the cytogenetic system utilized, there is a high level of exactness and reproducibility.

The present results showed that Am<sup>241</sup> induced chromosomal aberrations in

human lymphocyte culture. Similar result was obtained by (13) and (14). In the clonal descendants of hemopoietic stem cells after irradiating bone marrow with  $\alpha$ -particles show instability of chromosomal (15). Chromosomal distortion to be seen in fringe blood lymphocytes from people with fused  $\alpha$ -emanating radionuclides bringing about long haul ceaseless presentation.

The five type of chromosomal aberration breaks, dicentric chromosomes, fragments and 6% ring chromosomes when expose to Am radiation comparison with controle show that dicentric was high number comparison with other CA which is agreement with experiments for in vivo  $\alpha$ -particle exposure. (16, 17) Whenever disagreement with the one previously (18). The relative proportion of breaks and fragments (20%) and (18.5%) is low rate comparison with dicentric mean the lower rate of interstitial pieces proposes that disregarding the lifted generation of interstitial erasures after  $\alpha$ -particles, human lymphocytes repaired them all the more productively. (16). Lower ratio for ring 8.5% may be the low level of exposure for this radiation which have not a high number of metaphases cells is needed to distinguish the nearness of rings. There are many studies about the effect of genotoxic effect for alpha particle. The aftereffects of the vast majority of these studies (58%) show expanded harm to the nucleic acid (evaluated from breaks in DNA strand, occurrence of CA, MN and SCE) in cells presented to radiation contrasted and unexposed cells. Constructive results implies that epidemiological investigations of individuals presented to alpha radiation are prone to demonstrate expanded disease. The possibility of getting cancer is low at low doses, and increments as the dosage increments. (19). Americium releases alpha particles and gamma rays. Alpha particles can

produce damage to near distance of cells. Gamma rays may travel much longer distances and penetrate the body. (9)

So will be cause the damage of nucleic acid after a high-level exposure that can include one or more DSB as well as associated SSB, damaged locations, and DNA-DNA and DNA-protein cross links (6,7,8).

The mitotic index show highest value in exposed sample which agree with Mihaela that showed linear increasing of mitotic index and chromosomal aberration frequency with radiofrequency radiation treatment of increased exposure time (20) and Pesnya and Romanovsky significantly increased the mitotic index for bubls. Exposure to plutonium-239 have  $\alpha$ -particales which its induced for each aneugenic and clastogenic effectiveness (23). While Ozkan suggest the loss of a radiation sensitivity effect of Taxol in cell proliferation causes enhanced mitotic death rather than apoptotic death (21). And Bettega et al. show a slightly increased sensitivity in mid-G1 phase for the C3H 10T1/2. When cells preseted to alpha particles, it is not statistically significant (22).

There are many studies for epidemiology revealed these of radiation effects (24,25,26). Almost (19%) of other studies observe inconclusive. So it was presented to elevated amounts of radioactive americium as a result of inadvertent discharge at an assembling office, at an atomic plant, or in light of the fact that an atomic weapon has been harmed or exploded, take after the counsel of general wellbeing authorities who will distribute proper rules for decreasing introduction.

In conclusion, the effect of exposure to low  $\alpha$ -particales could caused chromosomal aberrations in human lymphocytes cultures. This effect may be due to expanded free radical action as a reaction to exposure.

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