

Genotoxic and Cytotoxic Effects of Cone Beam Computed Tomography on Exfoliated Buccal Epithelial Cells

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Abstract: Cone beam Computed Tomography (CBCT) is a valuable diagnostic aid. It provides three dimensional detailed images of the region of interest with good quality. It allows volumetric analysis and it has short scan times. Ionizing radiation emitted from different radiographic procedures may induce DNA damage and genomic alteration. Genomic damages can be assessed and evaluated by numerous methods. The present study was accomplished to evaluate genotoxicity and cytotoxicity in exfoliated buccal mucosa cells of adults following CBCT exposure. **Materials and Methods:** 30 healthy male patients were recruited from outpatient's clinic of Faculty of Dentistry Pharos University of age ranging from 27 to 43 years. All patients were submitted to CBCT. Exfoliated buccal cells were collected immediately before CBCT and after 10 days. The cytological smears were examined to detect micronuclei and other cytological alterations. **Results:** The mean frequencies of micronuclei and the other nuclear changes (pyknosis, condensed chromatin and karyorrhexis) showed a significant increase after CBCT exposure. Though, the increase in both parameters was not statistically significant in correlation with patients' age. **Conclusion:** From the current study, the authors concluded that CBCT has inevitable health side effects. Hence, it must be used following the ALARA principle.

Keywords: CBCT, condensed chromatin, DNA, karyorrhexis, micronuclei, pyknosis.

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I. Introduction

Radiographic assessment is a key diagnostic method for dental practitioners. It is of prime importance in various dental fields as it has an important role in diagnosis of different diseases, prediction of prognosis and monitoring the treatment progress.¹

The radiographs used in dentistry can be classified into intraoral as periapical, bitewing and occlusal and extra-oral as panoramic radiography, postero-anterior, water's view, lateral skull view, and lateral cephalometric examinations.² In addition, it can be categorized into two-dimensional imaging (2D) as all the previous types and three-dimensional imaging (3D) as computed tomography (CT) and Cone Beam Computed Tomography (CBCT) that viewed the morphologic structure in 3D.³

The application of CT is limited in dentistry since it has high radiation dose and high cost.⁴ CBCT was used widely in maxillofacial imaging, since it has lower cost and less radiation dose compared to the CT.^{5,6} On the other hand, radiation dose from CBCT is significantly higher than traditional dental radiography techniques.^{7,8} CBCT has many benefits, it provides three dimensional detailed images of the region of interest with good quality, allows volumetric analysis and it has short scan times.⁹

In spite of all these benefits, ionizing radiation emitted from CBCT can generate biologic damage.¹⁰ Many studies^{11,12} demonstrated that ionizing radiation may induce DNA damage and genomic alteration. DNA damage includes single and double strand breaks and DNA protein crosslinks which induce cellular death.¹³ Such genomic instabilities are considered as a major cause of developmental and degenerative disorders. Genomic damages can be assessed and evaluated by numerous methods. Among them, one of the most popular and sensitive methods is the micronucleus test.¹⁴

The micronucleus is a small extranuclear body formed during cell division, either due to chromosomal aberrations or improper function of the mitotic spindles.¹⁵ Therefore, micronucleus assay is a well-known technique in monitoring recent exposure of individuals to genotoxic agents, such as chemicals and ionizing radiation. This assay is used to detect the genotoxic damage in human peripheral lymphocytes. The use of other cells than lymphocytes, such as exfoliated buccal epithelial cells is of particular interest as it is cost-effective, noninvasive, and easily acceptable procedure by patients. In addition to micronucleus test, other cytological

changes such as nuclear alterations and different chromatin status have been characterized in exfoliated buccal cells as markers of cytotoxic effects.^{13,16}

The current study was accomplished to evaluate genotoxicity and cytotoxicity in exfoliated buccal mucosal cells of adults following CBCT exposure.

II. Material And Methods

Patient selection:

The present study was conducted on 30 healthy males. The patients were recruited from outpatient's clinic of Faculty of Dentistry Pharos University in Alexandria from May 2017 till December 2017 whom referred to CBCT for diagnostic purposes. The patients' age ranged between 27 to 43 years. Exclusion criteria included systemic disorders, patient taking medications, alcohol or tobacco consumers and patients subjected to CBCT one month prior to the study. Careful intraoral examination was performed including inspection and palpation of lips buccal mucosa, vestibular mucosa, hard and soft palate, tongue and floor of the mouth. Patient with red and/or white colored or pigmented lesions were excluded from our study. Informed consents were obtained from all the selected patients.

Scanning protocol:

Patients were subjected to CBCT using J Morita CBCT unit (J. Morita, Corporation, Kyoto, Japan), Imaging Software Included i-Dixel 2.0 software operated at 84 Kilovoltage (kVp) and 9–14 Milliampere (mA) with a voxel size of 0.16 mm, exposure time of 6 seconds, and field of view (FOV) of 80x100 mm for all patients.

Cell collection and slide preparation:

Exfoliated buccal cells were collected immediately before CBCT and after 10 days. Each patient was asked to rinse with tap water in order to remove any debris from the oral cavity. Exfoliated oral epithelial cells were collected by scraping the cheek mucosa with a moist wooden tongue depressor. The obtained cells were smeared on a sterile glass slide. Then, the smears were fixed with a 95% ethanol and stained with Papanicolaou stain.^{14,17}

Cytological analysis:

The smears were examined blindly by the authors in randomly selected microscopic fields at a magnification of x400 to detect the micronucleus and other cytological alterations. At least 1000 exfoliated epithelial cells in each smear were counted. The micronuclei were counted following Tolbert et al.¹⁸ parameters for identifying micronucleus. It is rounded membranous extranuclear body, which is one third the associated nucleus, with similar stain intensity as the nucleus and on the same focal plane. Other cytological changes were examined in terms of nuclear alterations and different chromatin status as pyknosis, condensed chromatin, karyorrhexis and karyolysis.

Statistical analysis

The Kolmogorov- Smirnov, Shapiro and D'agstino tests were used to verify the normality of distribution of variables. Normally quantitative data were expressed in mean \pm standard deviation (SD). The paired-samples *t*-test was used to compare the frequencies of micronuclei and other nuclear alterations among the samples before and after CBCT exposure. Pearson test was used to correlate the frequency with the patients' age. Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Significance of the obtained results was judged at the 5% level.

III. Result

The current study was performed on 30 male patients, with mean age of 34.27 ± 3.83 , (Table 1). The micronucleus was detected as extranuclear bodies smaller than the nucleus, with the same nuclear stain intensity. Concerning other nuclear alterations, pyknosis, condensed chromatin and karyorrhexis were observed. However, karyolysis was not observed after CBCT exposure, (Figure 1).

The mean frequencies of micronuclei and other nuclear changes were compared before and after subjection to CBCT *per* 1000 cells. Concerning the micronuclei, the frequency increased significantly after CBCT exposure from 0.026 ± 0.0062 to 0.030 ± 0.0068 , ($p < 0.001$). Correspondingly, the other nuclear alterations showed a significant increase from 0.013 ± 0.0039 to 0.027 ± 0.0072 , ($p < 0.001$) (Table 2). However, the increase in both parameters were not statistically significant in correlation with patients' age, ($p > 0.5$), (Table 3).

Table 1: Distribution of the studied cases according to age (n=30)

Age (years)	No. (%)
≤ 35	19 (31.7%)
> 35	11 (18.3%)
Mean \pm SD	34.27 ± 3.83

Table 2: Comparison of the frequency (%) of micronuclei and other nuclear changes (pyknosis, condensed chromatin, and karyorrhexis) before and after subjection to CBCT.

Frequency (%)	Before	After	p
Micronuclei (n=30)	0.026 ± 0.0062	0.030 ± 0.0068	<0.001*
Nuclear changes (n=30)	0.013 ± 0.0039	0.027 ± 0.0072	<0.001*

Normally quantitative data was expressed in mean ± SD

p: p value for **Paired t-test** for comparing between **before** and **after CBCT exposure** in each other group

*: Statistically significant at $p \leq 0.05$

Table 3: Correlation between age and frequency (%) (n=30)

Frequency (%)	Age (years)	
	r	p
Before	-0.193	0.306
After	-0.104	0.584
Difference	0.061	0.749

r: Pearson coefficient

*: Statistically significant at $p \leq 0.05$

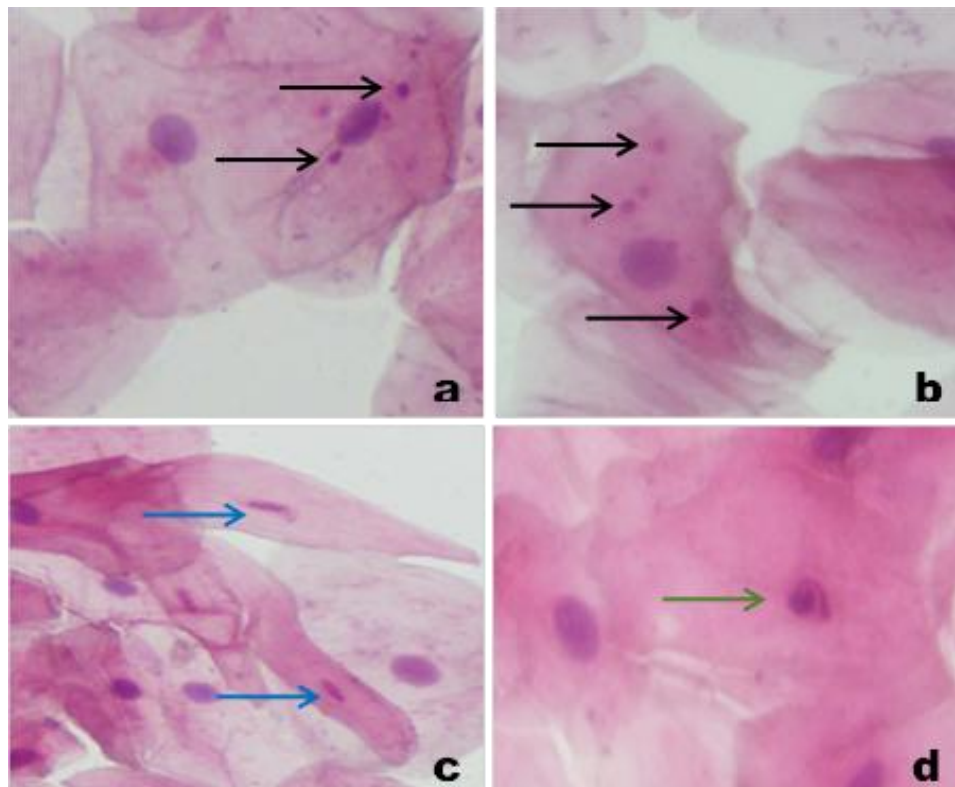


Figure 1: A photomicrograph of exfoliated buccal epithelial cells after CBCT exposure shows nuclear changes. a and b: micronuclei (black arrows), c: pyknotic nuclei (blue arrows), d: karyorrhexis (green arrow). (a-d: Pap stain, x400).

IV. Discussion

CBCT is an established diagnostic aid which offers numerous benefits compared to other available radiographic modalities. Shekhar *et al.*¹⁹ stated that CBCT is a gold standard diagnostic method comparing to periapical and panoramic radiography. However, the hazard of ionizing radiation from different dental radiographic process has been evaluated for several years and a number of guidelines have been recommended.^{20,21,22}

The radiation dose from CBCT and its adverse effects on living cells were considered as a major concern for many researchers.^{23, 24} In the present study we investigated the cytotoxicity and genotoxicity in patients subjected to CBCT. The genotoxic effects were evaluated by detection of micronuclei and other nuclear

alterations in exfoliated buccal epithelial cells. The effective dose differ from one CBCT unit to another according to the scanning protocol which include kVp, mA, voxel sizes and FOV.^{23,25} Consequently, we standardized the scanning protocol for all patients. Exfoliated cells were collected immediately before exposure to ionizing radiation from CBCT and after 10 days similar to other studies.^{26,27,28} Day 10 was chosen on the basis of the rapid turnover of the epithelial cells that take 7–16 days to emerge to the surface and to exfoliate.²⁹ The sample cells were collected from the buccal mucosa in consistent with the previous studies carried out by Agarwal *et al.*³⁰ and Filho *et al.*³¹. These studies indicated that buccal epithelial cells considered as a preferred target that accurately reflect the cytotoxic changes and genomic instabilities in epithelial tissues due to its high turnover rate that brings the cells to the surface.

The radiation hazard is greatly affected by age and gender.³² In addition, human genotoxic and cytotoxic biomonitoring are affected by different factors other than age and gender such as smoking and alcohol consumption.³³ In order to justify the results of our study, only non-smoker male subjects with a narrow age range of mean 34.3 years were recruited in the present study. Moreover, because each patient was considered to be his own control, any effect of other genotoxic agents must have been presented in the first cell count. Therefore, potential differences between first and second counts can be attributed to radiation.²⁹

On the basis of our results, the genotoxic biomonitoring revealed that the frequency of micronuclei in the exfoliated buccal cells were increased significantly after exposure to CBCT. These results were consistent with study by da Fonte *et al.*³⁴ who demonstrated a statistically significant increase in the frequency of micronucleated cells for both partial and total CBCT acquisition. The formation of micronucleus is attributed to chromosomal damage that could not be included in the daughter nuclei by the end of the mitotic process.^{14,35} According to Tolbert *et al.*¹⁸ the sensitivity of the micronucleus test is increased by recording degenerative nuclear alterations such as pyknosis, condensed chromatin, karyorrhexis and karyolysis, in addition to the micronucleus. In the present study, the cytotoxic nuclear alterations included pyknosis, condensed chromatin and karyorrhexis which indicated cellular death by apoptosis rather than necrosis. The frequency of the previously mentioned changes were increased significantly after exposure to CBCT. Our results were in agreement with Lorenzon *et al.*²⁹ and da Fonte *et al.*³⁴ who reported a significant increase in the nuclear alteration after exposure to CBCT.

Moreover, the results of the current study indicated that the increase in the frequency of micronucleus and nuclear alteration were not significantly related to age. These results confirmed those of previous studies performed on participants with homogenous age.^{26,29}

The significant increase of the micronuclei as a genetic damage biomarker after CBCT exposure highlights the consequences of accumulation of these genetic damages within the epithelial cells. It is well known that such alterations are considered as the fundamental cause of development of premalignant lesions and cancer.^{17,36} Buajeeb *et al.*³⁷ revealed an increase in micronuclei frequency in atrophic and erosive oral lichen planus, indicating genotoxic damage. Similarly, leukoplakia showed a significant increase in the micronuclei frequency as was reported by Kamboj and Mahajan³⁸. Moreover, Chaudhary *et al.*¹⁴ revealed that oral squamous cell carcinoma showed an increase in the micronuclei frequency.

Concerning the genotoxic and the cytotoxic effects of ionizing radiation emitted from other types of dental radiography, Angelieri *et al.*¹¹ indicated that panoramic radiography might not induce chromosomal damage, but it induces cytotoxic nuclear alteration. Whereas, Cerqueira *et al.*²⁶ reported that X-ray radiation emitted during panoramic dental radiography induces a genotoxic effect on epithelial gingival cells that increases the frequency of chromosomal damage and nuclear alterations. Furthermore, a study carried out by Kesidi *et al.*³⁹ reported that full mouth radiographs can induce cytotoxic and genotoxic effects in oral mucosa cells. Accordingly, ionizing radiation emitted from CBCT and different dental radiographic modalities potentiate both genotoxic and cytotoxic nuclear alteration.

V. Conclusion

Within the highlights of the results of the present study, the authors concluded that CBCT has inevitable health side effects that should be taken into consideration, and it must be used following the ALARA (As Low As Reasonably Achievable) principle, owing to the ability of CBCT to induce cellular death.

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