Studying the changes in the rate of formation micronuclei that are affected by exposure to dental x-rays

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Abstract:- The confirms IAEA It is necessary to use cytogenetic analysis, which is one of the best modern standards used in biological standardization to estimate the genetic damage caused by dental x-rays for workers. Radiographic examination is one of the principal diagnostic methods used in all fields of medical and dental services. The current research aimed to use cytogenetic indicator in blood lymphocytes of a sample of dental X-ray diagnostic workers in Al-Zahraa and Al-Karama Teaching Hospital as well as from Taiba Specialized Center in addition to dental clinics in Wasit. The study included (60) blood samples, (40) exposed , (33) males and (7) females, and the control group was (20) blood samples for healthy non-exposed subjects for comparison. The rate of micronuclei was used in this study of the changes in the rate of formation of micronucleus in the mitotic phase for workers in the field of dental x-ray diagnostics and the diagnosis of those changes resulting from continuous exposure to radiation. It was found through the study that there were significant differences between the four exposure groups. as was noted that there were no slight significant differences in the rate of small nuclei for age between the fourth groups as well as control group (mean ±SD)(0.03±0.001),(0.01±0.002),(0.034±0.001),(0.04±0.001) at level ($p<0.05$) (0.5),(0.98),(0.83),(0.36), As for the gender (male, female) there are significant statistically significant differences between first and third groups except for the second and fourth group (6-10) years,(16-20<) year at level ($p<0.05$)(0.08),(0.1 0).

Keywords— X-rays, frequency of micronucleui , ionizing radiation, dental radiographers , Human Blood

1. Introduction

X-rays were discovered by chance for the first time by scientist William Röttgen and are called X-rays [1]. They are ionizing rays capable of ionizing the medium by separating the electrons of atoms and molecules because they have a wavelength between (10 picometers-10 nanometers) and a frequency between (30 petahertz - 30 exahertz) and therefore have a shorter wavelength than ultraviolet rays and gamma rays [2]. They induce biological changes in the eukaryotic cells of the human body upon prolonged exposure to radiation workers [3]. This is due to the fact that exposure to radiation leads to the formation of reactive oxygen species in eukaryotic cells, which in turn causes damage to genetic material, lipids and proteins, which inevitably leads to cytotoxicity and genotoxicity in the long run [4]. Because of compliance with radiation protection measures (1) shielding (usually by lead) of unexposed areas, especially radiosensitive organs; (2) increased distance between the radiation source and patients of PEW; and (3) reduction of exposure time, however, even though each of these factors has been useful, they have serious limitations in clinical practice [5].
Countryman and Heddle described for the first time the PBL MN test in the Mid-1980s A big technological innovation. Adding cytochalasin B to the media blocked cytokinesis called CB cell without limiting nuclear division [6]. Ionizing radiation may cause chromosomal fragmentation and MA segregation. Fragments and complete chromosomes that can't connect with the spindle lag behind during anaphase and aren't included in the primary daughter nuclei hence the term micronucleus [7]. The micronucleus test detects substances that change chromosomal structure and segregation, causing micronuclei in interphase cells. Add cytochalasin B to cell cultures to test. This prevents cytokinesis in cultivated cells, allowing them to grow longer [8]. the possible to discriminate between cells that are proliferating (after the first mitosis) and those that are not, therefore MN should only be scored in bi-nucleate cells with surviving cytoplasm [9]. Bi-nucleate (BN) cells could be gathered and recognized as cells that had undergone one nuclear division; an MN could then be selectively and properly scored in these BN cells, excluding non-dividing mononuclear cells that are unable to express MN [10]. The micronucleus test may show genetic damage from cumulative exposure [11] . Method to measure chromosomal breakage and loss in nucleated cells Micronuclei (MN) may originate from acentric chromosomal segments not integrated during cell division. They are surrounded by a nuclear membrane and appear as micronuclei in the cytoplasm outside the primary daughter nuclei [12] . They occur from unrepaired DNA double-strand breaks caused by clastogenic chemicals. MN may comprise entire chromosomes that lag behind during anaphase during nuclear division [13] . the CBMN assay has become a standard cytogenetic approach for genetic toxicity assessment in human and animal cells, mn straightforward and speedy scoring makes it ideal for 1) large-scale genetic damage assessment in radiation workers receiving low dental radiation and 2) population triage in large-scale radiation accidents [14]. There is a close relationship between nuclear changes that

occur due to exposure to X-rays such as pyknosis, karyorrhexis, karyolysis and micronucleus formation[15]. Therefore, these nuclear changes that indicate cell death are associated with corneal haemorrhage, adrenal cortex and hemolysis[16].

2. Materials and Methods

This research was carried out from November to May in the Biology Laboratory, College of Education for Pure Sciences, Wasit University. collecting blood samples from workers who were exposed to dental x-ray in Al-Zahraa and Al- Karama Teaching Hospital as well as from Taiba Specialized Center in addition to dental clinics in Wasit. The study included (60) blood samples,(40) exposed, (33) males and (7) females, and the control group was (20) blood samples for healthy non-exposed subjects for comparison. Cytogenetic analysis of peripheral blood samples was performed using traditional cytogenetic methods according (short time culture), and the use of culture media (LymphoPrime Medium) and colchicine solution, reagents, and stains (KCL, PBS, fixative solution, trypsin solution, and others), many laboratory equipment and tools To make, 5 mg of cytochalasin B powder was dissolved in 1 mL of dimethyl sulfoxide (DMSO), and then the mixture was divided into 0.1 mL aliquots and stored in Eppendorf tubes at -20 C°. Prior to use, the volume was completed to 1mL, added to the medium, and the stock concentration was 500 µg /mL [17]. at 37 °C for 48 hours before harvesting. All of these procedures were carried out in an aseptic environment. At least 1000 cells were scored to assess the frequency of MN, the cells were classified as The formation multi-nuclei in cells exposed to dental X-rays were examined and studied using the microscope under the magnification power of (1000 x).. they observed that the multi-nuclei of the multiple types were formed as a positive result. The positive result from the in vitro micronucleus test indicates chromosome damage or damage in the cell division apparatus [18] .

2.1 Statistical analysis

Microsoft Office Excel version 2019 (Microsoft, USA) was used to process all data obtained. GraphPad Prism version 6 (GraphPad Software Inc., USA) All statistical analyses of all data in this study were performed using the ANOVA Test. Tukey's multiple comparisons test shows the significant differences between the totals of samples subject to the test and the control group, and Sidak's multiple comparisons test shows the significant differences between the totals of the samples subject to the test with each other In addition to the standard deviation and standard error of Mean.

3. Results and Discussions

The Cytokinesis Block Micronucleus Assay (CBMA) was used to assess cytogenetic damage in peripheral blood cells. The in vitro micronucleus assay is a mutagenic test technique for detecting substances that cause tiny membrane-bound DNA fragments to develop [19].

Table (1-1) above shows the rate of small nuclei in lymphocytes after examining 1000 lymphocytes for a sample of workers and the control group, as was noted that there were no slight significant differences in the rate of small nuclei for age between the fourth groups as well as control group (mean ±SD)(0.03±0.001),(0.01±0.002),(0.034±0.001),(0.04±0.001) at level ($p<0.05$)(0.55),(0.98),(0.83),(0.36), As for the gender (male, female) There are significant statistically significant differences between first and third groups except for the second and fourth group (6-10) years,(16-20<) year at level ($p<0.05$)(0.08),(0.1 0) Compared with each other as well as with the control group as per Sidak's test. MN are much easier to score manually or using automated systems and can therefore be considered as retrospective biomarkers of exposures [20].

The MN assay can also be viewed as an alternative method to dicentric MN is a specific biomarker of IR exposure the studies suggest that MN is one of the best discriminators between IR-exposed and unexposed medicine workers [19]. However, were shown hypersensitive to other factors (e.g., age, smoking habits, mode of exposure, diet and exposure to other clastogenic agents) that can influence their accumula-

tion and persistence [21]. the results present confirmed that IR-exposed medical workers had significantly elevated frequencies of blood lymphocytes with CA and/or MN. Particularly, dicentrics were shown reported to be “the biomarker and is a standard endpoint for radiation biodosimetry applications [22]. Because of its unstable nature and continuous renewal of PBL, the frequency of dicentrics decreases with time after exposure. This may explain why decreases in unstable CA frequencies were found in workers upon removal from IR exposure [23]. Interestingly, such periods without IR exposure (vacation, break or change to non-IR professional activities) may have contributed to the failure to show dose-response relationships for unstable CA in medical workers [23]. The results presented confirm the relevance of CA and MN as genotoxicity biomarkers that are consistently elevated in IR-exposed vs. unexposed workers [24]. In this review, we conclude that dental X-rays are not able to induce genetic damage but can promote cell death considering that micronucleus examination detects only chromosomal damage, It is important to employ methodologies in the future to assess genotoxicity caused by X-rays, not only to magnify the intrinsic risks but also to ensure the best safety of X-ray technicians, Although there was a slight significant increase in the level of($p<0.05$) In the rate of formation of small nuclei in lymphocytes, their rates are within the normal permissible limits according to the report of the IAEA No. 405 of 2001[25].

Table (1-1) Distribution Group Samples Dental Duration X-ray, According Micronuclus

	Groups of time duration		Micronu-cleus / cell MN(Mean ± SD)	Mean	Stander Deviation (S.D.)	P-Value
	Group(1) 1<year- 5	Age (25-35)	0.03±0.001	0.03	0.001	0.5
		Male	0.024±0.001	0.024	0.001	0.002
		Female	0.027±0.001	0.027	0.001	0.005
	Group (2) 6 years – 10 years	Age (35-45)	0.01±0.002	0.019	0.002	0.98
		Male	0.02±0.001	0.019	0.001	0. 08
		Female	0.02±0.001	0.021	0.001	0.09
	Group (3) 11 years 15	Age(45-55)	0.034±0.001	0.0335	0.001	0.8
		Male	0.032±0.001	0.032	0.001	0.0002
		Female	0.04±0.001	0.035	0.001	0.082
	Group (4) 16 years – 20 > years	Age(55 >- 65	0.04±0.001	0.039	0.001	0.36
		Male	0.04±0.001	0.038	0.001	0.19
		Female	0.04±0.001	0.040	0.001	0.20
	Control group (0 time duration	Age(20-70)	0.02±0.001	0.02	0.002	0.88
		Male	0.02±0.001	0.015	0.001	0.002
		Female	0.02±0.001	0.016	0.001	0.004

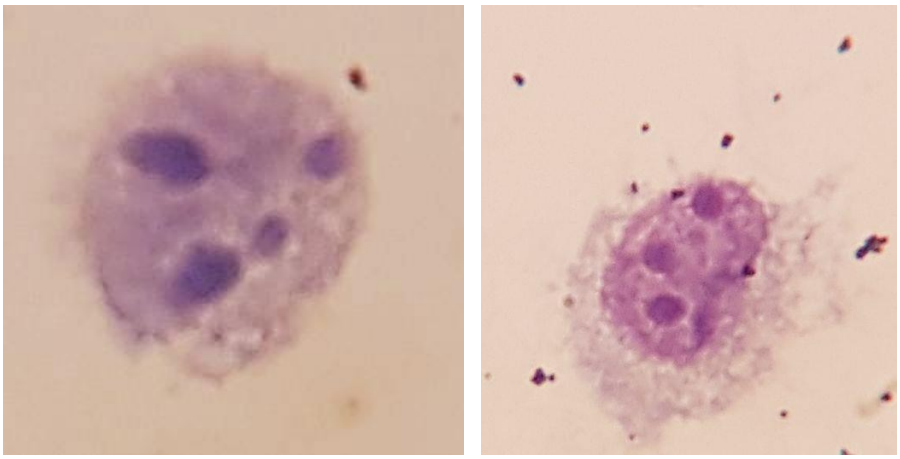

Fig.(1 -1) milt-nuclear and micronuclei (1000X).

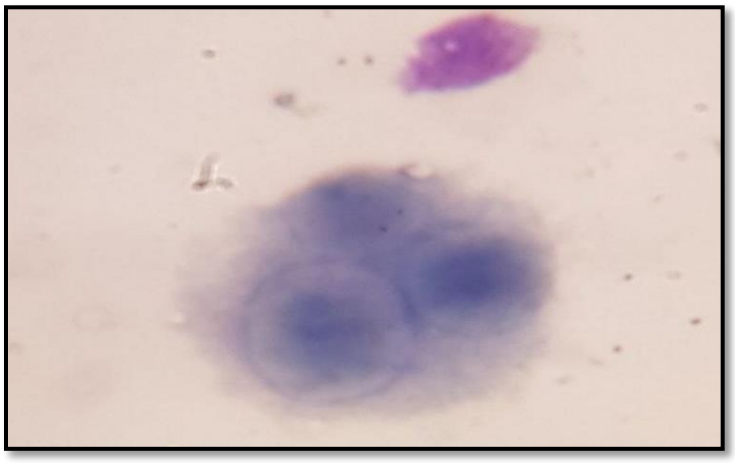

Fig.(1 -2) milt-nuclear and micronuclei (1000X).

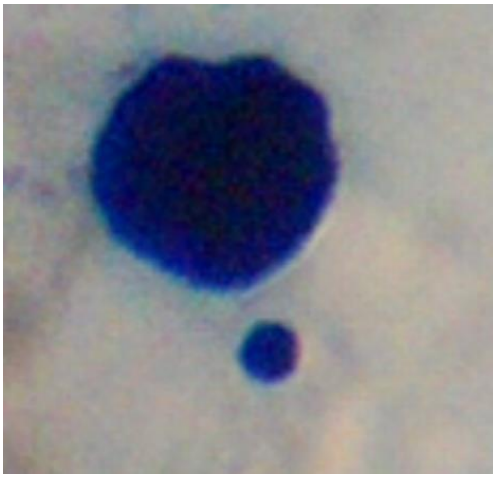

Fig.(1 -3) micronuclei (1000X).

4. Conclusion

from the result of this study that the formation of micronuclei was found in dental radiographers who were exposed to a duration of more than 10 yrs . The formation of micronuclei is an indicator of chromosomal damage caused by X-rays and the frequency of the micronucleus is related to telomere length (the end structures of linear chromosomes), which in turn is to protect the chromosomes and participates in the integrity of the genetic heritage Therefore, further studies involving an increasing number of individuals are necessary in order to obtain more reliable conclusions about the cytogenetic effect of dentists' chronic exposure to low levels of ionizing radiation, Thus, measuring telomere lengths in subjects exposed to X-rays has the potential to be a biomarker of the risk of cancer and other age-related diseases.

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