Republic of Iraq
Ministry of Higher Education
And Scientific Research
Al-Nahrain University
College of Science
Dep. Of Physics

Radioactive Detection On the Blood Samples of Cancer Patients Diseases by Using CR-39 Detector and its Effect on Cytogenetic

A Thesis
Submitted to the College of Science at
Al-Nahrain University in Partial Fulfillment of
The Requirements for the Degree of
Master of Science
in Physics

By

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March 2007 A.D.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة النهرين/ كلية العلوم

قسم الفيزياء

الكشف عن المستوى الإشعاعي في عينات دم المرضى المصابين بالإمراض السرطانية وتاثيرة على المورثات

رسالة

مقدمة الى كلية العلوم في جامعة النهرين

وهي جزء من متطلبات نيل درجة ماجستير علوم

في الفيزياء

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Acknowledgments

Praise be to ALLAH, Lord of the whole creation and peace be upon his messenger Mohammad.

I would like to express my sincere thanks and deep gratitude to my supervisors **Dr. Nada F. Tawfiq** and **Dr. Esmail K. Shubber** supervising the present work and for their support and encouragement throughout the research.

I am most grateful to the Dean of College of Science and Head and the staff of the Department of Physics at Al–Nahrain University. The assistance given by the staff of the library of the College of Science at Baghdad University is highly appreciated.

I wish acknowledge to Ministry of Science and Technology / Institution of Hazard Materials / Center of Bioradiation specially Dr.mithal, Dr. Ali Hussain, Dr. Nahei in the Center of Cancer Research, Mr. Mohammad AL-Aadami, and Mr. Ziad Gatee Al-Rekabi for this support.

I acknowledge the helpful comments assistance given to me at various stages of this work by Mr. Omar ,Mrs. Noor and Mrs.Lamay'

Finally, I most grateful to my **parents**, my **brothers** Sameer, Moneer, Mohammed and my **sister** Hadeel, and to my **husband** for their patience and encouragement throughout this work.

ATHEER

Abstract

The aim of this project is to measure the uranium concentrations in blood samples taken from control group and patient group taken from different governorates of Iraq , and measure the effect of Uranium in human chromosomes and cellular division, therefore this study was designed to investigate the effects of Uranium on the human through cytogenetic studies.

The first part contains the determination of Uranium concentration in blood samples using CR – 39 track detector.

The nuclear reaction used a source of nuclear fission fragments is (n, f) obtained by the bombardment of U – 235 with thermal neutrons from (Am – Be) source which has flux of (5×10^3 n cm^{-2} s^{-1}).

The concentrations values were calculated by a comparison with standard samples which prepared in our laboratory.

The result obtained show that the higher concentration of Uranium in blood samples was (1.89 ppb) in Diala Bridge, and minimum concentration of Uranium was (0.33 ppb) in Al – Zafraniya region.

The second part contains the study of three cytogenetic parameters were employed, which were including: chromosomal aberration analysis, mitotic index assay (MI), and blastogenic index assay (BI) for studying the biological effects of Uranium.

The result of this study showed that Uranium has a significant effect on the human chromosomes as represented by inducing chromosomal aberrations that include chromatid – type aberration (gap and break chromatid), and unstable structural chromosomal aberrations such as gap, break, and ring chromosome. Also dicentric, and acentric chromosome aberrations were obtained. These aberrations were scored with significant increasing as compared with control groups.

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Chapter One
INTRODUCTION

1.1 Radioactivity

The history of radioactivity really began with discovery of X-rays by Wilhelm Roentgen in 1895 [1].

In 1896 Henri Becquerel found that Uranium Salts could activate a photographic plate in the absence of light, also they could activate air so as to discharge an electroscope. He suggested the term 'radioactivity' by referring to the phenomenon of 'Radiation Activity' [2].

In 1898 Marie Curie found out a new radioactive elements, one she named polonium and the other radium, and also discovered the radioactivity of uranium with her husband [3].

Radioactivity is a number of disintegration per second, its unit for measurement is Becqurel (Bq) [4].

There are two main sources of radiation found in the environment; natural radioactivity sources (which include terrestrial, cosmic rays, and cosmogenic), and man-made radioactivity sources (which medical, fallout, and nuclear power) [5, 6, 7]

1.2 Natural Radioactive Sources

All natural elements with atomic numbers $Z \geq 83$ are radioactive, we have four distinct natural decay series [8] which are represented in table (1-1).

Table (1-1): Radioactive Decay Series [9]

Series	First Isotopes	Half-Life [Y]	Last Isotopes(Stable)
Uranium	^{238}U	4.49×10^{9}	^{206}Pb

Actinium	^{235}U	7.10×10^{8}	^{207}Pb
Thorium	^{232}Th	1.39×10^{10}	^{208}Pb
Neptunium	^{237}Np	2.14×10^{6}	^{209}Bi

There are three natural radioactive series, called Uranium, thorium and actinium series. Neptunium series is included in this table too, which dose not occur in nature because it's half life '2.1×10^{6} y' is much smaller than the age of the universe '3×10^{9}' .

With regards to depleted uranium, two series are important; the uranium series and the actinium series [9].

1.2.1 U-238 Series

This series begins with U-238 nuclei (half-life 4.49×10^{9} y) and gradually converted to the Pb – 206 which is a stable element through sequences of the emission of alpha and beta particles. All nuclides in this series are solid elements except Rn – 222 nuclei, which is gas. The elements of this series which are represented in table (1-2) are arranged according to the mass number indicated in (4n + 2) system [10].

Table (1-2): U – 238 Decay Series [11, 12]

Nuclide	Half – Life	Type of decay
U – 238	4.49×10^{9} y	Alpha
Th – 234	24.1 d	Beta
Pa – 234 m	1.18 min	Beta
U – 234	2.48×10^{5} y	Alpha
Th – 230	7.52×10^{4} y	Alpha
Ra - 226	1600 y	Alpha
Rn – 222	3.825 d	Alpha
Po – 218	3.05 min	Alpha
Pb – 214	26.8 min	Beta
Bi – 214	19.7 min	Beta

Po – 214	1.6×10^{-4} s	Alpha
Pb – 210	22 y	Beta
Bi – 210	5.01 d	Beta
Po – 210	138.4 d	Alpha
Pb – 206	Stable	-

1.2.2 Actinium Series

This series begins with U – 235 nuclei (half life 7.1×10^8 y), which is the longest half life comparing to other elements in this series and ends with Pb – 207, which is a stable element.

The elements of this series which are represented in table (1-3) are arranged according to the mass number indicated in (4n + 3) system [10].

Table (1-3): U-235 Decay Series [11, 12]

Nuclide	**Half – Life**	**Type of decay**
U – 235	7.10×10^8 y	Alpha
Th – 231	25.6 h	Beta
Pa – 231	3.98×10^4 y	Alpha
Ac – 227	22 y	Beta
Th – 227	18.17 d or 22 min	Alpha or Beta
Ra – 223	11.7 d	Alpha
Rn – 219	3.92 s	Alpha
Po – 215	1.83×10^{-3} s	Alpha
Pb – 211	36.1 min	Beta
Bi – 211	2.15 min	Alpha
Po – 211	0.52 s	Alpha
Ti – 207	4.79 min	Beta
Pb – 207	Stable	-

1.2.3 Th – 232 Series

Thorium was discovered by 'Berzelins', which is derived from the Scandinavian god 'Thor'.

This series begin with Th – 232 nuclei (half – life 1.39×10^{10} y) and end with Pb – 208 staple isotope.

The elements of this series which are represented in table (1-4) are arranged according to the mass number indicated in (4n) system [10].

Table (1-4): Th – 232 Decay Series [11, 12]

Nuclide	Half – life	Type of decay
Th – 232	1.39×10^{10} y	Alpha
Ra – 228	6.7 y	Beta
Ac – 228	6.13 h	Beta
Th – 228	1.9 y	Alpha
Ra – 224	3.64 d	Alpha
Rn – 220	54.5 s	Alpha
Po – 216	0.158 s	Alpha
Pb – 212	10.6 h	Beta
Bi – 212	60.6 min	Beta
Ti – 208	3.1min	Beta
Or		
Po – 212	3.0×10^{-7} s	Alpha
Pb – 208	Stable	-

1.2.4 Np – 237 Series

Np – 237 which a half – life (2.14×10^{6} y), which is much shorter than the geological age of the earth. Virtually all neptunium decayed within the first 50 millions of years after the earth was formed [10]. So ^{237}Np did not find in nature but it discovered in some stars spectrum [10].

1.3 Uranium

Uranium is a radioactive and chemical element, represent by (U) symbol. It was isolated in 1789 by a German chemist 'Martin Heinrich Klaproth' in a sample of pitchblends from Saxony. It was named after the planet Uranus, which had been discovered eight years earlier.

Natural Uranium contains 99.274% of U – 238, 0.7 of U-235 and 0.0055% of U – 234; they all have 92 protons in the nucleus but 146, 143 and 142 neutrons respectively. The half – lives of U – 238, U – 235, and U – 234 are 4.49×10^9 y, 7.10×10^8 y, and 2.48×10^5 y, respectively. The Natural Uranium is heavy element found in nature in different form and the human body contains (90 μg) as average result from food chain, about 66% are found in the skeleton, 16% in the liver, 8% in the kidneys, and 10% in the other tissues. The average annual intakes of uranium by adults are estimated to be (460 μg) from ingestion and (0.59 μg) from inhalation [13].

1.4 Depleted Uranium

Depleted Uranium is a toxic and radioactive by – product of the Uranium enrichment process. It was used as ammunition by USA and UK troops in an open environment for first time in history against Iraqi civilians and military target during (1991) [14, 15]. Uranium considered a new source of radioactivity that introduced into environment [16].

1.5 Solid State Nuclear Detectors SSNTDs

It is known as SSNTDs, the track recording properties of insulating solid were first reveled at AERE [17].

Solid state nuclear track detectors are insulating materials have the capabilities for measuring concentration and spatial distribution of isotopes

if they emit heavy nuclear particles, either directly or as a result of specific nuclear reactions [18] .

The damage of these particles along their path is called track (latent track), may become visible under an ordinary optical microscope after etching with suitable chemicals. There are two types of SSNTDs:

1. Organic detectors (polymers), and
2. Inorganic detectors (glasses or crystals).

These types differ in their sensitivity which increasing with increasing the atomic number of the incident particle than 20 [19] .

1.6 CR- 39 Detector

CR – 39 is the most sensitive of the nuclear track recording plastics . It was first discovered by Cartwright ,this detector consists of short polyallyle chains joined by links containing carbonate and die ethylene glycol groups into a dense three dimensional net work [20] . The chemical form of CR-39 is C12 H18 O7 it is illustrated in figure (1.1).

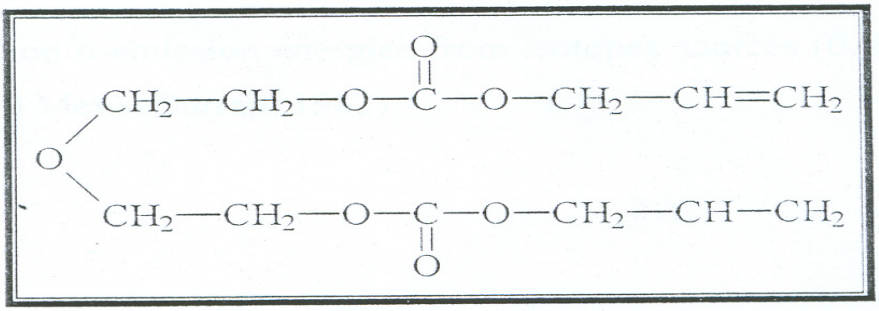

Fig. (1.1) the chemical form of CR-39 plastic [21] .

1.7 The Chemical Etching

Ionizing particles passing through polymeric track detectors produce latent track, which are trails of radiation damage [22]. The best means of observing the tracks is by etching the SSNTDs material with a chemical solution, which preferentially attacks the damaged material and

enlarges the original track to a size which is visible in the optical microscope [23].

The basic properties of the nuclear track etch technique are :

1. Adjustable hole diameter.
2. Single – particle drilling tool.
3. Uniform hole size, length and Orientation.
4. Adjustable hole density.
5. Applies to large variety of materials.

Essentially etching takes place via rapid dissolution of the disordered region of the track core, which exists in a state of higher free energy than the undamaged bulk material [24]. The reagent must be capable of slightly etching the bulk material, while at the same preferentially attacking the particle damage trails [25].

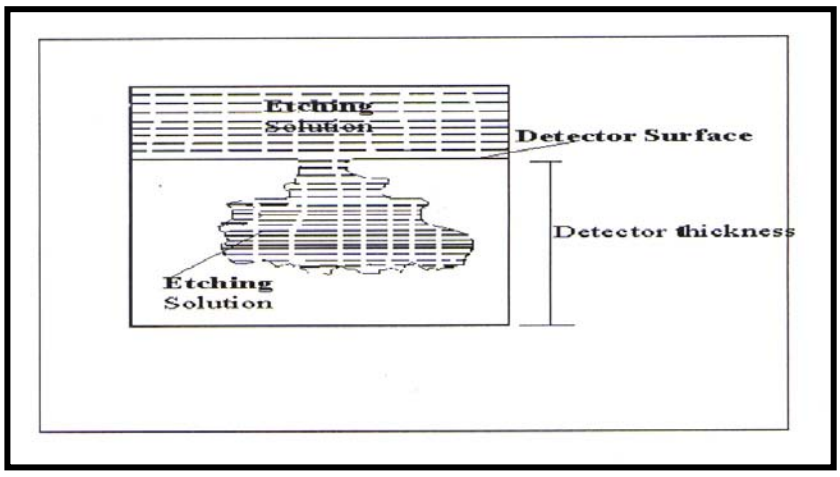

Fig. (1.2): shows the track etching process [25].

In fact the radiation damage trails produced by charged particles, consist of disordered structure which in turn are associated with a large free energy.

They therefore, represent a region of enhanced chemical activity. These regions get preferentially dissolve and their dimensions are enlarged when they are brought in contact with an etching solution. The etching conditions are optimized empirically for each detector material [24]. In general, etchants for polymeric detectors are frequently solutions of alkali

hydroxides such as NaOH or KOH with 1 - 12 N at 40 – 60°C [26, 27]. For glasses and minerals crystals such as quartz, mica, and certain pyroxenes etched in aqueous solutions of acids such as HF with ~ 48% concentration at 20°C [28, 29].

1.8 Track Geometry:

The geometry of track etching presented in the simplest case by the simultaneous action of two etching processes chemical dissolution along the particle track at a linear rate V_T and general attack on the etched surface and on the interior surface of the etched track at a lesser rate V_G as shown in fig (1.2)

There are many parameters used to describe the geometry of etched track, these are:

1. The full length of the latent track (L).
2. The thickness of the surface removed by etching (h).
3. The diameter of the etch pit (D).

The track length (L) at etching time (t) given by the following relation:

$$L = V_T . t \ldots (1\text{-}1)$$

The surface is also begin removed at a rate V_G, so that:

$$R = V_T . t - V_B . t \ldots . (1\text{-}2)$$

Where, V_T: track etch rate.

V_B: bulk etch rate.

When track etch rate (V_T) is constant and the particle penetrates normally, then the surface thickness (h) is given by:

$h = V_B . t$ … (1-3)

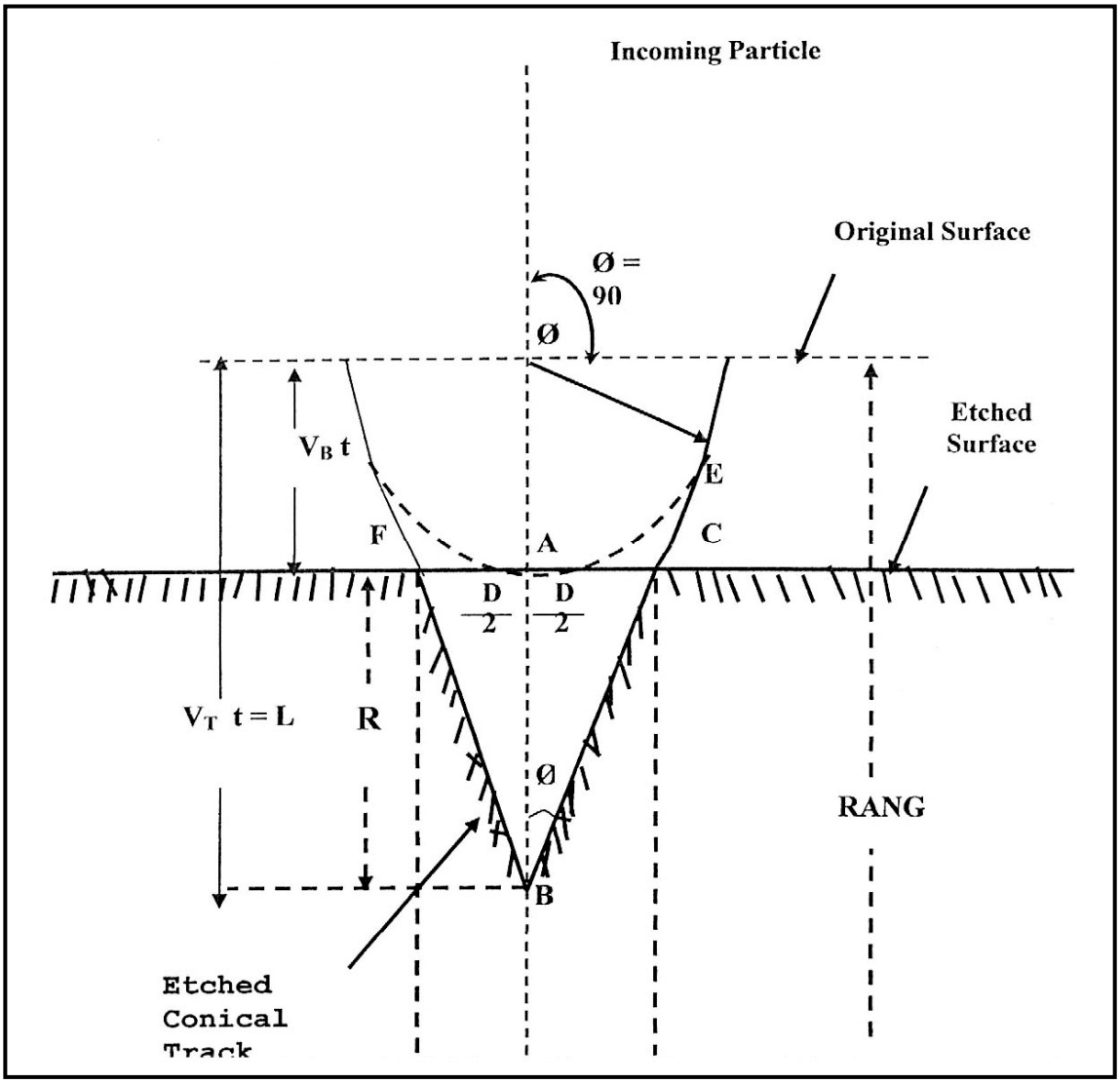

Fig (1-3): A track geometry for particle penetrates a detector material normally [3]

The diameter of etch pit is related to V_B and V_T according to the following equation:

$$D = 2V_G .t\left[(V_T - V_B)/(V_T + V_B)\right]^{\frac{1}{2}} \quad \ldots.(1.4)$$

Equation (1.4) shows that the diameter track (D) and the length of the track (L), depend essentially on the competitive effect of V_T and V_G.

When $V_T = V_B$, both (L) and (D) vanishes and then no track produces [3].

1.9 Biological Effects of Radiation

The Biological effects of radiation divide in to two groups: Somatic effects, which affect the irradiated person and genetic or hereditary effects, which affect the descendants of the irradiated individual, genetic effects are those related to the transmission of harmful hereditary information from one generation to the next [1].

The effects are also divided in two categories in term of the period between irradiation and appearance. (Short and long term effects) .

1.9.1 Long – Term Effects

Long – term effects may appear as a result of a chronic low – level exposure over a long period. These include genetic effects and other effects such as cancer, precancerous lesion, benign tumors, cataracts, skin changes, and congenital defects [30]

Evidence of injury from low or moderate doses of radiation may not show up for months or even years. For leukemia, the minimum time period between the radiation exposure and the appearance of disease (latency period) is 2 years. For solid tumors, the latency period is more than 5 years [31].

1.9.2 short ‑ Term Effects

May appear as a result of an acute irradiation. These include both immediate and delayed effects. High levels of a cute radiation exposure can result in death within a few hours, days or weeks [30].

An a acute exposure, if large enough, it can cause different health effects depending on the amount and the time to onset of exposure [32].

1.10 Radiation Induced Cancer

A long – term somatic effect is the damage of cells that are continually reproducing these cells are the most sensitive to radiation because any changes made in the parent cell's chromosome structure will be transmitted to it's daughters [33].

Also, radiation can affect the delicate chemistry of the cell causing changes in the rate of cell division or even the destruction of that cell. An event which causes a somatic cell to behave in this way is called a mutation.

The mutations in the reproductive cells translate the damage effects in to future generation. However, a mutation in a somatic cell has consequences only for the individual [33].

If the mutation in the somatic cell increases the rate of it's reproduction in an uncontrolled manner, then the number of daughter cells may increase rapidly on that area. In this case, daughter cells are often divided before reaching their mature state. The result then is an ever increasing number of cells that have no beneficial function to the body, yet are absorbing body nutrition at an increasing rate. The tissue could now be called a tumor [34].

If the cells remain in their place of origin and do not directly invade surrounding tissues, the tumor is said to be benign if the tumor invades neighboring tissue and causes distant secondary growths (called metastasis), It is known as malignant or cancer. Whether it is fatal or not depends on the tissue in which it is located, how rapidly it grows, and how soon it is detected [35] .

1.11 **Review of Previous Studies**

There are many studies have been performed to investigate and measure the concentrations of radioactive elements in biological samples (tissues, bone, blood, etc.) by using different techniques, some of these studies are abstracted as follows:

- A study has been presented by Welford & Baird in 1967 to measure the concentrations of uranium in human bones and tissues using CR-39 nuclear track detector to record the tracks of fission fragments, produced in the reaction $^{238}U(n, f)$ by bombarding the samples with neutrons emitted from neutron source. The concentration of uranium in bones and tissues was (0.12 ppm) [36].
- The studies that reviewed by Picer & Strohal in 1968 to determine the concentrations of uranium and thorium in the biological samples, included: bones, blood and urine by using neutron activation analysis. The results of the analysis of uranium and thorium contents for various samples were (4.1x10·l0gm/kg) and (2.3x10·^{10}gm/kg) for grind bones respectively, (5x10·^{10} gm/mol) and (5x10·^{10}gm/mol) for blood respectively, (3.1x10-10gm/mol) and (7.4x10-11gm/^{mol}) for urine respectively. As well as, the authors measured the gamma-ray emission of protactinium-233 and neptunium-239 by using NaI(Tl) scintillation counter [37].
- The studies have been reviewed by Nozaki, et al in 1970 to determine the concentrations of uranium in the bones of normal Japanese by neutron activation analysis technique using the $^{238}U\ (n,\gamma)^{239}U$ reaction. The concentrations of uranium ranged between (0.1-10 ppb) [38] .
- The studies that presented by Hamilton in 1970 to determine the concentrations of uranium in natural blood by using the delayed neutrons detection technique, where the average concentration was (0.84 ppb) [39].
- A study has been attempted by Hamilton in 1972 to determine the

concentrations of uranium in human tissues and bones by using the neutron activation analysis technique. The values of concentrations were between (0.16-0.6 ppb) in tissues, and (6.94 ppb) in bones [40].

- The research has been estimated by Koul & Chadderton m 1979 to measure the concentrations of uranium in the whole blood and plasma for healthy people and others injured by leukemia using the track etch technique. The concentrations for healthy people ranged between (0.35-0.6 ppb) in whole blood, and (0.11-0.82 ppb) in plasma. For casualty peoples, the concentrations ranged between (1.5-87 ppb) in whole blood, and (12-180 ppb) in plasma [41].
- The studies that reviewed by Parshad & Nagpaul in 1980 to determine the concentrations of uranium in natural blood of human by using track etch technique, where the concentrations ranged between (0.89-1.79 ppb) [42].
- The studies have been attempted by Igarashi, et al in 1985 to determine the concentrations of the natural uranium in tissues and bones samples for Tokyo city people by thermal neutrons irradiation method using mica detector. The lung tissue had the higher average concentration of uranium which equaled to (1 70x 10^{-7} ppm), and then the tissue of each of bone, heart, muscle, kidney, liver, cerebrum and spleen which reached ($85x10^{-7}$, $49x10^{-7}$, $43x10^{-7}$, $34x10^{-7}$, $24x10^{-7}$, $15x10^{-7}$, $13x10^{-7}$ ppm) respectively [43].
- The studies that presented by Singh, et al m 1986 to determine the concentrations of uranium in some vertebrate fossil bones that they used the alpha-auto radiographic method by usmg fission track technique. The concentrations of uranium ranged between (12.07-51.65 ppb) [44].
- The studies that reviewed by Segovia, et al in 1986 to determine the concentrations of uranium in whole blood and plasma samples from a gro_p of radiation exposed workers and another of leukemia patients by using the

track etch technique. The mean uranium concentration for the worker population was (0.98 ppb) in whole blood and (1.04 ppb) in plasma. For leukemia patients, the mean uranium concentration was (1.71 ppb) in plasma [45] .

- The studies have been reviewed by Reitz in 1995 to know the effect range of space radiation which absorbed by the space pioneers with deadly cancer diseases, and studied the possibilities to reduce this effect by increasing protection shielding thickness inside the spacecraft of between (1-30 cm), and this will still be very small in comparison with atmosphere thickness (-·-10 m water equivalent) that protect the earth from reaching these rays [46] .
- The research has been estimated by Al- Timimi in 2000 to measure the concentrations of depleted uranium in the human blood and tissues by using CR-39 nuclear track detector. The concentrations ranged between (0.0410.073 ppm) in the blood and (0.039-0.046 ppm) in the tissues [47].
- A study has been presented by Hussein in 2001 to determine the concentrations of depleted uranium in human tissues and bones samples by CR-39 nuclear track detector to record the tracks of fission fragments by using the irradiation method. The concentrations of depleted uranium in the selected samples ranged from (0.11-1.94 ppm) [48].
- A study has been attempted by Ibraheem in 2003 to determine the concentrations of depleted uranium in the injured human tissues by using CR-39 nuclear track detector, where the concentrations obtained ranged between (0.031-0.6 ppm) and the average concentration was (0.044 ppm) [49].
- A study has been presented by Al-Rubaii in 2004 to determine the concentrations of depleted uranium and investigate the radionuclide in biological samples; lung, kidney, stomach and colon collected before and after the 3 rd gulf war from cancered human living in Baghdad and neigh-

boring cities by CR-39 nuclear track detector using the natural exposure and irradiation method. For samples collected before war, the mean depleted uranium concentrations measured by natural exposure method for ovary, lung and kidney were (0.084 ppm), (077 ppm) and (0.075 ppm) respectively and the mean concentrations measured by irradiation method for the same samples ware (0.094 ppm), (0.094 ppm) and (0.073 ppm) respectively. While, for samples collected after war, the mean concentration measured by natural exposure were (0.976 ppm), (0.884 ppm) and (0.796 ppm) respectively and the mean concentrations measured by irradiation method were (0.991 ppm), (0.817 ppm) and (0.749 ppm) respectively. In the second part many radionuclides were investigated by using NaI(Tl) detector. These , nuclides included; K-40 having the highest specific activity, P-234 and U:235 giving evidence about depleted uranium, presence ofCs-137 and Cs-134 •. industrial radionuclides, Bi-214 short live decay product of Rn-222, Ac-228, ;TI-208 and Pb-212 [50].

1.12 Aim of the project

The purpose of the present work was radioactive detection in blood samples of control and patient groups taken from different locations of Iraq and measure effect of uranium in human chromosome and cellular division

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Chapter two
CYTOGENETIC ASPECT

2.1 History of Chromosome

Before 1950, it was generally believed that each human cell contained 48 chromosomes until (1956) when Tijo and Levan correctly concluded on the basis of their studies that the normal human somatic cell contains only 46 chromosomes.

They reached this conclusion according to results obtained from cell cultures of human lung embryonic tissue [51].

2.2 Cell Cycle

A eukaryotic cell cannot divide into two, the two into four, unless two processes alternate:

- doubling of its genome (DNA) in S phase (synthesis phase) of the cell cycle;
- halving of that genome during mitosis (M phase).

The period between M and S is called G1; that between S and M is G2. So, the cell cycle consists of:

- G1 = growth and preparation of the chromosomes for replication;
- S = synthesis of DNA and duplication of the centrosome;
- G2 = preparation for
- M = mitosis.

When a cell is in any phase of the cell cycle other than mitosis, it is often said to be in interphase [52] .

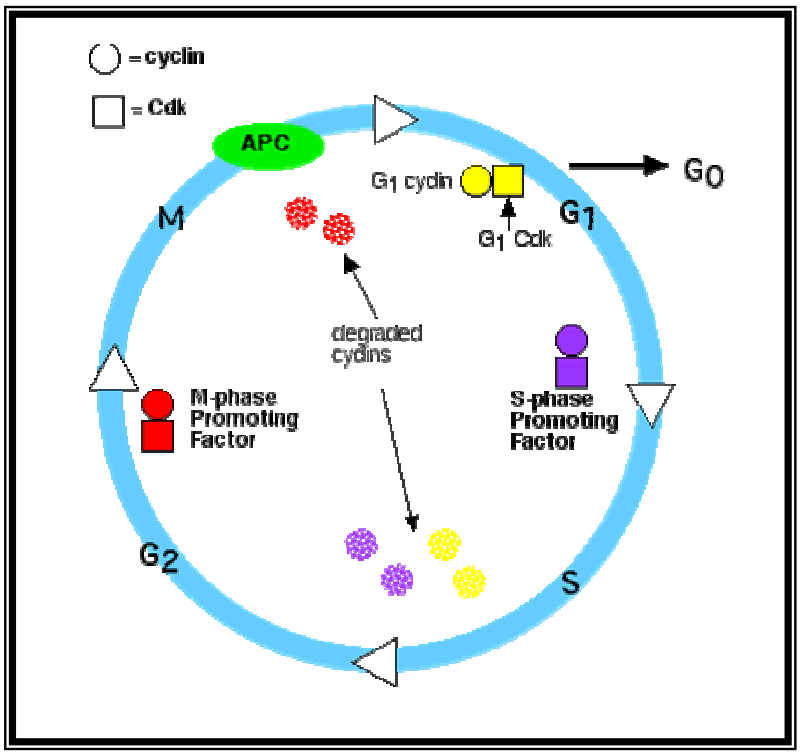

Fig (2-1): Cell cycle [52] .

2.3 Cytogenetic Study:

Cytogenetic analysis is widely used as an indicator system that allows detection of DNA damage induced by various mutagens and carcinogens. Therefore, the cytogenetic analysis is used for the detection of environmental, biological and industrial mutagens on the level of chromosome in man and animals .

Several parameters have been used in cytogenetic analysis including the chromosomal aberrations (CA), blastogenic index (BI), and mitotic index (MI). [53]

2.4 Chromosomes in the Cell:

Chromosomes words is drived from the Greek word means colored body where chroma (= color), soma (= body), this chromosomes are dense, long, thread – like bodies, located in the cell nucleus [54].

The chromosomes are the most critical part of the cell, since they contain the genetic information and instructions required for the cell to perform its function and to make copies of itself for reproduction purposes [55,56].

The chromosomes are a combination of DNA and basic protein known as 'histones', the DNA strands wind around the histones [54].

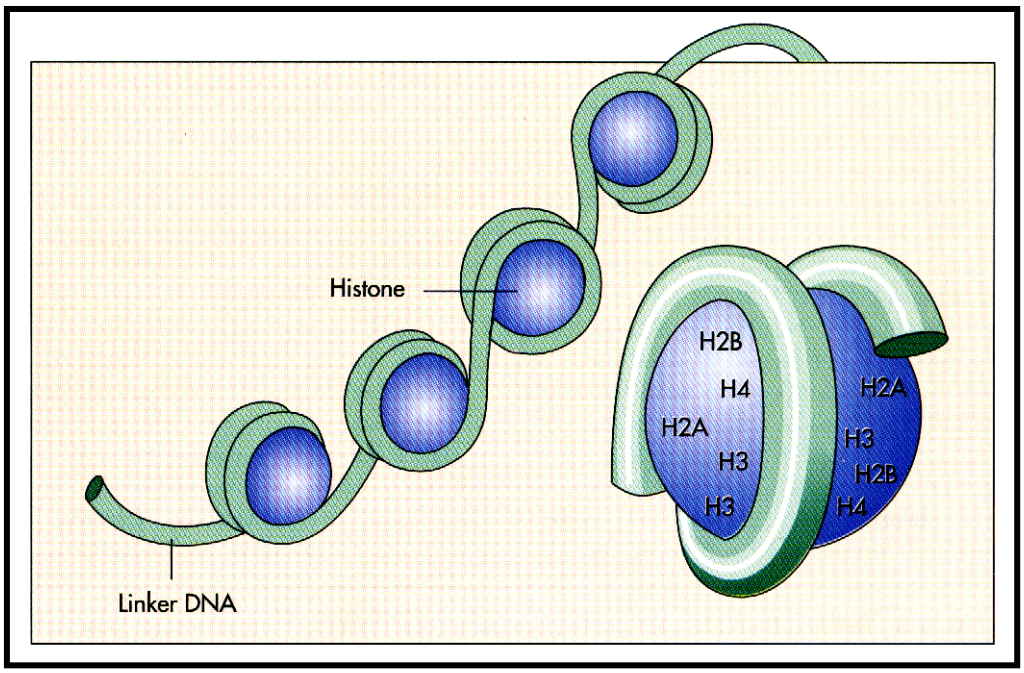

Fig (2-2): Winding Of DNA around Histones from A Helical Structure [57]

2.5 The Chromosome Study:

Any tissue with living nucleated cells, which undergo division, can be used for studying human chromosomes such as peripheral blood. Most commonly circulating lymphocytes from peripheral blood are used because they are convenient to obtain relatively long – lived, and can be removed from human subjects with minimal discomfort [58].

2.6 Effects of Ionizing Radiation on Chromosomes:

Many types of ionizing radiation produce similar effects in all living cells, which measurement in terms of dosage.

Genetic effect produced by ionizing radiations include increasing of chromosome damage become apparent in cellular division, the genes of the chromosomes may be locked, duplicated, inverted, or moved to a new position on the same chromosome [55,56].

Genetic damage to the chromosomes is observed as an alteration in chromosome structure [55,56].

2.7 Structural Chromosomes Aberrations:

Structural chromosomes aberrations that are caused by radiation this types of aberrations appeared on the whole chromosome, and it was the result of breaks that disrupt the continuity of one or more chromosomes, those occurring in somatic cells may increase the risk of cancer [59,60].

Structural chromosome abnormalities that are caused by radiation can be divided as the following: chromatid – type aberrations and chromosome – type aberrations [61].

2.7.1.1 Chromatid – Type Aberrations

These types of aberrations appeared on only single chromatid of chromosome. There are two important types of chromatid – aberration that are:

2.7.1.2 Gap Chromtid

Which appears as a non – staining and constricted region in the chromatid arm and the apparently 'broken' segments of the chromatid area in alignment.

2.7.1.3 Chromatid Breaks

Due to the discontinuity with displacement in the chromatid arm so that the broken chromatid ends are not aligned, the width of the non – staining region between the centric and a centric region is greater than the width of a chromatid the latter definition is used to distinguish between break chromatid and gab chromatid [61].

2.7.2 Chromosome – Types Aberrations

2.7.2.1 Ring Chromosome

Which appears when both ends of chromosome have been lost and the two broken ends have reunited to form a ring. There are two types of ring chromosome, which are

1. Acentric ring chromosome:

These are paired segments arise from interstitial deletion without acentromer region and which are joined to give aring [62].

2. Centric Ring chromosome:

These are paired segments arise from interstitial deletion with acentromer region and which are joined to give aring [63].

2.7.2.2 Dicentric or Polycentric chromosome

This arises from an exchange between two or more chromosomes, which results in the centric products reuniting in such a way to form a dicentric or polycentric structure. It is assumed that a dicentric will be accompanied by an acentric fragment and a tricentric by 2 a centric fragments [64].

2.8 Chromosomes Aberration Assay

Chromosome aberration are regarded as the most sensitive biological indicator of radiation induced damage and have been widely used over the past decades to estimated the risk associated with radiation exposure [65]. This procedure has been used to assess the radiation exposures in human populations ranging from occupational groups to the atomic bomb survivors in Japan [66].

2.9 Blastogenesis Index Assay

A blast cell is a relatively large cell with abundant RNA in the cytoplasm, a nucleus containing loosely arranged chromatin, and a prominent nucleous. Blast cells are active in synthesizing DNA and contain numerous polyribosomes in the cytoplasm of the lymphocytes [67].

Many of the known lymphocytes activators can be classified into non – specific activators (mitogens) which stimulate large number of lymphocytes and specific activators or antigens [68].

The blastogenic index is a ratio of blast cells to interphase nuclei in 1000 cell as in the following equation [69].

$$\mathrm{BI} = \frac{\mathrm{No.of\ non\ mitotic\ cells}}{1000\,\mathrm{cells}} \times 100 \ldots \text{(2-1)}$$

2.10 Mitotic Index Assay

It is used to express genotoxicity of chemical and physical agent .This assay shows whether the agent is a mutagen, in this case the amount of MI will be reduced, or the agent is a mitogen and in this case the amount of MI will be increased [70].

The mitotic index (MI) is the ratio of nuclei in the mitotic stage to interphase nuclei in a thousand cells as in the following equation [68].

$$\mathrm{MI} = \frac{\mathrm{No.of\ dividing\ cells}}{1000\,\mathrm{cell}} \times 100 \ldots \text{(2-2)}$$

Chapter three
MATERIALS AND METHODS

Introduction

This chapter expresses the methods to preparation of samples (blood) , contain materials , instrument and electric devise which used in this research for physics part and Biological part.

3.1 Physical part

3.1.1 Collection of Samples

Blood Samples were collected from different sites in Baghdad for control group, but the samples of patient group collected from different cities in Iraq, as shown in table (3-1) and (3-2).

Table (3-1): Location of Blood Samples for Control Group

Number of samples	City	Location
1	Baghdad	Al- ghazaliya
2	Baghdad	Al – zafraniya
3	Baghdad	Diyala – Bridge
4	Baghdad	Al – twitha
5	Baghdad	Al – twitha
6	Baghdad	Al – Baya
7	Baghdad	Al – twitha
8	Baghdad	Al – zafraniya
9	Baghdad	Al – Doura
10	Baghdad	Al – Doura
11	Baghdad	Diyala – Bridge
12	Baghdad	Al – zafraniya
13	Baghdad	Al – zafraniya

Table (3-2): Location of Blood Samples for Patient Group

Number of blood samples	City	Location
1	Baghdad	Doura
2	Baghdad	sadr city
3	Al - Kut	nomaniya
4	Dahuk	-
5	Al – Kut	nomaniya
6	Dahuk	-
7	Baghdad	Sadr city
8	Dahuk	-
9	Baghdad	Doura
10	Baghdad	Diyala – Bridge
11	Al – Kut	Al-Izah
12	Al – Kut	Al-Izah
13	Baghdad	Doura
14	Irbil	-
15	Basrah	Al – Ashaar
16	Baghdad	sadr city
17	Basrah	Al – Ashaar
18	Al – Kut	Al-Izah
19	Dahuk	-
20	Baghdad	Diyala – Bridge

3.1.2 Material and Apparatus

I. The Track Detectors: CR-39 track detector of thickness (250 μm) made by pershore modeling LTD. U.K. was used to record fission tracks.

II. Oven: Used to dry and ashing the blood samples the Oven of type *Thelco*

III. Sensitive Balance: Balance of type (Mettler carantia AE 163) Industrialization in Switzerland was used.

IV. Chemical Etching Solution: Chemical etching process was done by using 6.25 normality sodium hydroxide (NaOH) with temperature (60°C), which prepared as [71]:

$W = Weq \times N \times V$ ….(3.1)

Where:

W = the wight of NaOH in grams

Weq = equivalent wight of NaOH = addition of atomic wight of Na, O and H = 40

V = volume of distilled water = 250 ml

N = normality = 6.25

V. Water Bath: water bath of type 'Labsco' (Germany), which can operated over a rang of 20°C to 110°C, was used in the present work.

VI. Optical Microscope: To viewing and counting the etched track,the optical microscope was used (Bausch and Lomb, Japan), it is capable of giving magnifications of (100×).

VII. The Irradiation source: The irradiation source consists of a rod of (Am – Be) surrounded by a paraffin wax, the paraffin wax was used to moderating the fast neutrons to thermal neutrous energies. an (Am – Be) source which used for irradiation the blood samples with flux 5×10^3 n/cm^2.s. It emits fast neutrons from the (α, n) reaction such as:

$$^{9}\text{`Be} + \propto \rightarrow {}^{12}\text{C} + {}^{1}_{\circ}\text{n} + 5.76\,\text{Mev} \ldots(3.2)$$

3.1.3 Experimental Details for Uranium Concentration Measurement

In the present work, the measurement of Uranium concentrations of blood samples (control, patient) groupby using irradiation methods are:

3.1.4 Experimental Procedure for Blood Sample

Blood samples were taken from different cities of Iraq regions, as shown in table (3-1), (3-2).

Blood samples were dried by using oven. 0.5g of blood powder samples were mixed with 0.1g of methylcellulose (C_6 H_{10} O_5) used as a binder. The mixture was pressed into a pellet of 1 cm diameter and 1.5 mm thickness. The pellets were covered with (CR – 39) detector and put in a plate of paraffin wax at a distance of (5 cm) from the neutron source as shown fig. (3.1), with flounce of thermal neutron (3.024×10^9 n.cm^{-2}), obtain induced fission fragments from the equation (3-3) [72].

$$^{235}U + {}^{1}_{\circ}n_{Thermal} \rightarrow {}^{236}U + {}^{174}Kr + {}^{87}Ba + 0.85Mev \ldots \text{(3-3)}$$

After the irradiation time (7d), (CR – 39) detectors were etched in (N=6.25) NaOH solution at temperature of 60°C for (6h), the induced fission tracks density were recorded using the optical microscope.

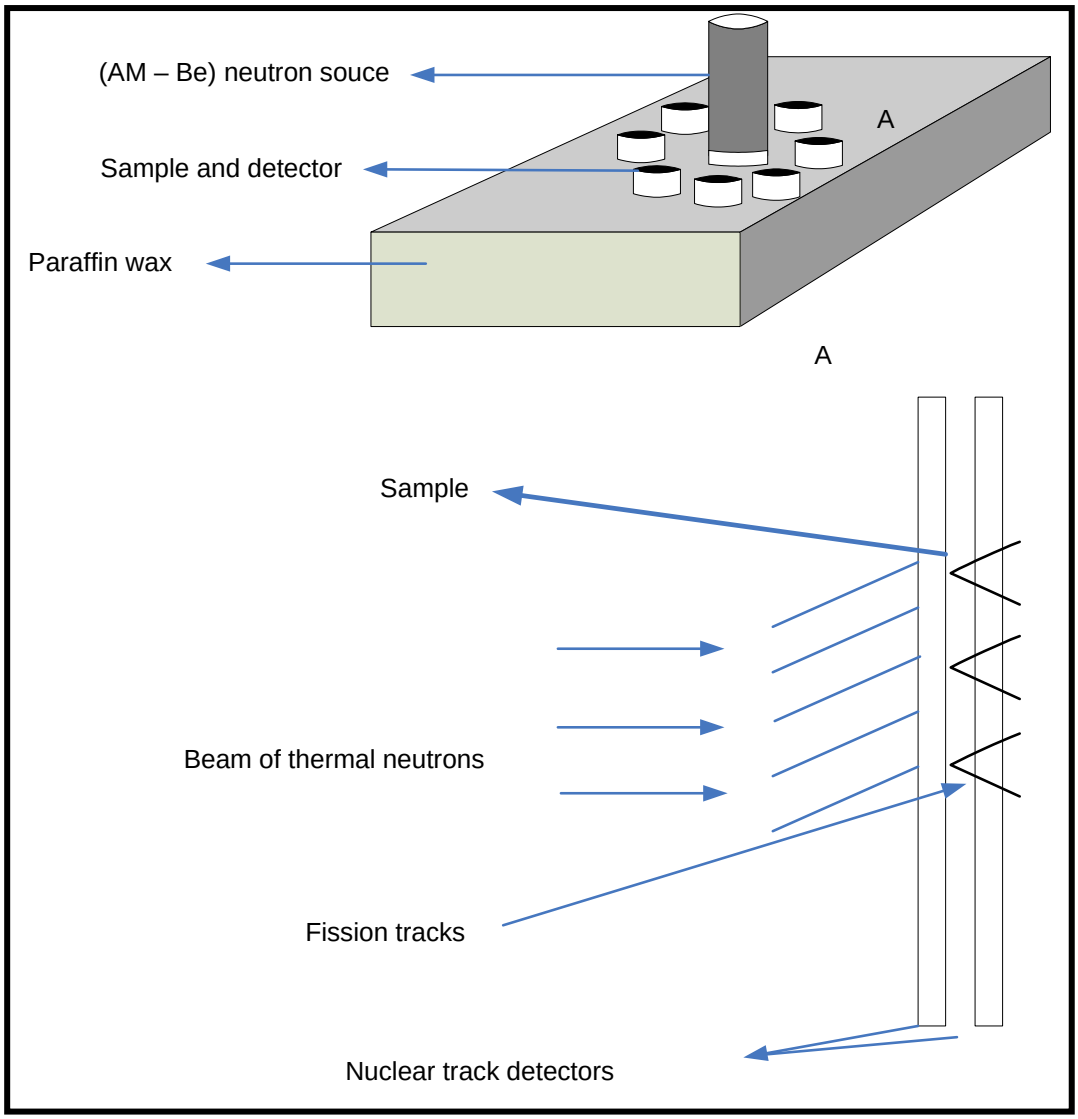

Fig. (3-1): The irradiation of detectors and samples to the neutron Source [72]

The density of the fission tracks (ρ) in the samples was calculated according to the following relation [73].

$$\text{Track density } (\rho) = \frac{\text{Average number of total pits (track)}}{\text{Area of field view}} \quad \ldots (3\text{-}4)$$

The Uranium concentrations in the blood samples were measured by comparison between track densities registed on the detectors of the sample pellet and that of standard geological sample pellets from the relation [19, 20, 74]:

$$C_x \text{ (sample)} / \rho_x \text{(sample)} = C_s \text{ (standard)} / \rho_s \text{ (standard)} \quad \ldots (3\text{-}5)$$

$$C_x = C_s \cdot \left(\frac{\rho_x}{\rho_s} \right) \ldots (3\text{-}6)$$

Fig (3.2) shows this relation for instance bloods samples.

Where

C_x: Uranium concentration in unknown sample (ppb).

C_s: Uranium concentration in standard sample (ppb).

ρ_x: track density of unknown sample (tracks/mm^2).

ρ_s: track density of standard sample (tracks/mm^2).

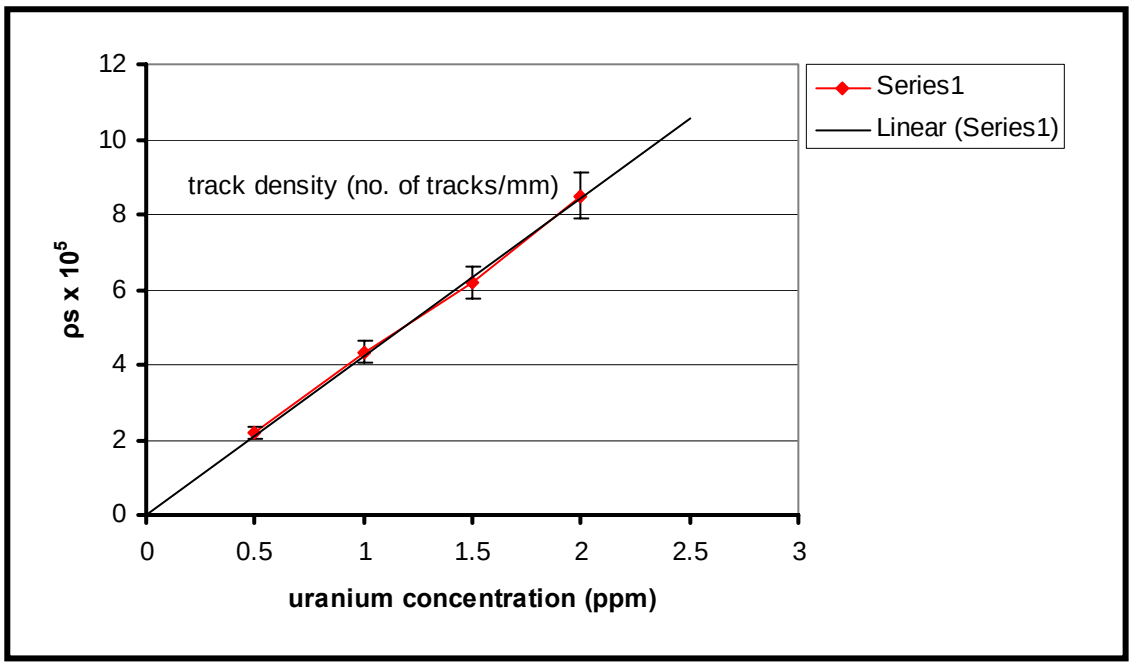

Fig. (3-2): The relation between track density and uranium concentration (ppb) for standard blood samples

3.2 Cytogenetic part

There are several material and instrument which used in this work.

3.2.1 Electric Instrument

1. Centerfuge.
2. PH meter.
3. Sensitive Blance.

4. Incubator.
5. Magnetic stirrer.
6. Optical microscope.

3.2.2 plastic and Glass Instrument

1. Slides.
2. Cover slides.
3. Nalgene filter.
4. Disposable syringe 5ml, 10ml, 1ml.
5. Plastic disposable test tube.
6. Maslc.
7. Gloves.

3.2.3 Chemical Materials

1. Human serum.
2. Phytohemoaggluthin (PHA).
3. Penicillin.
4. Streptomycin.
5. RPMI 1640 culture media powder with L – clutamin.
6. Heparin.
7. Colchicine.
8. Glatial Acetic Acid (CH_3 COOH).
9. Gimsa stain.
10. Dople distal water (D. D. W)

11. Potassium chloride (KCℓ).

12. Absolute methanol (CH_3 OH).

13. Oil immersion.

3.2.4 Preparation of Matters

3.2.4.1 Preparation of RPMI 1640 Medium.

a. RPMI 1640 (1000 ml)
b. Penicillin (1.0 ml)
c. Streptomycin (0.5 ml)
d. Human plasma (200 ml)

This matters above were mixed in a container of volume (1 letter) in a laminar flow cabinent the resulting culture media was filtered through a (0.22) μm pore size filter (Nalgen filter). At final I divided in disposable test tubes by estimate 4.5 ml for each tube and stored at – 20°C.

3.2.4.2 Preparation of Colchicine

One tablet of colchicin was dissolved by 10 ml of sterile deionized distilled water by using magnetic stirrer as stocks and (1 ml) of stock colchicine dissolved by (9 ml) of sterile deionized distilled water and store at – 20°C untile use.

0.2 ml for each tubes was added at last two hours (70) from acubation.

3.2.4.3 Preparations of Potassium Chloride Solution. Kcl (0.075) M (Hypotonic Solution)

Which is prepared from adding (5.587) gm of potassium chloride to (1000 ml) of sterile deionized distilled water to give constration of 0.0758M and it stored at 4°C.

3.2.4.4 Fixative Solution

Fixative solution was prepared on the same day by using three volumes of absolute methanol (CH_3 OH) with one volume of glacial acetic – acid (CH_3 COOH)

3.2.4.5 Preparation of Slides

The slides were washed at first with water in one direction to remove oil layer from these surfaces after that clear with cold water (D.D.W) and put in clear container that contain deionized water and store at – 20°C until used.

3.2.4.6 Preparation of Phytohemagglutinin Solution (PHA)

The preparation of this solution from dissolving (1 Vial) of (PHA) in (10 ml) of sterial deonized distilled water and store at – 20°C.

3.2.4.7 Prparation of Human Chromosomes [Cytogenetic Analysis]

1. Blood sample were collected from control group and patient group by disposable syring (5ml) contain the heparin to avoid clotting.
2. Blood culture: Blood samples were cultured inside Laminar air flow to prevent the contamination. Each tube contain 4.5 ml. of RPMI – 1640 culture medium, 0.5 ml of whole blood was in these tubes and 0.2 ml of PHA was added to each tube. Tubes were then tightly closed by a screw cap and were incubated in incubator at 37°C for 71 hours. The tubes were frequent shaking for one time at least every 24 hours.

3. Harvesting: At the end of 71 hours of the incubation period, the cell were treated with colchicine to arrest them in the metaphase stage by adding 0.1 ml of colchicine to each tube with mild shaking and transferred back to the incubator to incubate for 1 hour to complete the period of incubation (72 hours) at 37°C.

4. Treatment with 0.075 M KCℓ Hypotonic solution
    I. Culture tubes were removed from the incubator and centerfuged at 1500 rpm for 10 min. The cells were setteled at the bottom of the tubes and the supernatant was clear.
    II. By using pastor pipette, the supernatant was gently removed and the pellet was left in the bottom of tube with small amount of culture medium.
    III. The pellet was well skaken by using vortex and re – suspended in approximately 5 – 10 ml of hypotonic solution with continuously shaking.
    IV. The tubes were incubated in water bath at 37°C for 20 min.

5. Fixation: At the end of the period of water bath incubation the fixation process was as follows:
    I. The tubes were centrifuged at 1500 rpm for 10 min then the supernatant was discarded.
    II. By using the vortex the pellet was mixed and 5ml of freshly made fixative solution (Methanol acetic acid 3:1 volume/ volume) was added drop by drop with initial mixing.
    III. The tubes were centrifuged at 1500 rpm for ten min and then the supernatant was discarded.
    IV. The step 2 and 3 were repeated to 3 - 5 times in order to get a good fixation.

V. One ml of the fixation liquid was added to the cells after the last wash.

6. Slides preparation and staining

I. The cells were re-suspended and then dropped on the slides (2-3) from a suitable hight. The slides were then dried at room temperature for one day.

II. The slides were stained with Geimsa stain, where the slides covered with the stain and left for ten min and then washed with disttiled water.

7. Slides Screening

The slides were examined by using optical microscopic with magnification 10X to determine the number of mitotic cells and transforming cells. And by using magnifying power 100X with oil immersion the slides were examined to determine the chromosome aberration of Chromosomes.

8. Microscopically Examination

By using optical microscope, the mitotic cells at metaphase state were determined by using oil immersion at magnifying power 100 X where each chromosome examined in details and the banding for each chromosome.

I. Chromosomal Aberration (CA): Chromosomal aberrations were determined in 100 cells in the metaphase stage of cell cycle and the average is determined.

II. Mitotic Index Analysis (MI): The mitotic index was determined as a ratio of mitotic cells to interphase nuclei in 1000 cells as in equation (2-2).

III. Blastogenic Index Analysis (BI): The blastogenic index was determined as a ratio of blast cells to interphase nuclei in 1000 cell as in equation (2-1).

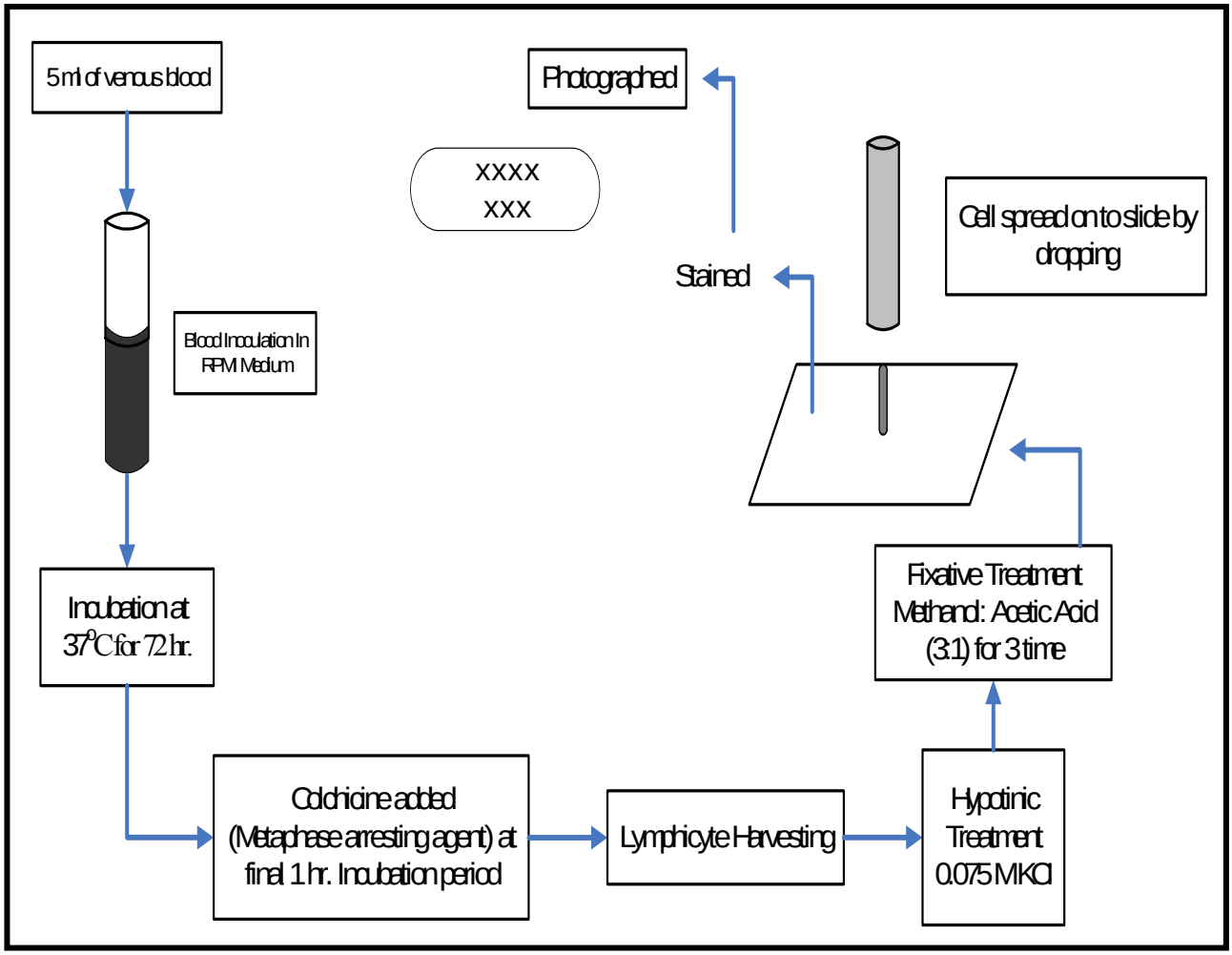

Fig (3-3): The schematic of the cytogenetic method for preparation of human chromosomes from peripheral blood

3.3 Statistical Analysis

All data in this study were analyzed by using the statistical program spss, Duncan test was carried out to know the significant differences among the means in this study.

Chapter Four
RESULTS AND DISCUSSION

4.1 Uranium Concentrations in Blood of Control Group

Table (4-1) and fig. (4-1) present uranium concentrations in irradiated blood samples measured by using the (CR – 39) detector. This samples collected form different sities of Baghdad.

Table (4 -1): Uranium Concentrations in Blood Samples of Control Group

No. of Samples	**Density of tracks (No. of tracks/mm^2)x10 ^{5}**	**Concentration of Uranium Cx (ppb)**
1	3.5 ± 0.5	0.933 ± 0.8
2	1.6 ± 0.4	0.506 ± 0.8
3	3.06 ± 0.4	0.816 ± 0.4
4	1.8 ± 0.5	0.48 ± 0.5
5	5.16 ± 0.4	1.3 ± 0.4
6	3.1 ± 0.4	0.82 ± 0.8
7	4.77 ± 0.4	1.27 ± 0.5
8	1.38 ± 0.5	0.36 ± 0.6
9	1.2 ± 0.8	0.33 ± 0.8
10	1.5 ± 0.8	0.4 ± 0.6
11	5.3 ± 0.8	1.41 ± 0.6
12	1.89 ± 0.8	0.504 ± 0.6
13	2.46 ± 0.5	0.656 ± 0.8
		Average = 0.7 ± 0.37

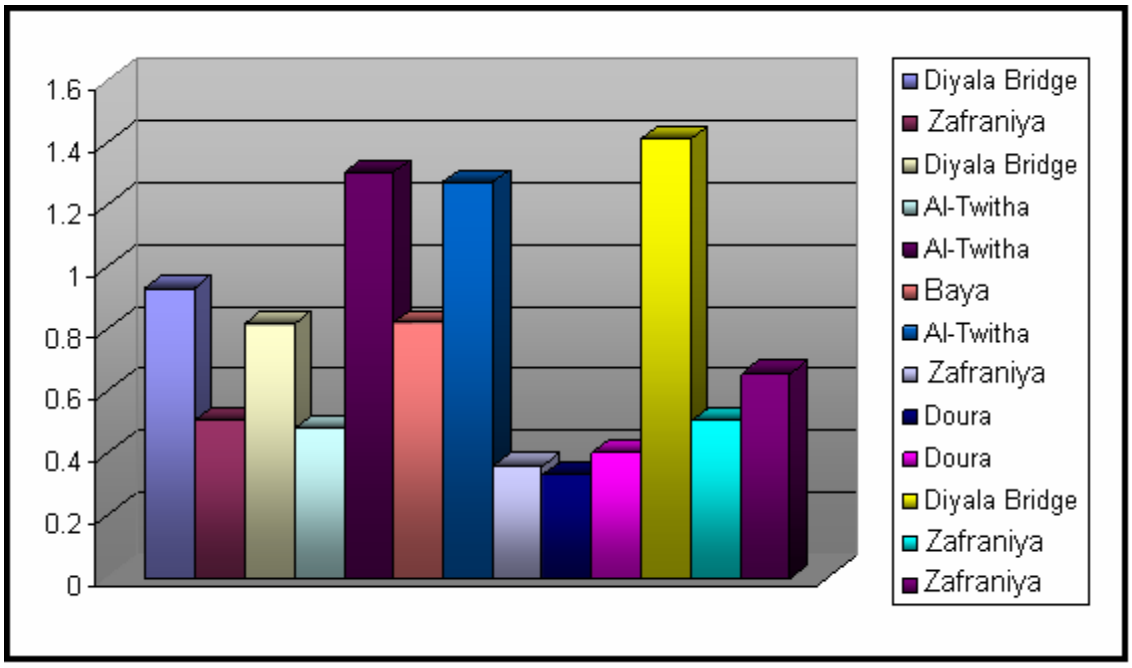

Fig. (4-1): Uranium Concentration in Blood Samples of Control Group

4.2 Uranium Concentrations in Blood of Patient Group

Table (4-2) and fig. (4-2) present Uranium concentrations for blood samples measured by using the irradiation method for (CR – 39 detector). This samples collected from different cities of Iraq.

Table (4-2): Uranium Concentrations in Blood Samples of Patient Group

Number samples	Density of tracks (No. of tracks/mm^2) ×10^5	Concentration of Uranium Cx (ppb)
1	5.2 ± 0.3	1.38 ± 0.4
2	6.83 ± 0.3	1.82 ± 0.5
3	7.0 ± 0.4	1.86 ± 0.4
4	5.75 ± 0.1	1.53 ± 0.3
5	6.02 ± 0.3	1.605 ± 0.6
6	5.5 ± 0.3	1.46 ± 0.6
7	6.2 ± 0.4	1.65 ± 0.5
8	4.0 ± 0.4	1.07 ± 0.5
9	5.9 ± 0.5	1.5 ± 0.6
10	7.1 ± 0.4	1.89 ± 0.7

11	5.58 ± 0.4	1.4 ± 0.4
12	5.52 ± 0.6	1.47 ± 0.6
13	5.38 ± 0.5	1.55 ± 0.5
14	2.89 ± 0.6	0.77 ± 0.4
15	5.38 ± 0.6	1.43 ± 0.4
16	6.00 ± 0.5	1.6 ± 0.6
17	7.04 ± 0.5	1.87 ± 0.4
18	4.97 ± 0.6	1.32 ± 0.4
19	2.18 ± 0.6	0.58 ± 0.4
20	6.3 ± 0.6	1.68 ± 0.6
		Average = 1.46 ± 0.33

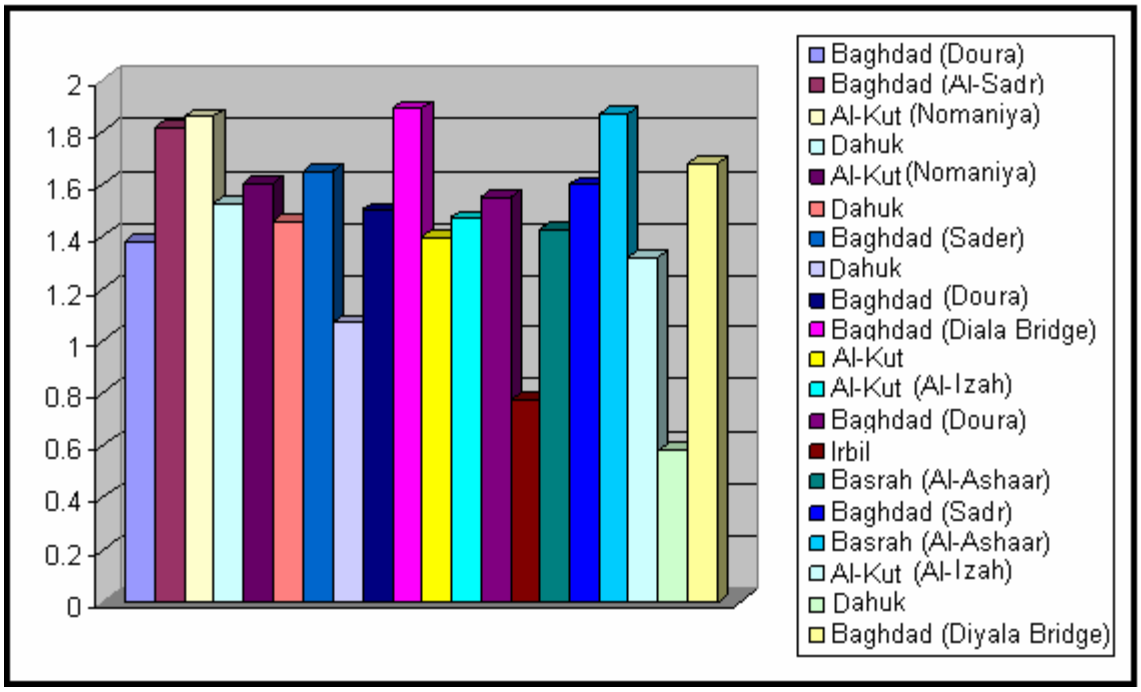

Fig. (4.2): Uranium Concentration in Blood Samples of Patient Group

4.3 Groups of Samples

We rearranged the control and patient samples in four groups as in table (4-3) .

Table (4-3): Uranium Concentrations in four Group

Number of sample	Type	Concentration of Uranium Cx (ppb)	
2	Control	0.506	Group 1 6 samples concentrations of Uranium (0 → 0.5 ppb)
12	Control	0.504	
4	Control	0.48	
8	Control	0.36	
9	Control	0.33	
10	Control	0.4	
1	Control	0.933	Group 2 6 samples concentrations of Uranium (0.6 →1 ppb)
3	Control	0.816	
6	Control	0. 82	
13	Control	0.656	
14	Patiant	0.77	
19	Patiant	0.58	
5	Control	1.37	Group 3 11- samples concentrations of Uranium (1.1 → 1.5 ppb)
7	Control	1.27	
11	Control	1.41	
18	Patient	1.32	
15	Patient	1.43	
12	Patient	1.47	
11	Patient	1.4	
9	Patient	1.5	
8	Patient	1.07	
6	Patient	1.46	
1	Patient	1.38	
20	Patient	1.68	Group 4 10 – samples
17	Patient	1.87	

16	Patient	1.6	concentrations of Uranium (1.6 → 2.0 ppb)
13	Patient	1.55	
10	Patient	1.89	
7	Patient	1.65	
5	Patient	1.605	
4	Patient	1.53	
3	Patient	1.86	
2	Patient	1.82	

4.4 Uranium and Total Chromosomal Aberrations (CA)

The cytogneic analysis that performed in blood samples of control and patient groups had indicated a highly significant difference among the total of the chromosomal aberrations of control group and patient group as shown in table (4-4).There were some types of chromosomal aberrations, which were not observed in the control groups, while it was observed in the patient group and other types of chromosomal aberrations appeared in both groups with significant difference among them.

To evaluate the effects of uranium on human chromosomes. Results showed that there were highly significant difference among the mean of total of chromosomal aberrations of Uranium concentrations. There was a clear gradual increase in the total of mean CA with increasing concentrations of uranium

Table (4-4): Total Chromosomal Aberrations.

Group	**Concentration of uranium (ppb) ± SD**	**No.**	**Mean** $\frac{\text{CA}}{100\,\text{Cell}}$ **± SD**
1	0.0 ± 0.0 - 0.5 ± 0.1	6	0 ± 0.0 c
2	0.6 ± 0.1 – 1.0 ± 0.2	6	0.175 ± 0.08 c
3	1.1 ± 0.3 - 1.5 ± 0.2	11	0.704 ± 0.11 b
4	1.6 ± 0.1 – 2.0 ± 0.07	10	1.143 ± 0.03a

- Different small letters in any picture (a, b, c...) in the same table refer to there are significant differences among the mean in this study.

4.4.1 Uranium and Gap Chromatid

To evaluate the effects of uranium on the gap chromatid, as shown in table (4-5) and Fig. (4-3) . there was a high correlation between increasing of uranium and gap chromatid. The gap chromatid for the 4th group concentration was significantly higher than the mean value of the 3rd group concentration, that show a significant increasing higher than the mean value of 2nd group concentration, and the later was higher than the mean value of the first concentration. Gap chromosome did not appear in the 1st group concentration .

Table (4-5): Gap Chromatid Aberrations

Group	Concentration of Uranium (ppb)	No.	Mean ± SD gap chromatid/ 100 cell
1	0 ± 0.0 - 0.5 ± 0.1	6	0.0 ± 0.0 d
2	0.6 ± 0.1 – 1.0 ± 0.2	6	11. 54 ± 5.9 c
3	1.1 ± 0.3 - 1.5 ± 0.2	11	25.704 ± 0.11 b
4	1.6 ± 0.1 – 2.0 ± 0.07	10	43.84 ± 0.03a

4.4.2 Uranium and Break Chromatid

Break chromatid did not appear in the 1st group of concentration as shown in table (4-6), and Fig. (4-3). but appeared in other concentrations, there was increasing of uranium concentrations and occurrence of break chromatid. The mean of break chromatid aberration of 4th group concentration has a significant increase higher than the mean of 2nd and 3rd groups concentration, respectively. And the mean of break chromatid aberration of the 3rd group concentration higher than mean of the 2nd group concentration and there was no significant difference between the first two concentration

Table (4-6): Break Chromtid Aberration

Group	Concentration of uranium (ppb)	No.	Mean ± SD break chromatid/ 100 cell
1	0 ± 0.0 - 0.5 ± 0.1	6	0.0 ± 0.0 c
2	0.6 ± 0.1 – 1.0 ± 0.2	6	1. 003 ± 0.5 c
3	1.1 ± 0.3 - 1.5 ± 0.2	11	2.29 ± 0.4 b
4	1.6 ± 0.1 – 2 ± 0.07	10	3.87 ± 0.1a

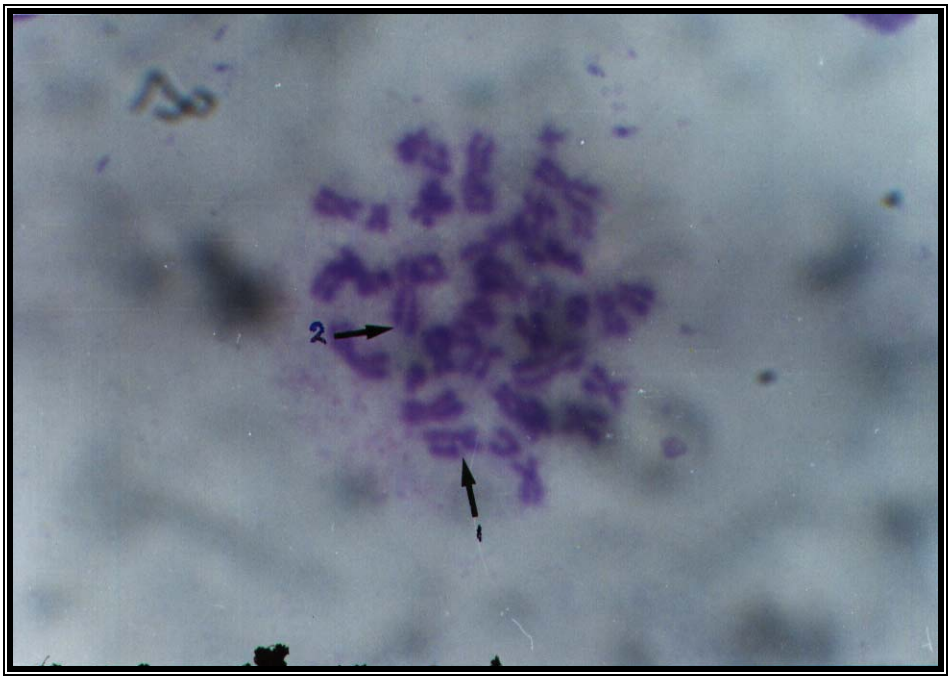

Fig. (4-3): Refer To The Chromosomes of female 46, xx in The Metaphase State, The Arrow (1) indicates the Gap Chromatid in Long Arms and Arrow (2) Indicates The Break Chromatid in the Long Arm of Chromosome.

4.4.3 Uranium and Gap Chromosome

The results of the effects of uranium on human chromosomes, as shown in table (4-7). that there was a high significant difference among the mean values of gap chromosome aberration . there was a clear significant increase in the mean value of gap chromosome of 4^{th} group concentration, which was significantly higher than the 2^{nd} and 3^{rd} groups concentrations, respectively.

There was no significant difference between the mean values of the 1^{st} and 2^{nd} groups concentrations.

Gap chromosome did not appear in 1^{st} group concentration,

Table (4-7): Gap Chromosome Aberration

Group	Concentration of uranium (ppb)	No.	Mean ± SD Gap chromatid/ 100 cell
1	0 ± 0.0 - 0.5 ± 0.1	6	0.0 ± 0.0 c
2	0.6 ± 0.1 – 1.0 ± 0.2	6	2.63 ± 1.5 c
3	1.1 ± 0.3 - 1.5 ± 0.2	11	7.89 ± 1.5 b
4	1.6 ± 0.1 – 2.0 ± 0.07	10	13.4 ± 0.3a

4.4.4 Uranium and Break Chromosome

To evaluate the effects of uranium in the break chromosome, the results of 4th group concentrations of uranium showed that there were high significant differences among the mean values of the break chromosome aberration as shown in table (4-8) . There was a clear significant increase in the mean value of the 4th group concentration, which was higher then the mean value of the 2nd ,3rd groups concentration, and this value was higher significantly than the mean value of 1st group concentration. While there were no significant differences between the break chromosome of 2nd and 3rd groups concentrations

Table (4-8): Break Chromosome Aberration.

Group	Concentration of uranium (ppb)	No.	Mean ± SD break chromosome/ 100 cell
1	0 ± 0.0 - 0.5 ± 0.1	6	0± 0.0 c
2	0.6 ± 0.1 – 1.0 ± 0.2	6	10.3 ± 1.4 b
3	1.1 ± 0.3 - 1.5 ± 0.2	11	16.9 ± 2.0 b
4	1.6 ± 0.1 – 2.0 ± 0.07	10	26.3 ± 0.7 a

4.4.5 Uranium and Ring Chromosome

Ring chromosome did not appear in the 1st group of concentration. The mean of ring chromosome aberration of 4th group concentration was

exhibited a significant increase higher than the mean of 3rd , 2nd and 1st group concentration as shown in table (4-9) and Fig (4-4) .

Table (4-9): Ring Chromosome Aberration.

Group	Concentration of uranium (ppb)	No.	Mean ± SD Ring chromosome/ 100 cell
1	0 ± 0.0 - 0.5 ± 0.1	6	0.0 ± 0.0 d
2	0.6 ± 0.1 – 1.0 ± 0.2	6	2.60 ± 0.4 c
3	1.1 ± 0.3 - 1.5 ± 0.2	11	5.41 ± 2.0 b
4	1.6 ± 0.1 – 2.0 ± 0.07	10	6.7 ± 0.17 a

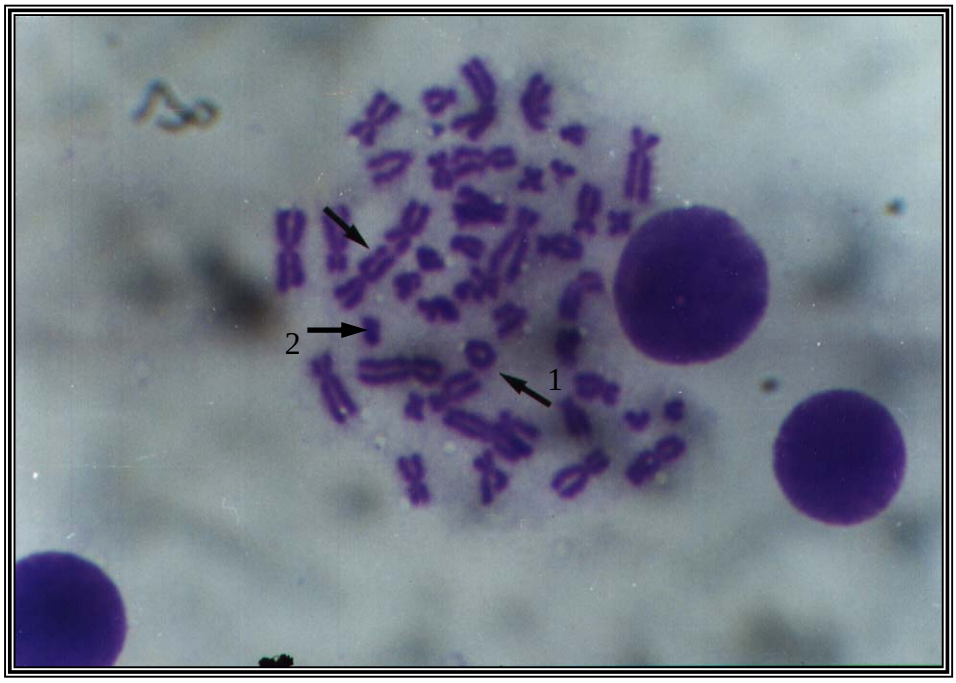

Fig (4-4 A): Refer to the Chromosomes of Male 46, Xy in The Metaphase, The 1st Arrow Indicates The Ring Chromosome, and 2nd Arrow Indicates Acentric Chromosome.

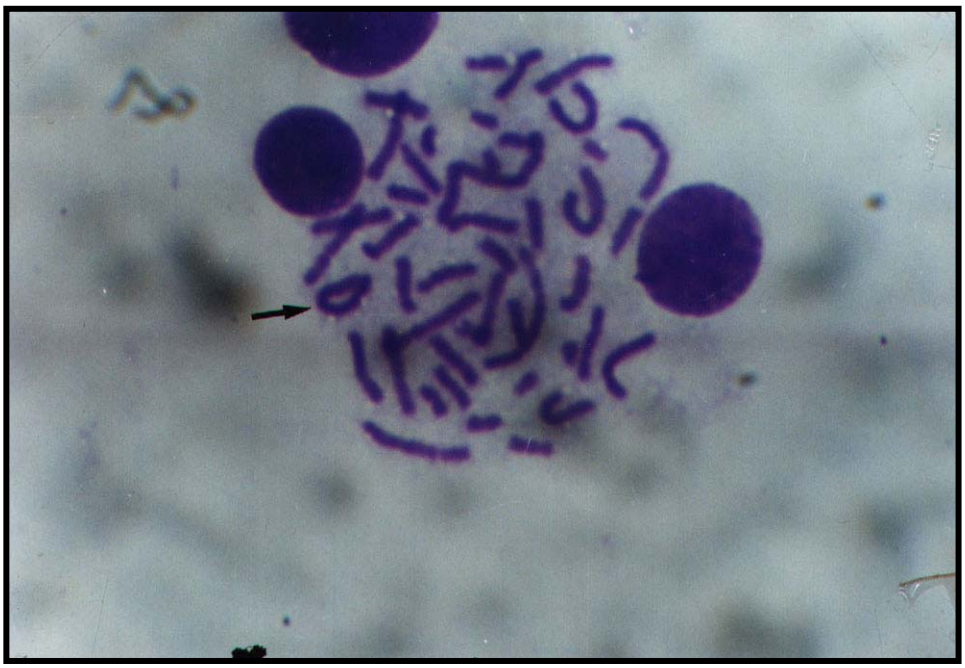

Fig (4-4 B): Refer to the Chromosomes of Male 46, Xy in The Metaphase State, The Arrow Indicates The Ring Chromosome.

4.4.6 Uranium and Dicentric Chromosome

The dicentric chromosome had progressive increment in its mean values with increasing of uranium concentration except for the 1st group of concentration of uranium as shown in table (4-10) and Fig (4-5) .The mean value of dicentric chromosome of 4th group concentration exhibited a significant increase higher then the 3rd and 2nd groups concentration . This type of aberration did not appear in the first group of concentration , so that uranium effected at a level (0.6 - 2.0). and there was no significant difference between the mean value of 1st group concentration and the 2nd group concentration .

Table (4-10): Dicentric Chromosome Aberration

Group	Concentration of uranium (ppb)	No.	Mean ±SD Dicentric Chromosome/100Cell
1	0 ± 0.0 - 0.5 ± 0.1	6	0.0 ± 0.0 c
2	0.6 ± 0.1 – 1.0 ± 0.2	6	1.3 ± 0.82 c
3	1.1 ± 0.3 - 1.5 ± 0.2	11	5.77 ± 1.13 b
4	1.6 ± 0.1 – 2.0 ± 0.07	10	9.28 ± 0.64 a

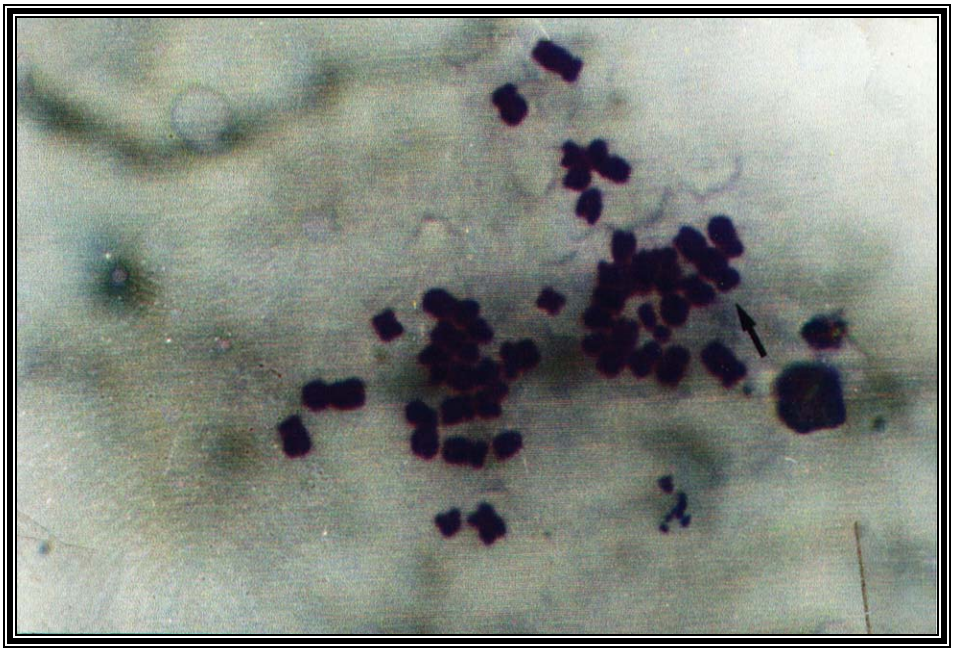

Fig (4-5): Refer to Chromosomes of female 46, xx in the Metaphase State, the Arrow Indicated the Dicentric Chromosome.

4.4.7 Uranium and Acentric Chromosome

Acentric chromosome did not appear in the 1st group of concentration as shown in Table (4-11) and Figure (4-4 A). But appeared in other higher Uranium concentrations. The mean value of acentric chromosome of 4th group concentration exhibited a significant increase higher than the mean values of 3ed and 2nd groups concentration, and there was no significant difference between the mean value of 1st and 2nd group concentration .

Table (4-11): Acentric Chromosome Aberration

Group	Concentration of U. (ppb)	NO.	Mean ±SD Acentric chromosome/ 100 cell
1	0 ± 0.0 - 0.5 ± 0.1	6	0 ± 0.0 c
2	0.6 ± 0.1 – 1.0 ± 0.2	6	1.33 ± 0.84 c
3	1.1 ± 0.3 - 1.5 ± 0.2	11	5.77 ± 1.15 b
4	1.6 ± 0.1 – 2.0 ± 0.07	10	9.98 ± 0.64 a

4.5 Uranium and Mitotic Index (MI)

To evaluate the effects of uranium on the mitotic index, for the 4th group of concentration of uranium showed there were no significant difference for three 1st group among the means values of the MI, but at the 4th group it was significant difference, as shown in table (4-12).

Table (4-12): Mitotic Index

Group	Concentration of uranium (ppb) ± SD	NO.	Mean $\frac{\text{MI}}{1000\,\text{Cell}}$ ± SD
1	0 ± 0.0 - 0.5 ± 0.1	6	0.316 ± 0.03 b
2	0.6 ± 0.1 – 1.0 ± 0.2	6	0.616 ± 0.12 b
3	1.1 ± 0.3 - 1.5 ± 0.2	11	1.136 ± 0.09 b
4	1.6 ± 0.1 – 2.0 ± 0.07	10	2.88 ± 0.46 a

4.6 Uranium and Blastogenic Index (BI)

The results show a high significant increase for the first three groups concentration of uranium, but at the 4th group there is a significant decreasing in the mean BI of them. As shown in table (4 - 13).

Table (4 -13): Blastogenic Index

Group	**Concentration of uranium (ppb)**	**No.**	**Mean** $\frac{\text{BI}}{1000\,\text{Cell}}$ **±SD**
1	0 ± 0.0 - 0.5 ± 0.1	6	8.67 ± 0.01 d
2	0.6 ± 0.1 – 1.0 ± 0.2	6	150.03 ± 89.3 c
3	1.1 ± 0.3 - 1.5 ± 0.2	11	492.8 ± 95. b
4	1.6 ± 0.1 – 2.0 ± 0.07	10	356.6 ± 9.4 a

4.7 Discussion

4.7.1 Uranium Concentration in Blood and Chromosomal Aberrations

The uranium concentrations it is found that of control group as with average is (0.7 ±0.37ppb). The maximum uranium concentrations were (1.41 and 1.3ppb) in Diyala Bridge and Al-twitha in Baghdad respectively as shown in fig.(4.1) .which indicate that this sites are higher contamination with uranium than other regions because its nearest from IAEA . The minimum concentration of control group in Al-Zapharania with concentration (0.33ppb).

The uranium concentrations for blood samples of patient group were with average (1.46 ±0.33ppb).The maximum of uranium concentration were (1.89 and 1.86ppb) in Diyala Bridge and Al-Kut as shown in fig. (4.2) which indicate that this sites are higher contamination than other regions and the minimum concentration were 0.55ppb in Dahuk.

In this study, there was a significant increase in the total chromosomal aberrations (CA) for patient group . These increments were increased with increasing of uranium concentrations, which proved that the capacity of effects the genetic material. The uranium causes damage chromosomes is further complicated by the concurrent exposure to both the chemical aspect (heavey metal) and the radiological aspect because uranium is radioactive and chemically toxic.

4.7.2 Uranium and the Induction of Chromatid – Types Aberrations:

First control group exhibit no significant difference in break chromatid but the increase in the (2^{nd}, 3^{rd}, 4^{th}) groups with uranium concentration increase.

The gap chromatid also scored a highly significant increase than the break chromatid.

Chromatid – type aberrations of the chromosomes may occur because:

1. The chemical behavior of uranium on it, where chromosome aberrations induced by chemical agents are almost produced during S – phase. However, the majority of aberration will be of the chromoatid – type [75,76].
2. The radiological effects of uranium on the cell, where it believed that the ionizing radiation causes chromatid – types aberration when it hits the cell during the G2 stage of cell cycle [77].

4.7.3 Uranium and the Induction of Chromosome – Types Aberrations

The (2^{nd}, 3^{rd}, 4^{th}) groups showed a significant increasing in the gap and break chromosome as compared with the first group. These aberrations may occur because of the ionizing radiation emitted from uranium which

hits the cell during G1 stage of cell cycle, where the ionizing radiation cause these types of chromosomal aberrations [78] .

these types of structural chromosomal aberrations (gap and break chromatid, gap and break chromosome) had appeared in the control groups, that may be due to the exposure of the persons to the ionizing radiation from different sources such as diagnostic x- rays, cosmic rays, or exposure to chemical agent during their life [79].

4.7.4 Ring Chromosome

This type of aberrations appeared in (2^{nd}, 3^{rd}, 4^{th}) groups with a gradual significant increase as the concentration increase except for the 1^{st} group it was no significant in the mean value of this aberration. This may indicate that this type of chromosomal aberration had a threshold level of uranium concentration to occur, this aberration may have occurred due to the action of ionizing radiation on the cell during G_1 stage of cell. This type of aberration appeared in people who lived in the contaminated areas in Iraq [78].

4.7.5 Dicentric Chromosome

Dicentric aberration did not appear in the first group of concentration of uranium, but it exhibit a gradual highly significant increase with increasing the concentration of uranium, which was started with 2^{nd} group. This may indicate that this type of chromosomal aberration had a threshold level of uranium concentration to occur.

4.7.6 Acentric Chromosome

This aberration did not appear in the first group, and exhibit a gradual significant increase with increasing of uranium concentrations which beginning with 2^{nd} group. This may indicate that this type of chromosomal aberrations had a threshold level of Uranium concentration to occur. This

aberration may have occurred due to the action of ionizing radiation on the cell during G_1 stage of cell .

4.7.7 Effects of Uranium on the Mitotic Index (MI).

The mitotic index assay was used to evaluate the genotoxicity of physical and chemical material, so we used this assay to evaluate the effect of uranium concentration on the human Lymphocyte cell.

For the patient the uranium causes a high difference in the mitotic index as compared with control groups. There was a significant gradual increase in the mitotic index 3^{rd} and 4^{th} group of uranium .

4.7.8 Effects of Uranium on the Blastogenic Index (BI).

We used this assay to evaluate the effects of different uranium concentrations on the lymphocyte cells. The uranium causes a high significant difference in the Blastogenic index compared to the control group. For patient group in the present study noticed that there was a significant increase in the Blastogenic for the first three groups. This behavior of uranium is similar to behavior of mitogene agents (non – specific activators) which stimulates large number of lymphocyte cell to form blast cells. while the highest concentration of uranium, there was a clear significant decrease in BI in blood samples. This indicate that the behavior of uranium of the higher concentrations is same as the behavior of specific activator or antigens that stimulate for fewer cells to transform to the blast cells [67].

4.8 Conclusions

The discussion of the results, which are obtained from this study leads to the following conclusions:

1. Effects of uranium on the human chromosomes increase with increasing uranium concentrations.
2. Uranium has cytogentic effects in lymphocyte cells of human peripheral blood.
3. Uranium induced different types of structural chromosomal aberrations.
4. Chromosomal aberrations had a threshold level of uranium to occur (Level= 0.6) .

4.9 Future Studies

1. Study the effects of uranium on the micronuclei in human peripheral blood samples.
2. Study the effects of uranium on the sister chromatid exchange.

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الخـــلاصة

الهدف من البحث هو حساب تراكيز اليورانيوم في نماذج من الدم أخذت من مجموعتين (أصحاء و مرضى) وتم قياس التأثير الإشعاعي على الكروموسومات ولانقسام الخلوي.

يتضمن البحث جزئين:

1. الجزء الأول: إيجاد تراكيز اليورانيوم باستخدام كاشف الأثر CR – 39 حيث استخدم التفاعل U – 235 (n, f) U – 236 كمصدر لشظايا الانشطار النووي وذلك بقصف نواه اليورانيوم U – 235 بالنيوترونات الحرارية من المصدر النيوتروني (Am – Be) بفيض نيوتروني حراري ($5 \times 10^3 n\ cm^{-2} s^{-1}$). وتم مقارنة التراكيز المحسوبة لتلك النماذج مع نماذج قياسية تم تحضيرها. ووجد إن أعلى تركيز لليورانيوم هو (1.89 ppb) في منطقة جسر ديالى، واقل تركيز لليورانيم كان في منطقة الزعفرانية حيث بلغ (0.33 ppb).
2. الجزء الثاني: تضمنت دراسة ثلاثة معايير وراثية مستخدمه وهي تحليل الانحرافات الكروموسوميه (CA)، اختيار معامل الانقسام الخلوي (MI)، واختيار معامل التحول (BI)، لدراسة التأثيرات البايولوجية لليورانيوم حيث أظهرت نتائج الدراسة الحالية إن اليورانيوم له تأثيراً معنوياً على الكروموسومات البشرية كما هو متمثل باستحثاث التغيرات الكروموسومية والتي تشمل التغيرات الكروماتيدية (Gab and Break Chromatid Aberration) والتغيرات الكروموسومية التركيبية (Gab Break and Ring Chromosome Aberration) وكذلك (Dicentric, acentric Chromosome Aberration) هذه التغيرات سجلت زيادة معنوية مع مجاميع السيطرة.

من ناحية أخرى إن اليورانيم يؤثر وبشكل معنوي على الخلايا الليمفاوية لدم الإنسان من خلال تأثيره على معاملات الانقسام وتحول الخلايا (MI and BI) حيث كان هناك زيادة معنوية في MI، BI مما يعطي مؤشراً على قابلية اليورانيوم للعمل كالعوامل المحفزة للانقسام عند التراكيز (0.5 – 1.5 ppb) بينما يعطي التركيز الأخير (1.6 – 2 ppb) انخفاضاً معنوياً في MI، BI مما يعطي مؤشراً على قابلية اليورانيوم للعمل كالعوامل المطفره عند هذه التراكيز.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين/ كلية العلوم
قسم الفيزياء

الكشف عن المستوى الإشعاعي في دم المرضى المصابين بالإمراض السرطانية باستخدام كاشف الأثر وتاثيرة على مورثات الخلية

رسالة
مقدمة الى كلية العلوم في جامعة النهرين
وهي جزء من متطلبات نيل درجة ماجستير علوم
في الفيزياء

إعداد
أثير قاسم مريوش الربيعي
(بكالوريوس ٢٠٠٤)

ربيع الأول ١٤٢٨ هـ
آذار 2007 م