Electron Microscopic Ultra Structural Study of Alteration in Leukocytes of Patients Having Leukemia Disease

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الخلاصة

صممت الدراسة الحالية لتسليط الضوء على التغيرات في التركيب المستدق لخلايا الدم البيض عند المرضى المصابون بمرض ابيضاض الدم . أوضحت الدراسة وجود خلايا بيض غير ناضجة مع حصول تغيرات عديدة في التركيب الخلوي المستدق فضلا عن ظهور حالة الالتصاق في مجموعة من خلايا الدم .معظم الدراسات الأخيرة بينت وجود علاقة بين التعرض لمادة اليورانيوم والمضاعفات الصحية.

Abstract

This study was design to put a light on the ultra-structural changes in leukocyte of patient having leukemia disease. The result demonstrates a presence of immature leukocyte with several changes in their structure besides adherence phenomena in group of leukocyte cells was seen. Most studies reported in 2020 showed a positive association between uranium exposure and adverse health outcomes.

Key word: Leukemia , Leukocyte, Ultra structural, uranium, exposure.

Introduction

Basrah is the second largest city in Iraq; It has been exposed to massive environmental pollution during past years including depleted uranium (DU) as a consequence of military conflicts. Many studies have reported an increase in incidence of cancer in Basrah**(1)**.It is challenged with a range of environmental problems that are both immediate and severe; some can be directly linked with the effects of recent military struggle **(2).** According to the Pentagon, about 300 tons of depleted uranium weapons were fired in the Gulf war of 1991**(3)**. DU decays mainly through emission of alpha particles that affect internal body cells (which are more susceptible to the ionizing effects of alpha radiation) when DU is ingested or inhaled. Risks for other radiation induced cancers, including leukemia, lung cancer**(4)**. Despite this, many studies suggested that the use of DU during 1991 and 2003 had resulted in a significant increase in the incidence of malignant disease in Basrah**(5)**. In another study by Abdalhadi *et. al*.(2017) results showed that there were immature leucocytes present with a varied structure as compared to their normal structure. Adherence phenomenon was observed in this group of leucocytes **(6).** Most studies reported in 2020 showed a positive association between uranium exposure and adverse health outcomes**(7).** To the best of our knowledge about the effects of Gulf war on Iraqi people, there is no evidence concerning its effects on the ultrastructure of leukocytes have been taken into account, this study was design to put a light on its effects on leukocytes.

Materials and Methods

Blood for transmission electron microscopy study were collected from a total of 4 leukemic children (from 8 to 10 years old) presented to the Basra Hospital during 2002. Samples for ultrastructural studies were prepared by centrifuging heparinized blood samples at 1000 rpm for eight to ten minutes according with procedure in **(8)** with some modification , aspirating the buffy coat, the cells were washed in phosphate buffered saline solution. The cell pellet was suspended in 2.5%phosphate buffered glutaraldehyde at pH 7.4, fixed for 3h at 4^5C. Additionally, the pellet samples were washed twice in phosphate buffered saline solution, post-fixed with phosphate buffered 1 % osmium tetroxide, dehydrating in graded alcohol, and propylene oxide. After infiltration with an araldite propylene oxide mixture for 24 h., the specimens were embedded in araldite according to the procedure**(8)**. Silver-gold interference sections were obtained on a Jung Ultra microtome, placed on a mesh grid, stained with uranyl acetate and lead citrate, and examined with Philips 210 transmission microscope in Al-Nahreen University.

Results:

Transmission electron microscopic examination display several changes in the blood of patients represented by the followings:

1-Agglomeration of leukocytes were observed (fig1, 2, 3, 4) .

2-Immature leukocytes cells (blast cells) were typified by large cells with high nuclear to cytoplasmic ratios, Nuclei were large and varied from oval to irregular in shape The chromatin was finely granular. **A** narrow rim of condensed chromatin accentuated the nuclear outline, finely granular chromatin, prominent nucleolus with high electron density chromatin, and relatively few but variable numbers of cytoplasmic organelles such as mitochondria, ribosomes, Golgi complex and endoplasmic reticulum (fig 2).

3-Progressive nuclear indentation and segmentation, and the appearance of cytoplasmic granules and glycogen aggregates were observed in myeloid cells (fig 3).

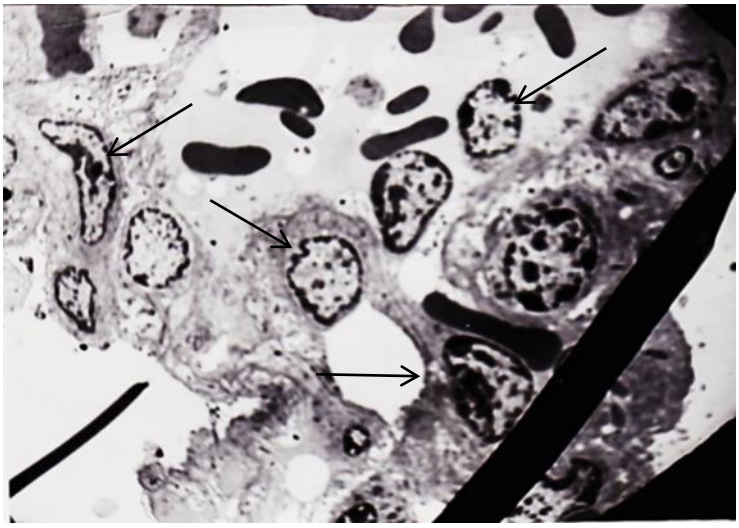
4- Cells were usually round, but had several irregular surface projections or microvilli with increment of electron density of mitochondria and increment of nucleolus number (fig4).

5-Immature leukocyte was modified into phagocytic cells since RBC was found in their cytoplasm (fig 5).

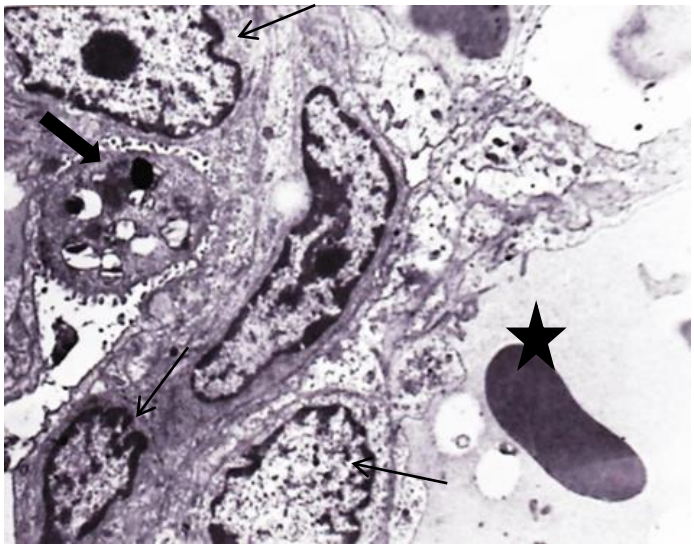
5- Long cisternae of dilated endoplasmic reticulum were seen in some cells (most of the cells), a few small to medium-sized dense granules were found in most cells. Also apoptotic leukocyte was found (fig 6).

6-Formation of chromatin bridges between two different leukocytes besides lipid droplets and cytoplasmic vacuoles present (fig 8) .

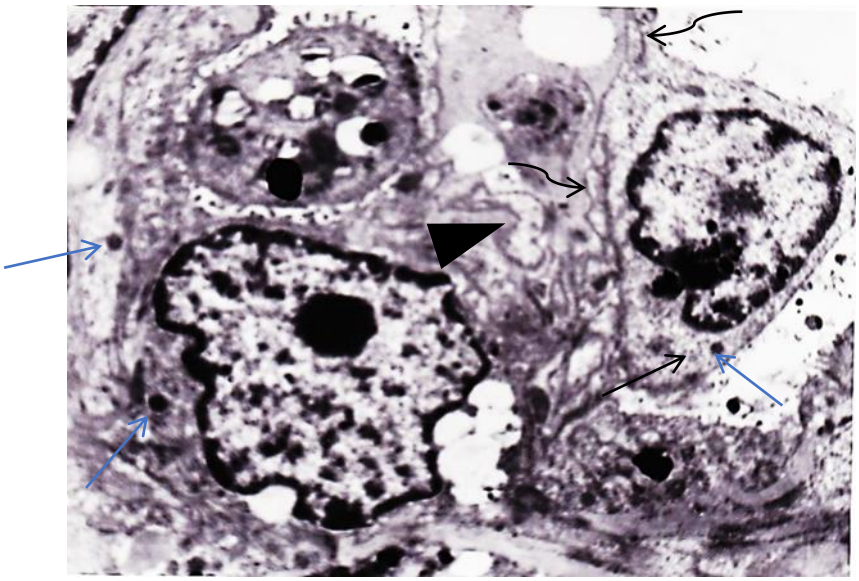
7- Erythroid cells had cytoplasmic ferritin aggregates and increased cytoplasmic density that corresponded to hemoglobin synthesis (fig 9).



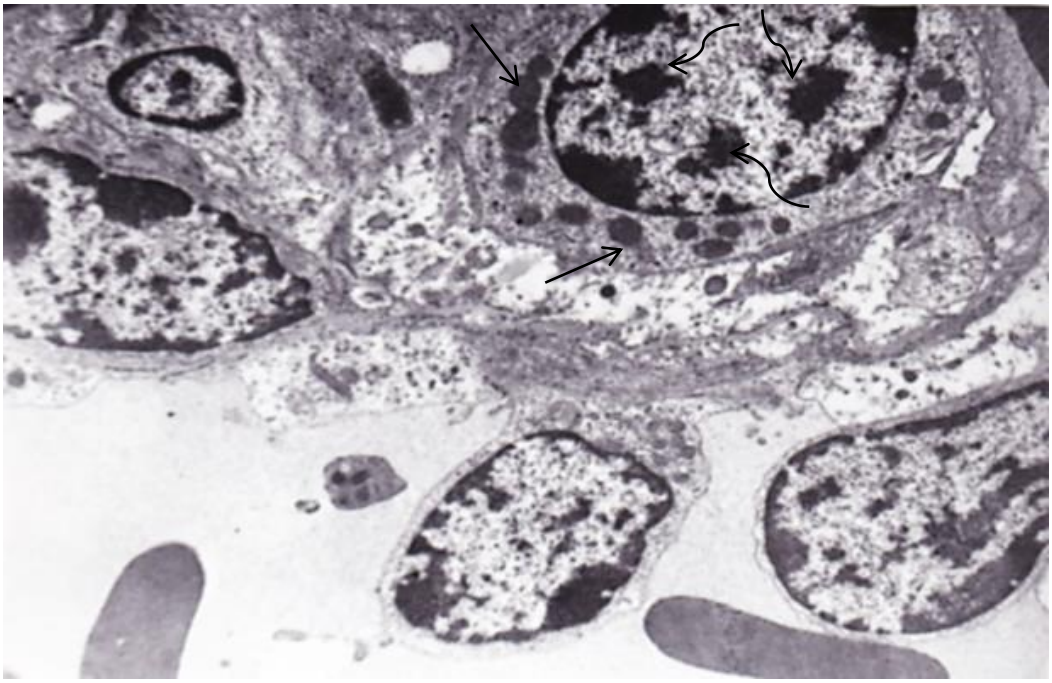
F1g(1):General view of immature Agglomerate leukocyte(→) in blood of patients having leukemia disease. Uranyl acetate and lead citrate, (2600x).



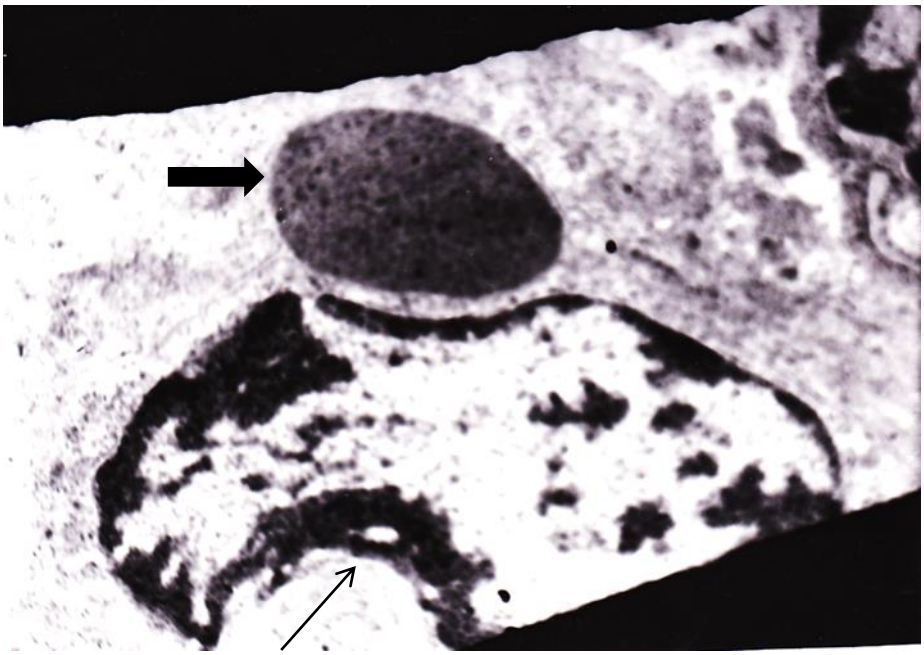
Fig(2):Immature agglomerate leukocyte in blood of patients having leukemia disease. Large nucleus within dented nuclear envelope (→), platelets (➡), R.B.C (★).Uranyl acetate and lead citrate,(4600x)



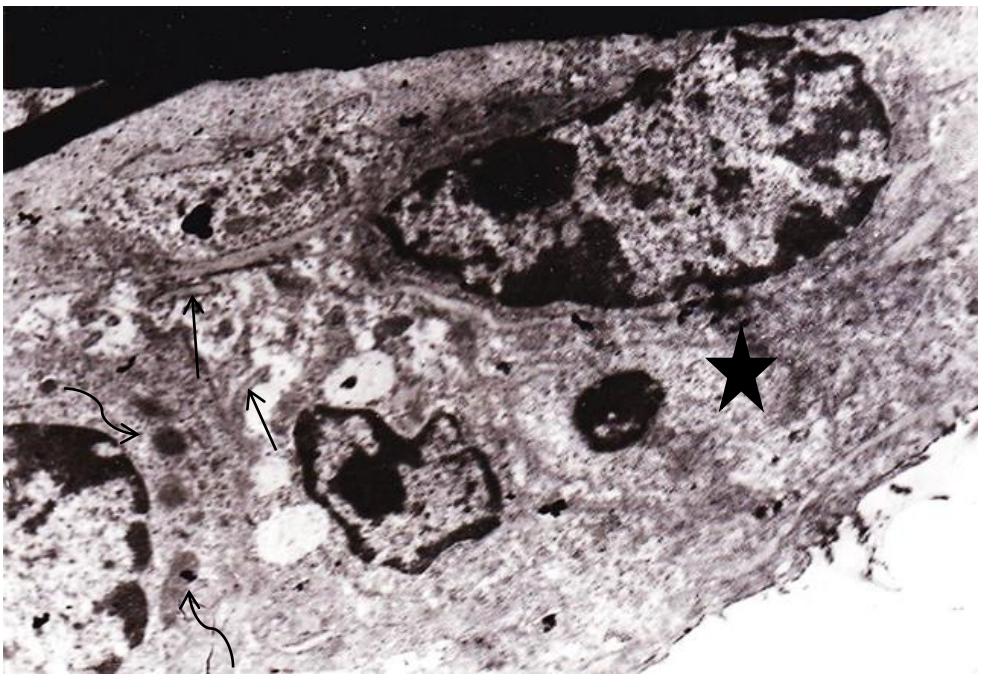
Fig(3):Immature agglomerate leukocyte in blood of patients having leukemia disease. Note the increment in nuclear cytoplasmic ratio, granular cytoplasm ( ) , glycogen granule ( ), microvilli projection ( ),dilated endoplasmic reticulum ( ).Uranyl acetate and lead citrate,(5800x)



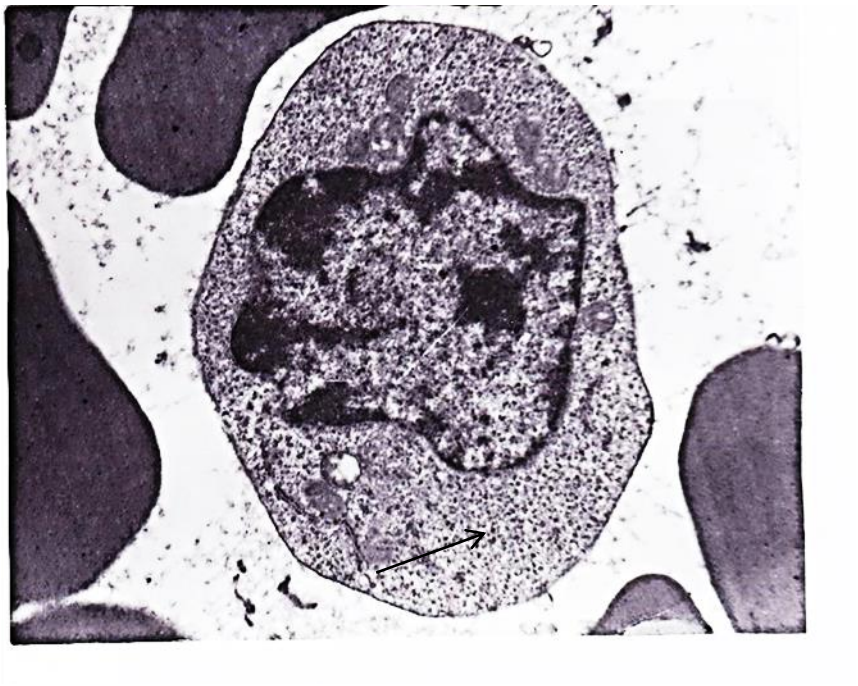
Fig(4):): Immature agglomerate leukocyte in blood of patients having leukemia disease. Mitochondria with high electron density( ), increment in nucleolus number ( ). Uranyl acetate and lead citrate, (4600x)



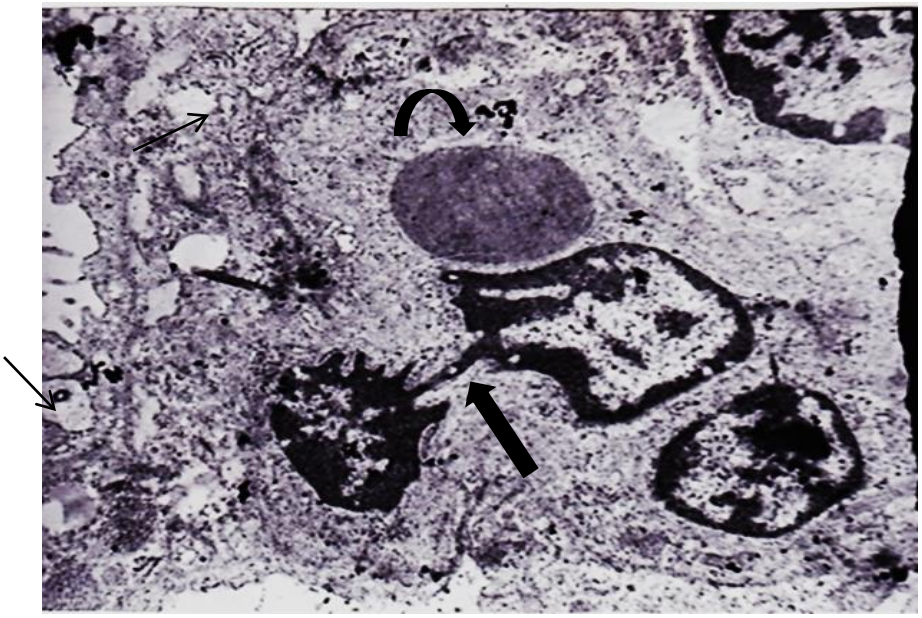
Fig(5):Immature leukocyte in blood of patients having leukemia disease. Leukocyte modified into phagocytic cell. RBC with ferritin ppt.(➡), irregular nucleus (↗) .Uranyl acetate and lead citrate,(7900x)



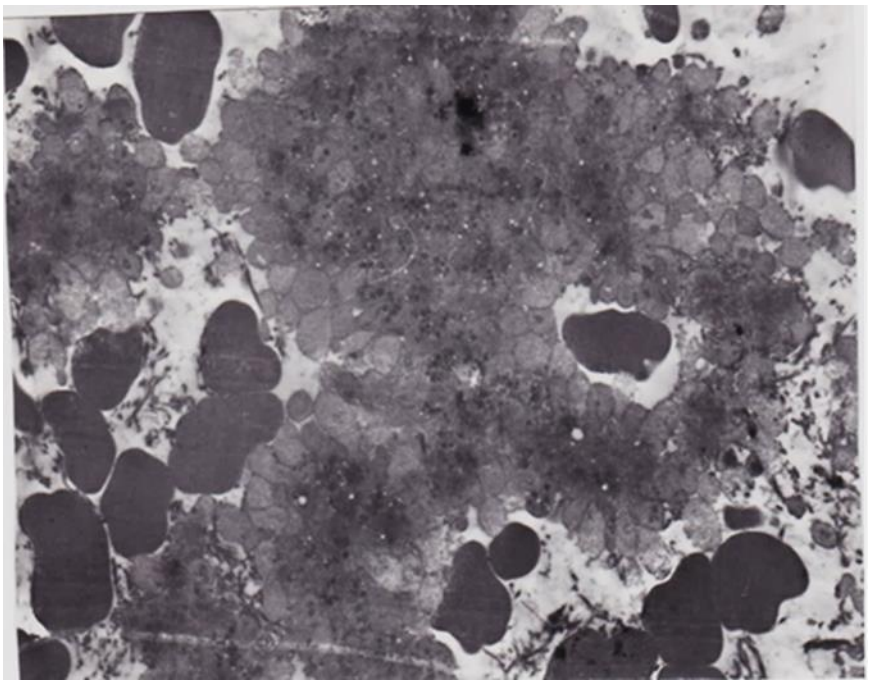
Fig(6):Immature agglomerate leukocyte in blood of patients having leukemia disease. Apoptotic leukocyte (★), dilated cisternae of endoplasmic reticulum (↗), mitochondria with high electron density (↷),Uranyl acetate and lead citrate,(5800x)



Fig(7):Immature agglomerate leukocyte in blood of patients having leukemia disease. Note the granular cytoplasm (⟶).Uranyl acetate and lead citrate, (7900x)



Fig(8):Immature agglomerate leukocyte in blood of patients having leukemia disease. Formation of chromatin bridge between two different nuclei type (➡), lipid droplet (↷), vacuole (⟶),Uranyl acetate and lead citrate, (4600x)



Fig(9):Erythroid cells with cytoplasmic ferritin aggregates in patients having leukemia disease,), Uranyl acetate and lead citrate,(2600x)

Discussion:

Leukemia is cancer of the white blood cells. It develops in the bone marrow and spreads into the blood and to other organs. There are several types of leukemia **(9)**. They are grouped in two ways: by how rapidly the disease develops: :(acute and chronic leukemia) and by the type of blood cell involved (lymphocytic and myeloid leukemia being the most common) **(10)**. In present paper all patients having acute myeloid leukemia since most of leukocytes are immature and are unable to function normally **(11)**.These changes in leukocytes we can said its due to that the environment in Iraq and its population have been exposed continuously to DU weaponry or its contaminating remains, since 1991.Its well-known fact that millions of Iraqi's have received higher doses of radioactivity than ordinary background levels. As a result a multi-fold increase of low level radiation exposure related diseases have been registered since 1995. Thousands of Iraqi children and their families are suffering from different low level radiation (LLR) related diseases such as congenital malformations, malignancies, congenital heart diseases, chromosomal aberration and multiple malformations. Women in the contaminated areas suffered of sterility**(12)**.

The shift of leukemia incidence rates towards younger children during the recent years, and its association with geographically distributed contaminated areas, offers strong evidence of the correlation between low level radiation (LLR) exposure and resulted health damage. The UK military used about 1.9 tons of depleted uranium in Basrah **(13).** DU decays mainly through emission of alpha particles that do not penetrate the external skin layers but may affect internal body cells (which are more susceptible to the ionizing effects of alpha radiation).Despite this, many studies suggested that the use of DU during 1991 and 2003 had resulted in a significant increase in the incidence of malignant

disease in Basrah **(14)**. In present paper leukemic cells exhibit adhesion phenomena which could be explained Leukemic cells produce a complex array of extracellular matrix (ECM) molecules consisting of proteoglycans and their constituent **(15)**. In addition stromal cells also synthesize other matrix molecules such as fibronectin, laminin also provide a source of many hematopoietic cytokines, and these compounds allow interactions between hematopoietic and stromal cells by means of cell adhesion molecules. Evidence is emerging that, in addition to hematopoietic cytokines, adhesion molecules are involved in mediating signal transduction in hematopoietic precursors and hence play an important role in cell proliferation that extends beyond brokering hematopoietic cell contact and adhesion to stromal cells and ECM elements **(16).** During Gulf war in 1991,and throughout the occupation Iraqi military operations in 2003,US and UK armed forces used of Depleted Uranium (DU)radioactive munitions in populated residential areas specially in southern Iraq **(17).** The results of 1991-war risk assessment in a highly populated area of about 1200Km^2 including the cities of Safwan , Zubair and west of Basrah are presented .Residents of these areas were exposed to high radioactive doses due to DU contamination. Results indicated that the most important sources of exposure was the inhalation of uranium during first months of the military operations of 1991. The calculated effective annual doses from this path were found to be 435mSv for the armed forces and 167mSv for the population in the city of Safwan . The annual whole body effective dose the population of Zubair and western Basrah city were exposed to is 268.6 mSv. The second highest effective radiation dose resulted from inhalation of resuspension-emanation of (DU)radionuclides destroyed tanks and other military artilleries sites. With each dust and sandstorm , the population in these areas kept receiving extra doses from DU pollution. Residents of the study area have been exposed to 200 times the annual radiation dose any person would receive from the natural background in most parts of the world, which is only 2.4mSv**(18)**.

Conclusion:

1- The American administration still claims that the biological and chemical agents of hydrocarbon smoke of oil field fires in southern Iraq are the main causes and not the exposure to the DU. This is very false and misleading information.
2- It is only fair to conclude that the environment in Iraq and its population have been exposed continuously to DU weaponry or its contaminating remains, since 1991.
3- The shift of leukemia incidence rates towards younger children during the recent years, offers strong evidence of the correlation between LLR exposure and leukemia.

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