Chromosomal aberrations in the peripheral blood lymphocytes of Dental Radiographers who exposed to diagnostic X-ray in Erbil city / Iraqi Kurdistan region

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Abstract
Radiographic examination is one of the principal diagnostic methods used in all fields of medical and dental services. The incidence of chromosomal aberrations was evaluated in the peripheral blood lymphocytes of 50 individuals who worked in different dental colleges and clinics in Erbil City. This research was donning between March to September 2016. Blood samples were collected also from 20 individuals as control group, that were not exposed to any diagnostic radiations. An attempt was done to find the relationship between the frequency of chromosomal aberrations and smoking habit. The radiographers showed a significant increase of chromosomal aberrations as compared to control group at both probability level ($P< 0.01$, $P< 0.05$), but significant decreases in mitotic index were shown. The highest value of chromosomal aberrations was chromatid gap and lowest value of mitotic index were found both observed in dental radiographers who were smoker with duration of exposure for more than 10 yrs.

Keywords: Chromosome aberration, Mitotic index, dental radiographers, Occupational exposure, X-rays, Smokers.

1. Introduction
The X- ray used in diagnostic of many diseases and have the ability to cause mutations and inducing chromosomal aberrations, so they act on DNA molecule [1].Radiation induces mutation in genetic material in experimental animals and human [2]. The effects of X- ray on female fertility was observed by [3] in adult rats. Leukemia and chromosomal aberrations also were founded by [4] in the mice exposed to X-rays.

Cytotoxic effects of X-rays in workers who were occupationally exposed were recorded in several earlier studies [5], who observed high frequency of chromosomal aberrations such as acentric and dicentric chromosome in peripheral blood lymphocytes of workers who handled diagnostic X-ray machines. Cytogenetic analysis can play an integral role in retrospective dose reconstruction of chronic exposure in epidemiological studies of exposed populations [6].

Chromosome changes play a major role in carcinogenesis [7] and there is increasing evidence that their presence in peripheral blood lymphocytes provides a marker of cancer risk [8].

Tobacco smoke is contain many of potentially hazardous chemicals including radioactive agents[9], the formation of free radicals from radioactive and nonradioactive chemicals is one of the major pathways by which tobacco smoke causes genetic damage , chromosomal aberrations and cancer[10].

The ionizing radiation classified as direct or indirect radiation .X-ray are indirectly ionizing radiation, they not produce biological or chemical changes themselves, but when they are absorbed by those subjects which they pass through . so they give up their energy to produce very fast – moving charged molecules [11]. The present study was conducted to determine chromosomal aberrations and mitotic index in dental radiographers in Erbil City/ Iraqi Kurdistan Region.

2. Materials and Methods
In Erbil city a large groups of dental radiographers who not specialists in radiology . This fact lead us to select this group for cytogenetic study .The subjects were consist of 50 dental radiographers and the study of frequency of chromosomal aberrations based on smoking habit and duration of work (less than ten years and more than ten years), their age groups range from (25-55) years, and 20 controls (non- workers/ non – smokers).

A special questionnaire form were used in order to collect all the important information's. The questions included age, smoking and alcohol habits and total working hours/ day , were filled in through direct interviews with them.

2.1 Blood sampling:-

Five ml of blood were collected from 50 dental radiographers, using sterile disposable syringes. Then, the blood was put in a special tube for chromosomal study (Lithium Heparin tube). Heparinized blood were collect from each dentist and processed at Research Center /Salahaddin University. Cytogenetic analysis was performed by using the protocol of Iraqi center for cancer and medical genetic research (ICCMGR).

2.2 Blood culture and harvesting

About one ml or 6 – 7 drops of heparinized blood was cultured in 5 ml RPMI – 1640 culture medium, then supplement 0.3 ml of PHA .Culture tubes were incubated at 37 C° for 72 hours, after 71 hours of incubation 0.2 ml of colchicines was added to the culture tube with mild shaking and then incubated at 37 C° for next 1 hour. Then after many steps of centrifugation and adding of fixatives, 3 to 4 drops of cell suspension were dropped evenly from appropriate distance (typically 30 cm) on to a wet chilled and grease free slide, then the slide was dried at room temperature. The slide was stained with freshly prepared giemsa stain (Giemsa stain 1:4

Sorensone buffer solution) for 2-3 minutes. Then the slide was washed by Sorensone's buffer and left to dry at room temperature. Excess buffer was removed by slanting the slide on filter paper. After processing the cultures and preparation of slides, the slides were stained by Giemsa stain [12].

2.3 Microscopic analysis

The slides were examined at a magnification of 1000 X. A total of 100 cells for each individual in each group and all different type of chromosomal aberrations were classified.

2.4 Mitotic index assay

The mitotic index calculated MI= number of mitosis (metaphase cells) / total number of cells × 100 .

2.5 Statistical Analysis

Performed, using SPSS version 18 software application to study the chromosomal aberrations and mitotic index in different groups.

3. Results and Discussion

Figure (1) shows the total number of dental radiographers were categorized into two groups based on smoking habit represent (20 control –non workers-non smokers), (20 dental radiographer – smoker) and (30 dental radiographer –non smoker),by random sampling, while figure (2) shows the characteristic of population represent years of exposure and smoking habit for dental radiographers in which whom non-smoker and exposed to x- ray for less than 10 years represent (20%) , non-smoker exposed to x- ray for more than 10 years represent (40%) and whom smoker exposed to x- ray for less than 10 years represent (20%) exposed to x- ray for more than 10 years represent (20%) with their age groups range from (25-55) years.

The results of the present study was supported by [13] who suggest that people who work in dental care specially who work with x-ray or other imaging test may exposed to radiation at work . The Exposure have limited to an effective dose of 100 msv over five years .

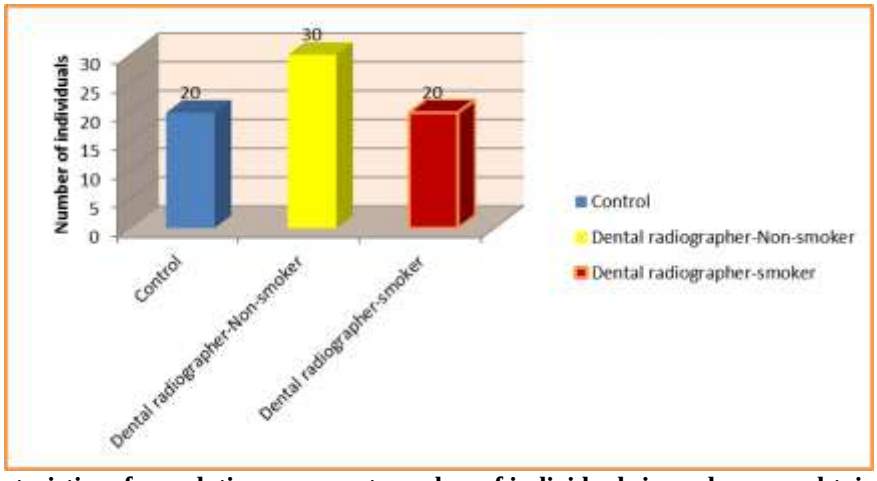

Fig. (1) Characteristics of population represent number of individuals in each group obtained by random sampling

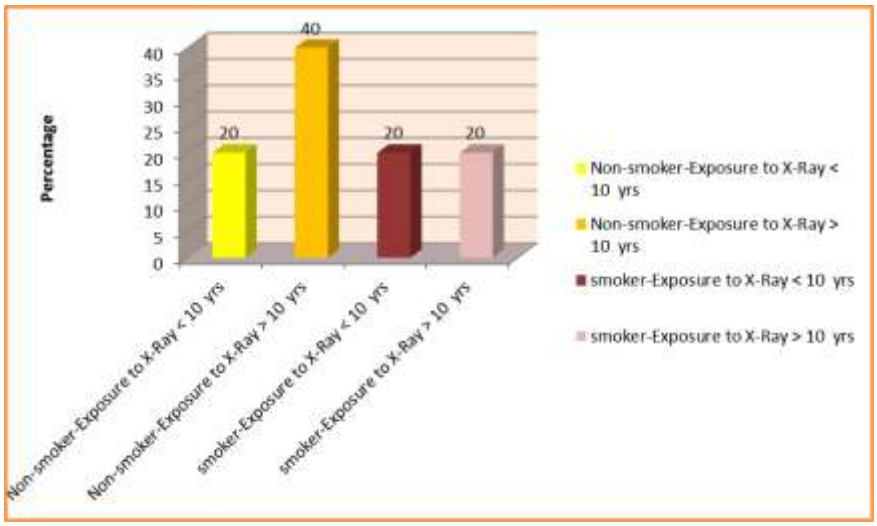

Fig. (2) Characteristic of population represent years of exposure of dental Radiographers (smoker and non-smoker)

From study the frequency of chromosomal aberrations (CAs) and mitotic index in dental radiographers, the results shows different types of chromosomal aberrations in blood sample of

radiographers., including (centromeric break, centromeric gap,dicentric chromosome and chromatid gap) .In table (1) E1 was control while E2 represent workers (radiographers), and study of smoking habit which included non- smokers and smokers represent (S1 and S2), while study of duration of exposure which included work for less than 10 years and more than 10 years represent (D1< 10 yrs and D2> 10 yrs), respectively.

Table (1) shows highly significant effect at level (P<0.01). in both control and exposed individuals on different types of chromosomal aberrations like (Centromeric break, centromeric gap ,dicentric chromosome and chromatid gap), and also mitotic index as shown in figure (4,5 and 6) .The different between mean values were clear as shown in table (2). Smoking habit, have highly significant effect on all types of chromosomal aberrations but not significant on mitotic index. Also duration of work have highly effect on all types of aberrations but only significant on mitotic index.

In table (2) we observed that in both the highest value of CAs was (dicentric chromosomes) (5.750 ±0.144) which occurred in exposed radiographers in which mitotic index were decreased compare to control (8.583± 0.144). In case of study smoking habit the high value of chromosomal aberrations was (chromatid gap) (4.500 ± 0.156) which found in smoker person, while mitotic index showed high value in non –smokers (10.750±0.144) . In case of duration of exposure the highest value of CAs was (dicentric chromosome) (4.000±0.144) which occurred in radiographers who work for more than 10 years while mitotic index show high in radiographers who work for less than 10 years. The effects of interaction between control, radiographers, smoking habit, and duration of exposure on chromosomal aberrations and mitotic index, finally the highest value of chromosomal aberration was (chromatid gap) (6.333±0.312), and lowest value of mitotic index (6.667±0.289) were found in dental radiographers who were smoker with duration of exposure for more than 10 yrs .

Numerous studies have been conducted on the cytogenetic effects of radiation on the occupationally exposed workers in medical field. But studies conducted on exposed workers in a dental set up are few. [14] concluded that chromosomal aberrations , specially dicentric chromosomes can be used as good indicator of exposure to radiation.

[15] Observed in his study on effect of dental X ray radiation in averted cheek pouch of Chinese hamsters which were exposed to 0.25R, 2.9R and 5.4R radiation dose found significant amounts of chromosomal damage for all doses of radiation. [16] concluded in their studies in radiation exposed groups working in various medical fields compared to controls, there were increases in the rate of chromosomal aberrations.[17] concluded from cytogenetic study on Brazilian dentists who occupationally exposed to low dose of X radiation that there were no significant difference between the dentists and the unexposed controls.

The evaluation of the potential of both physical and chemical agents in producing many dangerous effects on all cells can de doing by determination the proportion of (metaphase cells) and calculating of mitotic index. Decreases of mitotic index is a result of reducing in the rate of cell division [18]. [19] concluded from a study of chromosomal aberrations in the peripheral lymphocytes of workers exposed to diagnostic X-ray, that there were significant increase in chromosomal aberrations when compared to control group also they funded that chromosomal aberrations increased with duration of exposure, those aberrations included chromatid gap, fragments, dicentric and break.[20] founded high frequency of centromere positive and centromer negative in blood sample of radiographers .

High frequency of ring type of chromosomal aberrations, dicentric and acentric chromosome were observed in the peripheral blood lymphocytes of medical staff who were exposed to x- ray [21] .[22] concluded that chromosome damage is associated with low levels of radiation exposure from diagnostic X-ray examinations, which including dose scores of nearly about 50 and lower, suggesting the possibility of long-term adverse health effects. [5] concluded that an increased frequency of acentric fragments as a function of years of employment in exposed groups, more in medical radiographers than in radiologists..

Ionizing radiation is a potent mutagenic agent capable of inducing both mutation and chromosomal aberrations. Non-lethal doses of ionizing radiation may induce genomic instability favoring carcinogenesis [23].Strong mitotic delays could be observed, which depended on both the irradiated volume and the applied dose[24]. Long term occupational exposure to low doses IR contributes to the development and increased frequency of specific CA like dicentrics [25].

A smoker is exposed to a variety of carcinogenic constituents present in cigarettes, making it necessary to analyze the cells at metaphase as these can be a health hazard to the future generations, an increased frequency of chromosome breaks has been demonstrated to be an initial event in carcinogenesis [26]. [27] showed that the frequency of CA was significantly higher in smokers than in non-smokers showing the highest number of Chromosomal Aberrations (CA) among heavy smokers (>20 pack-years. [28] concluded from a study of cytogenetic biomonitoring of workers in hospital who exposed to low level of ionizing radiation, that there were a significant differences in the incidence of cells with chromosomal aberrations between smoker and non smoker people .

Table (1):Analysis of variance to study the chromosomal aberrations in dental radiographers who exposed to diagnostic x- ray in Erbil City

		Mean square(MS)				
Source of variation	**dd.f**	**Centromeric break**	**Centromeric gap**	**Dicentric chromosome**	**Chromatid gap**	**Mitotic Index**
Case: Control(E1) Exposures(E2)	**1**	60.167 **	84.375 **	170.667 **	150.000 **	104.167 **
Smoking: Non-Smokers(S1) Smoker(S2)	**1**	10.667 **	35.042 **	24.000 **	60.167 **	0.167
Durationof exposure: (D1. < 10yrs) (D2 . > 10yrs)	**1**	6.000 **	9.375 **	20.167 **	13.500 **	1.500 *
Case/ Smoking(E/S)	**1**	13.500 **	30.375 **	20.167 **	42.667 **	10.667 **
Smoking/ Durationof exposure (S/D)	**1**	2.667 *	3.375 **	0.020	1.500 *	0.667
Case/Duration of exposure (E/D)	**1**	8.167 **	18.375 **	16.667 **	10.667 **	6.000 **
Case/Smoking/Duration of exposure (E/S/D).	**1**	1.500 *	2.042 *	0.167 **	2.667 **	8.667 **
Error	**4**	0.333	0.250	0.250	0.292	0.250
Total	6	**24**				

Table (2): Mean±S.E to study chromosomal aberrations in Dental radiographers who Exposed to diagnostic x- ray in Erbil city

Factors	**Chromosomal aberrations**				
	Centromeric break	**Centromeric gap**	**Dicentric chromosome**	**Chromatid gap**	**Mitotic Index**
Control(E1)	0.417±0.167	0.417±0.144	0.417±0.144	0.417±0.156	12.720±0.144
Exposure(E2)	3.583±0.167	4.167±0.144	5.750±0.144	5.417±0.156	8.583± 0.144
L.S.D (0.05)-(0.01)	0.411 0.607	**0.356** **0.527**	**0.356** **0.527**	**0.385** **0.568**	**0.356** **0.527**
Non-Smoker(S1)	1.333±0.167	1.083±0.144	2.083±0.144	1.333±0.156	10.750±0.144
Smoker(S2)	2.667±0.167	3.500±0.144	4.083±0.144	4.500±0.156	10.583±0.144
L.S.D	0.411 0.607	**0.356** **0.527**	**0.356** **0.527**	**0.385** **0.568**	-
Durationof exposure < 5 yrs.(D1)	1.500±0.167	1.667±0.144	2.167±0.144	2.167±0.156	10.917±0.144
> 5yrs (D2)	2.500±0.167	2.917±0.144	4.000±0.144	3.667±0.156	10.417±0.144
L.S.D	**0.411** **0.607**	**0.356** **0.527**	**0.356** **0.527**	**0.385** **0.568**	**0.356** **0.527**
E1S1	0.500±0.236	0.333±0.204	0.333±0.204	0.167±0.220	12.167±0.204
E1S2	0.333±0.236	0.500±0.204	0.500±0.204	0.667±0.220	12.333±0.204
E2S1	2.167±0.236	1.833±0.204	3.833±0.204	2.500±0.220	9.333±0.204
E2S2	5.000±0.236	6.500±0.204	7.667±0.204	8.333±0.220	7.833±0.204
L.S.D	**0.581** **0.860**	**0.504** **0.743**	**0.504** **0.743**	**0.544** **0.803**	**0.504** **0.743**
S1D1	1.167±0.236	0.833±0.204	1.167±0.204	0.833±0.220	11.167±0.204
S1D2	1.833±0.236	2.500±0.204	3.167±0.204	3.500±0.220	10.667±0.204
S2D1	1.500±0.236	1.333±0.204	3.000±0.204	1.833±0.220	10.333±0.204
S2D2	3.500±0.236	4.5000±0.204	5.0000.204	5.500±0.220	10.500±0.204
L.S.D	**0.581** **0.860**	**0.504** **0.743**	-	**0.544** **0.803**	-
E1D1	0.500±0.236	0.667±0.204	0.333±0.204	0.333±0.220	12.500±0.204
E1D2	2.500±0.236	2.667±0.204	4.000±0.204	4.000±0.220	9.333±0.204
E2D1	0.333±0.236	0.167±0.204	0.500±0.204	0.500±0.220	13.000±0.204
E2D2	4.667±0.236	5.667±0.204	7.500±0.204	6.833±0.220	7.833±0.204
L.S.D.	**0.581** **0.860**	**0.504** **0.743**	**0.504** **0.743**	**0.544** **0.803**	**0.504** **0.743**
E1S1D1	0.667±0.333	0.667±0.289	0.333±0.289	0.000±0.312	12.667±0.289
E1S1D2	0.333±0.333	0.667±0.289	0.333±0.289	0.667±0.312	12.333±0.289
E1S2D1	1.667±0.333	1.000±0.289	2.000±0.289	1.667±0.312	9.667±0.289
E1S2D2	3.333±0.333	4.333±0.289	6.000±0.289	6.333±0.312	9.000±0.289
E2S1D1	0.333±0.333	1.000±0.289	0.333±0.289	0.333±0.312	11.667±0.289
E2S1D2	0.333±0.333	0.333±0.289	0.667±0.289	0.667±0.312	14.333±0.289
E2S2D1	2.667±0.333	2.667±0.289	5.667±0.289	3.333±0.312	9.000±0.289
E2S2D2	6.667±0.333	8.667±0.289	9.333±0.289	10.333±0.312	6.667±0.289
L.S.D	0.822 1.216	0.712 1.053	0.712 1.053	0.770 1.139	0.712 1.053

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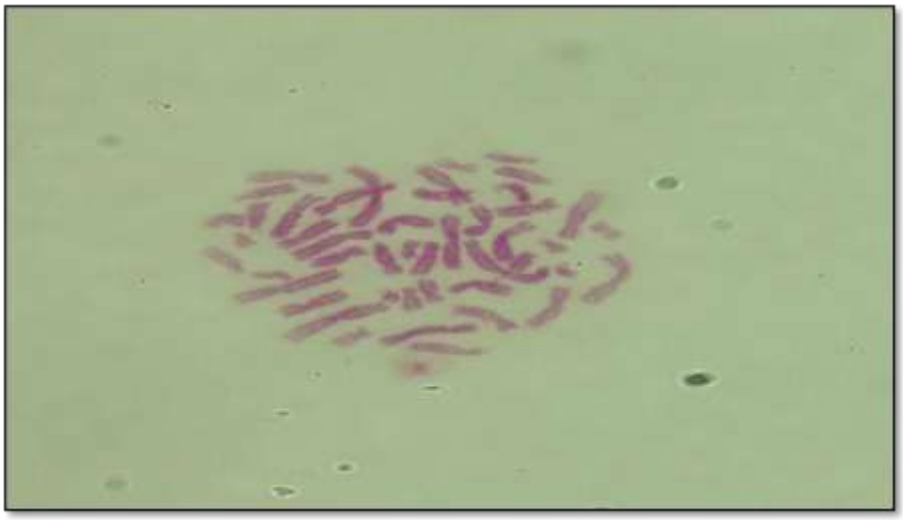

Figure (3): Normal distribution of human chromosome (1000 X, Giemsa stain

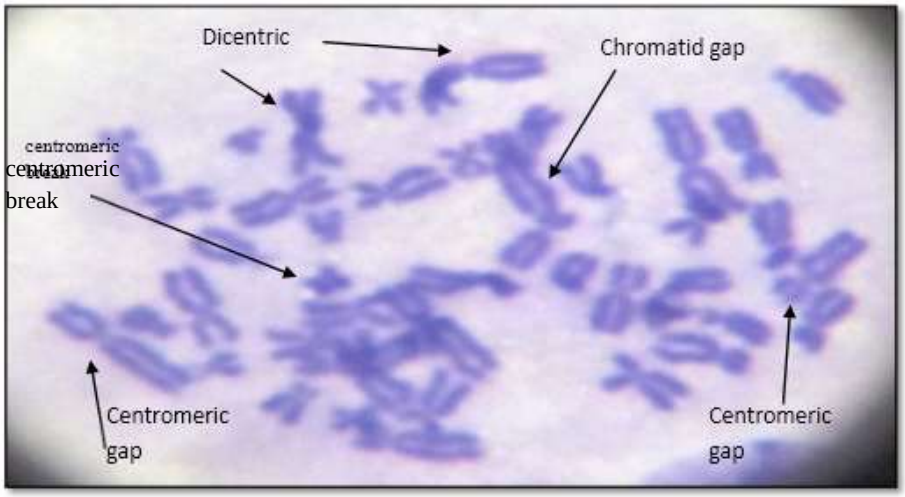

Figure (4): Chromosome aberrations in lymphocytes of dental radiographers who exposed to diagnostic x- ray in Erbil City.(1000 X, Giemsa stain)

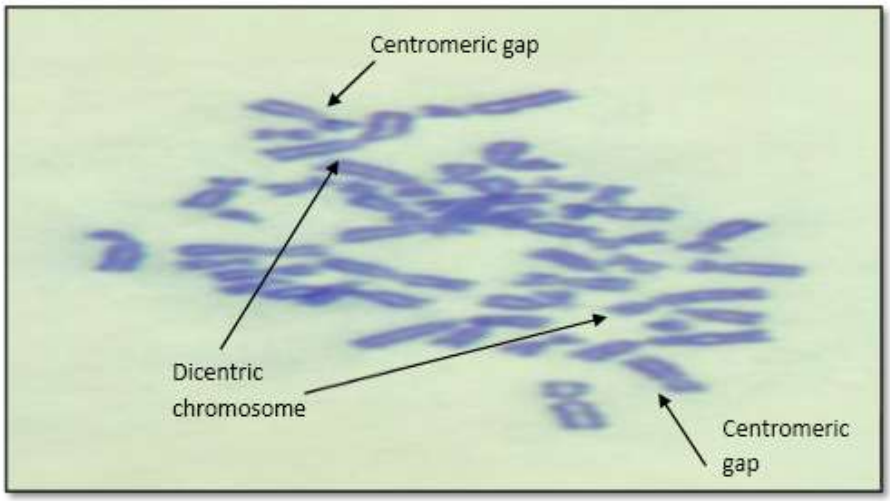

Figure (5): Chromosome aberrations in lymphocytes of dental radiographers who exposed to diagnostic x- ray in Erbil City.(1000 X, Giemsa stain)

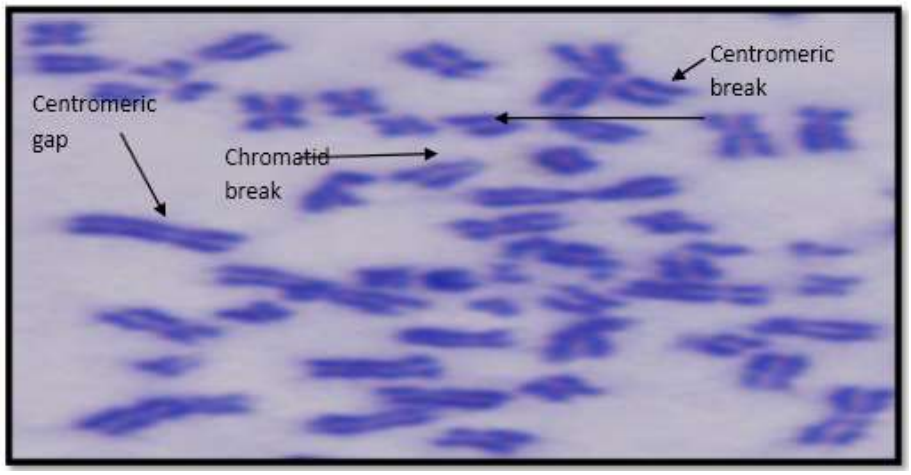

Figure (6) : Chromosome aberrations in lymphocytes of dental radiographers who exposed to diagnostic x- ray in Erbil City.(1000 X, Giemsa stain).

Conclusions

From the results of this study we concluded that chromosome aberrations was occurred in dental radiographers who exposed to diagnostic x- ray, included (centromeric break, centromeric gap, dicentric chromosome and chromatid gap). The highest value of chromosomal aberrations was chromatid gap was found in dental radiographers who were smoker with duration of exposure for more than 10 yrs. In case of mitotic index the results shows that lowest value of mitotic index were found in dental radiographers who were smoker with duration of exposure for more than 10 yrs.

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التشوهات الكروموسومية في الخلايا اللمفاوية في الدم المحيطي لفنيون اشعة الاسنان المعرضين الى اشعة اكس التشخيصية في مدينة اربيل / اقليم كردستان العراق

كةذالّ محمد سليمان

قسم علو م الحياة ، كلية التربية ، جامعة صلاح الدين ، اربيل ،العراق

الملخص

ان الفحص الاشعائي هي واحدة من الطرق الاساسية التي تستخدم في كل المجالات الطبية وخدمات الاسنان. ان حدوث التشوهات الكروموسومية قد قدرت في خلايا الدم المحيطي ل 50 اشخاص يعملون في عيادات و كليات طب الاسنان في مدينة اربيل. ان هذا البحث قد اجريت بين شهر اذار الى شهر ايلول 2016. ولاجراء المقارنة تم جمع 20 عينة دم من اشخاص كمجموعة سيطرة (غير معرضين الى اية اشعة تشخيصية). وتم محاولة لاجراء علاقة بين التشوهات الكروموسومية والتدخين ان النتائج تبين بانه توجد زيادة معنوية في التشوهات الكروموسومية لدى فنيون اشعة الاسنان مقارنة بمجموعة السيطرة عند مستوى الاحتماليه ($P< 0.01, P< 0.05$) ولكن توجد انخفاض معنوي في قابلية انقسام الخلايا، ان اعلى نسبة للتشوهات الكروموسومية هي التشوه ثلم الكروماتيدي وان اقل قابلية انقسام الخلايا لوحضت في فنيون اشعة الاسنان وهم يعملون اكثر من 10 سنوات في هذه المهنة وهم ايضا مدخنين.