Cytogenetic Study among Chemical Bombardment Survivors in Shekh Wasan & Balisan Valley Kurdistan Region-Iraq

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Abstract: This study was performed randomly on 40 individuals selected within a specific period, between January 2014 to October 2014 in order to a certain whether or not a sample of chemical weapons used on Shekh Wasan & Balisan valley in 16 April 1987, have incurred genetic damage. The following results were obtained:

The chromosomal aberration is a very sensitive and widely applied assay used as a bio indicator of genetic damage induced by an environmental agent or clastogen. In current study a group of (40) chemical bombardment survivors there were (15) individuals have different types of chromosome aberrations, including (ring chromosome %52.5, dicentric chromosome %30, chromosome break with fragment %57.5, chromatid interchange (quadriradial) %27.5, chromatid interchange (triradial) %27.5.

Keyword: Chromosomal Abberation, Chemical Weapons, Clastogen

1. Introduction

For centuries extremely toxic chemicals have been used in wars, conflicts, terrorists, extremists and dictators activities, malicious poisonings and executions. One of the earliest forms of chemical warfare agents (CWAs) were natural toxins from plants and animals which were used to coat arrowheads commonly refer to as "arrow poisons" (Gupta, 2009).

The population of towns in Kurdistan region-Iraq, especially the Shekh Wasan & Balisan valley is exposed to chemical weapons more than once. The Shekh Wasan & Balisan valley poison gas attack was a genocidal massacre against the Kurdish people that took place on April 16, 1987. The Kurdish inhabitants of Shekh Wasan & Balisan valley were aerially bombarded with a cocktail of chemical weapons, including mustard gas, nerve agents sarin, tabun, and hydrogen cyanide (HRW, 1993).

Sulphur mustard is genotoxic because of its reactions with DNA, which is an important first step in carcinogenesis (Mood & Hefazi, 2005). Human exposure to nitrogen mustard resulted in chromosomal breakage (Scott & Fox, 1974).

Changes in the genes and chromosomes do not usually produce an immediate health hazard, they

may go undetected for a life time or even for several generations, Yet, the human gene pool can become insidiously polluted (Margery & Shaw, 1970).

The toxicant that enters into human body cause disturbance to the normal state and behavior of the chromosomes which in turn lead to reshuffling of hereditary material causing chromosomal aberration and gene mutation in somatic and germ cells (Higginson et al, 1992)

The frequency of chromosomal aberrations produced by mustard gas can be expected to depend on initial extent of alkylation, removal and repair of lesions prior to replication, and the extent and accuracy of post replication repair. The persistent lesions are largely responsible for the clastogenic effects of mustard gas. It has long been accepted that cross-linking of DNA by bifunctional mustards is largely responsible for the production of chromosomal aberrations, since much higher extent of monofunctional DNA alkylation is required to produce chromosomal damage (Roberts et al, 1971).

2. Materials and Methods

2.1 Collection of Samples

2.3 Blood Sampling

Two ml of blood were collected from each patient having health problems, using sterile disposable syringes. Then, the blood was put in a special tube for chromosomal study (Lithium Heparin Tube).

2.4 Blood Culture

Lumphocyte were separated from Whole blood cultures were initiated by the addition of 5 mL RPMI-1640 medium with hepes and L- glutamin containing 10% fetal bovine serum (Sigma-Aldrich, United Kingdom) , penicillin (100 U/mL) and streptomycin (100 U/mL), , and phytohemagglutinin (2%). Duplicate cultures for each case were carried out for seventy-two hours at 37 C°. Colcimide (final concentration, 10 mg/mL) was added and was incubated for forty-five minutes before the end of the culture.

The cells were harvested and slides were prepared under standard conditions (incubated with 0.075M KCL for twelve minutes and then cells were fixed with methanol:acetic acid 1:3), and the suspension was dropped onto clean slides and stained with Giemsa (Freshney, 2008).

2.5 Statistical Analysis

Complete Randomize Design (CRD) used in analysis the data also means± stander error (M±SE) were computed, All statistical analysis were carried out manually (Sokal & Rohlf, 1987)

3. Results & Discussion

This study included the targeted group of (40) individuals from Shekh Wasan & Balisan valley, all of them are exposed to chemical weapons and (10) individuals non-exposes to chemical weapons. In this study the exposed individuals were selected in order to determine the effects of chemical weapons used on Shekh Wasan & Balisan valley in 16 April 1987.

Out of (40) chemical bombardment survivors there were (15) individuals have different types of chromosome aberrations, including (ring chromosome %52.5, dicentric chromosome %30, chromosome break with fragment %57.5, chromatid interchange (quadriradial) %27.5, chromatid

interchange (triradial) %27.5.

Table (1): Analysis of Variance for the effect of sex on the chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley (Mean square)

Source of variance	d.f	Chromosome aberrations
		Mean square
Between treatments	1	6.4*
Within treatments (Error)	8	9.95
Total	9	

*($p<0.005$)

The result of the present study represent different types of chromosomal aberrations in peripheral blood lymphocyte of patients, including both sexes, the values of the Table 1 shows analysis of variance for the effect of sex on the chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley, statistically there is non-significant difference ($p<0.05$) for sex factors, mean that males and females are equally affected.

Similar result were obtained by Sulaiman, (2013) in the study on Halabja city population, 215 patient's having different health problems, 215 among them having different type of aberrations (dicentric chromosome, chromatid interchange (triradial), Ring chromosome, fragment, centromeric break, hyperdiploidy, chromatid interchange (quadriradial) and hypertriploidy, also 45 patients having different types of cancer (20 patients Lung cancer, 10 patients Leukaemia, 9 patients Breast cancer and 6 patients Colon cancer).

Our results were supported also by Scott & Fox (1974) suggested that human exposure to nitrogen mustard resulted in chromosomal breakage. They also concluded that following the seven years of treatments with nitrogen mustard, a significant aberration rate (especially chromosome 5 and 7) and chromosome breakage (especially chromosome 5, 7 and 9) developed.

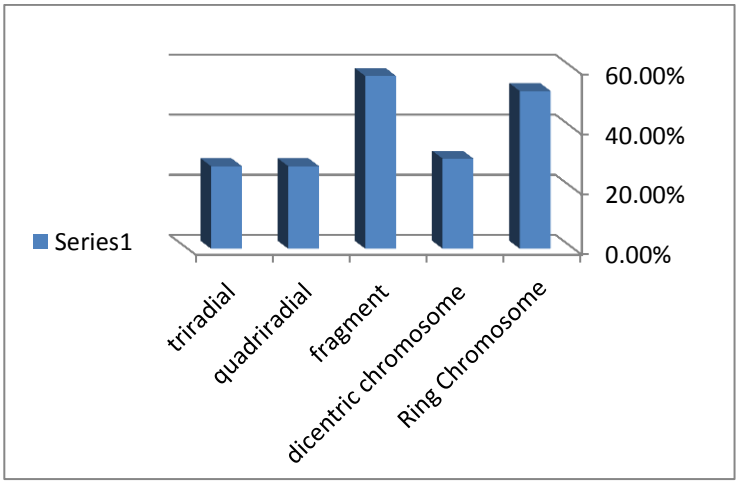

Figure (1): Shows the percentage of all chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley

Table (2): Mean ± S.E for the effect of sex on the chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley

Sex	Ring	Dicentric	Fragment	Qudriradial	Triradial
Female	1.428±0.368	0.714±0.285	1.285±0.285	0.857±0.142	0.857±0.404
Male	1.375±0.283	0.875±0.294	1.75±0.25	0.5±0.188	0.75±0.25

Table 2 shows Mean ± S.E for the effect of sex on the chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley, the highest value was ring chromosome (1.42±0.368) and the lowest value was dicentric chromosome (0.714±0.285) in females, while in males the highest value was fragment (1.75±0.25) and the lowest value was chromatid interchange (Quadriradial) (0.5±0.188).

Similar results were obtained by Sulaiman, (2013) in the study on Halabja city population, the highest value of chromosome aberrations was ring chromosome (1.395±0.434) in females, while in males the highest value was (chromatid interchange: triradial) (9.333±0.333), this similarity is due to that the same gas used in Halabja city and Shekh Wasan & Balisan valley (Dizaye, 2012).

Table (3): Analysis of Variance for the effect of age (3 groups) on the chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley (Mean square)

Source of variance	d.f	Chromosome aberrations
		Mean square
Between treatments	2	8.6*
Within treatments (Error)	12	6.86
Total	14	

*($p<0.05$)

Table 3 shows analysis of variance for the effect of age (3 groups) on the chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley, statistically there was non-significant difference ($p<0.05$) for 3 age groups factors, means that the first age group (27-36) , second age group (37-49) and third age group (50-59) are equally affected.

Mutation can be caused by two distinct mechanisms: (a) misreplication resulting from insertion of an incorrect base opposite an alkylated purine with altered base-pairing properties. Replication errors will give rise to point mutation; and (b) misrepair resulting from the operations of error-prone system, in addition to point mutation, repair errors can give rise to deletions, abnormal recombination, and structural damage to chromosome (Papirmeister et al, 1992).

Sulphur mustard is genotoxic because of its reactions with DNA, which is an important first step in carcinogenesis (Mood & Hefazi, 2005). Human exposure to nitrogen mustard resulted in chromosomal breakage (Scott & Fox, 1980).

Changes in the genes and chromosomes do not usually produce an immediate health hazard, they may go undetected for a life time or even for several generations, Yet, the human gene pool can become insidiously polluted (Margery & Shaw, 1970).

Significant enhancement in the frequency of sister chromatid exchanges has been reported in peripheral lymphocytes obtained from fishermen who exposed to SM by leakage of the agent from World War II shells recovered from the Baltic sea (Genderen et al, 1985).

Other cytogenetically detected chromosomal changes that have been reported to be caused by mustard exposure (mostly in studies performed with HN2) include gaps, deletions, exchanges, chromosomal stickiness, segregation errors, variable chromosome numbers, micronuclei, and chromosome "shattering" (Scott & Fox, 1974).

The frequency of chromosomal aberrations produced by mustards can be expected to depend on

initial extents of alkylation, removal and repair of lesions prior to replication, and the extent and accuracy of post replication repair. The persistent lesions are largely responsible for the clastogenic effects of mustards. It has long been accepted that cross-linking of DNA by bifunctional mustards is largely responsible for the production of chromosomal aberrations, since much higher extent of monofunctional DNA alkylation are required to produce chromosomal damage (Roberts et al, 1971).

The mutagenic and chromosome damaging effects of SM are of interest. SM was one of the first chemical compounds to demonstrate mutagenic activity in Drosophila and subsequently, in variety species (Fox & Scott, 1974).

Chronic exposure of mice to vaporized SM (0.1 mg/m3) over a 52-week periods produced a cumulative increase in dominant lethal mutations. Such mutations are thought not to be point mutation; rather, they are thought to result from chromosome breakage (Herriott, 1951).

Table (4): Mean ± S.E for the effect of age (3-groups) on the chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley.

Age	Ring	Dicentric	Fragment	Quadriradial	Triradial
27 -36	1.50± 0.499	0.666 ± 0.210	1.333 ± 0.333	0.666 ± 0.210	1.166 ± 0.307
37-49	1.25 ± 0.25	0.78 ± 0.478	1.75 ± 0.478	0.75±0.25	0
50-59	1.4 ± 0.244	1.000 ± 0.447	1.600 ± 0.244	0.600±0.244	1.000 ± 0.447

Table 4 shows Mean ± S.E for the effect of age (3-groups) on the chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley, the highest value for first group age (27-36) was ring chromosome (1.50± 0.499) and lowest value for the age group was quadriradial (0.666 ± 0.210), while the highest value for the second age group (37-49) was fragment (1.75 ± 0.478) and lowest value for the same group was quadriradial (0.75±0.25), and the highest value for the third group age (50-59) was fragment (1.600 ± 0.244) and lowest value for the same group was quadriradial (0.600±0.244).

In this study we can noticed that the highest rate of chromosomal aberrations is for the last age group between (50-59), Also similar result was obtained by Sulaiman, (2013) in study on Halabja city we also getting the highest rate of chromosomal aberrations in same age group (50-58), this is may be due to that the people become older in age their DNA repair system and also immune system cannot able to repair all the accumulated mutations in chromosome so high number of aberrations was found in older age group.

In the 2013 and 2014, the Ministry of Martyrs and Anfal in the Kurdistan Region-Iraq established the Commission of all medical specialties and were examined (937) people on four groups that exposed to chemical weapons in Khoshnawaty areas including (Shekh Wasan & Balisan valley) and proved that (626) actually still suffering from the effects of chemical weapons after more than 28 years later

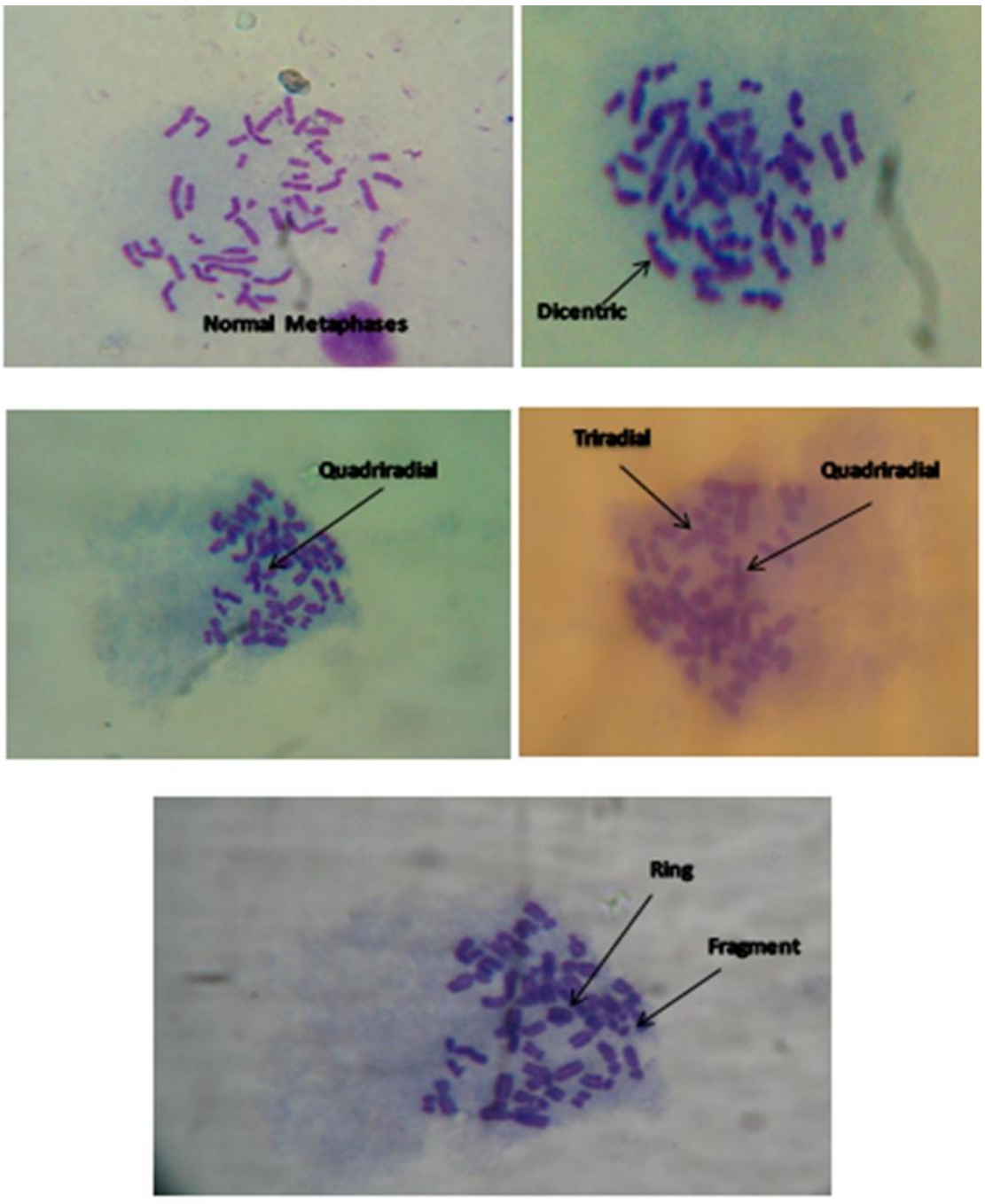

Figure (2): Shows structural chromosomal aberrations among chemical bombardment survivors in Shekh Wasan & Balisan valley (100X, Giemsa stain).

References

Dizaye, K. (2012). Victims of the Long Term Effects of Chemical Weapons on Health in Kurdistan of Iraq. *Middle East Journal of Internal Medicine*, 5(4),27-35.

Freshny, G. J. (2008). *Cultures of Animal Cells*. (3rd Edition). Wiley- Liss. USA.

Genderen, V., Mol, J., & Wolthuis, O. L. (1985). On the development of skin models for toxicity test. Fund. Appl.Toxicol.5:S98-S111.(Cited from Papirmeister, B. ; Feister, A. J.; Robinson, s. i. and Ford, R. D.1992)

Gupta, C. R. (2009). *Handbook of Toxicology of Chemical Warfare Agents*. First edition. Elsevier, USA.

Herriott, R. M. (1951). Nucleic acid synthesis in mustard gas-related E.coli .B. *The Journal of General Physiology*, 34(6), 761-764.

Higginson, J., Muir, C. S., & Munoz, N. (1992). Human cancer: epidemiology and environment causes. In Cambridge Monographs on Cancer Research. Cambridge University Press, Cambridge UK. 377-387.

HRW (Human Rights Watch): (1993): GENOCIDE IN IRAQ THE ANFAL Campaign Against the Kurds, A Middle East Watch Report, Library of Congress Card Catalog Number: 93-79064, Nework, USA.

Margery, W., & Shaw, M. D. (1970). Human Chromosomal Damage by chemical Agents. *Annual Review of Medicine*, 21,409-432.

Mood, B.M., & Hefazi. M. (2005). The pharmacology, Toxicology, and medical treatment of sulfur mustard poisoning in severely intoxicated Iranain veterans. *Fundam. Clin. Pharmacol*,19, 713-721.

Papirmeister, B., Feister, A. J., Robinson, S. I., & Ford, R. D. (1992). Medical Defense Against Mustard Gas Toxic Mechanism & Pharmacologist Implication.P:142. Chapter:6. Mutagenic and chromosome Damaging.

Roberts, J.J., Pascoe, J.M., Plant, J.M., Sturrock, J. E., & Crathorn, A. R. (1971). Quantitative aspect of the repair of the alkylated DNA in cultured mammalian cell. The effect of HeLa and chines hamster survivor of alkylation cellular macromolecule. *Chem. Biol. Interact*, 3,29-47.

Scott, D., & Fox, B.W. (1974). The relationship between chromosomal aberrations, survival and DNA repair in tumour cell lines of differential sensitivity to X-rays and sulphur mustard. *Mutat Res*, 22, 207–221.

Sokal. R. R. & Rohlf, J. F.(1987). Introduction to Biostatistics. 2nd edition.P:109.

Sulaiman, K. M. (2013).Genetic variation and long term effects of chemical exposure on the Halabja population. PH. D. University of Salahaddin. Iraq, Erbil.