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Original Article

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

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Abstract

Background: Cone beam computed tomography (CBCT) and multidetector computed tomography (MDCT) are frequently used in dental and maxillofacial problems. This study aimed to assess the genotoxicity and cytotoxicity effects of CBCT and MDCT radiographies on exfoliated buccal epithelial cells during dental examinations.

Methods: This prospective experimental study was conducted at Babol University of Medical Sciences (Babol, Iran) from March 2021 to April 2021. Buccal mucosa smears were collected bilaterally pre-exposure and 12 days after CBCT or MDCT examinations. To compare the frequency of micronuclei and other cytotoxic cellular changes such as pyknosis, karyolysis, and karyorrhexis, the paired sample *t* test and Wilcoxon test were used. In addition, independent sample *t* test, Mann-Whitney, and Chi square tests were used to investigate the differences between the imaging methods and between men and women. All statistical analyses were performed using the SPSS software, and $P \leq 0.05$ was considered statistically significant.

Results: The current study included 60 adult patients (30 patients in each group), ranging in age from 21 to 50 years. The micronuclei and the other cytotoxic cellular changes increased significantly after CBCT and MDCT radiographic examinations on the 12th day compared to the pre-exposure results ($P<0.001$). MDCT had statistically higher cytotoxic and genotoxic effects than CBCT (9.4%, 23.1%, and 40% higher values in micronucleus frequency, the mean frequency of micronuclei, and other cytotoxic changes, respectively). There were no significant differences between men and women in the two examination methods ($P=0.46$ and $P=0.49$, respectively).

Conclusion: Dental examinations with CBCT and MDCT can increase cytotoxicity and chromosomal damage in both men and women. Due to its lower radiation toxicities, CBCT can be recommended as an alternative to MDCT for dental examinations.

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Keywords ● Cone beam computed tomography ● Multidetector computed tomography ● DNA damage ● Mouth mucosa

What's Known

- Cone beam computed tomography (CBCT) and multidetector computed tomography (MDCT) are investigative scans frequently employed by dental practitioners in dental and maxillofacial imaging.
- Although several studies reported the toxic effects of dental CBCT; no previous study reported the chromosomal changes in buccal mucosa after MDCT dental examinations

What's New

- CBCT and MDCT dental examinations can worsen cytotoxicity and chromosomal damage.

Introduction

Dental imaging is one of the medical procedures used for evaluating dental and maxillofacial disorders.[1] Dental radiology

systems commonly use ionizing radiation (X-ray) to diagnose dental diseases and plan dental surgeries.[2] Cone beam computed tomography (CBCT) is a dental imaging method that can provide three-dimensional (3D) anatomical images from maxillofacial regions.[3] CBCT also offers high-resolution volumetric data for a variety of conditions, including maxillary sinus evaluation, oral surgery, temporomandibular joint evaluation, orthodontic evaluation, implant planning, and craniofacial trauma assessment.[4, 5]

Computed tomography (CT), particularly multidetector CT scan (MDCT) with specialized dental software, is a standard method that provides lots of information on both soft and hard tissues of the oral and maxillofacial region, as well as surrounding tissues.[6] CBCT typically exposes patients to higher radiation doses than conventional dental radiographic methods, ranging from 3-20 times higher than panoramic to 100-150 times higher in intraoral periapical radiography.[7-9] MDCT imaging delivers higher radiation doses than CBCT (approximately 40% higher effective dose in dental imaging).[10] However, several studies published recently suggested that MDCT could provide acceptable image quality at low radiation doses.[2, 11] The conical exposure geometry of CBCT results in lower doses. In addition, the majority of CBCT units utilized pulsed emission rather than continuous emission, which reduced exposure duration.[12]

Ionizing radiation emitted from MDCT or CBCT can cause biological damage. Micronuclei formation and cytotoxic changes are sensitive biomarkers for assessing ionization radiation adverse effects.[13] Genotoxicity refers to factors that cause DNA or chromosomal damage, such as micronucleus formation.[1] Micronucleus are microscopic nuclei composed of acentric chromosomal fragments or lagging chromosomes. They are typically produced during the metaphase-anaphase transition due to chromosomal damages.[6, 14] Cytotoxicity refers to toxic factors that usually cause fatal damage or necrosis. These damages cause nuclear changes such as pyknosis (chromatin condensation), karyorrhexis (fragmentation of Pyknotic nuclei), and karyolysis (dissolution of chromatin).[13]

The genotoxic and cytotoxic effects can be found in exfoliated buccal cells. Since the buccal mucosa is located in the path of MDCT and CBCT dental X-radiations, evaluating these cells is an appropriate method for measuring genotoxicity and cytotoxicity effects.[15] Several studies reported the toxic effects of dental CBCT;[15-18] however, no previous study reported the chromosomal changes in buccal mucosa following MDCT dental examinations. Thus, the present study aimed to assess the genotoxicity and cytotoxic changes in exfoliated buccal mucosal cells after dental examinations caused by MDCT compared to CBCT. Furthermore, these outcomes were contrasted between men and women adult patients who underwent dental CBCT and MDCT scans.

Patients and Methods

This prospective cross-sectional study was recruited among patients who were referred to the Department of Oral and Maxillofacial Radiology, Babol University of Medical Sciences (Babol, Iran) from March 2021 to April 2021. The study was conducted in compliance with all the ethical considerations of Helsinki 1964 related to human studies. The study protocol was approved by the Ethics Committee and National Research Ethics Board of the Babol University of Medical Sciences (IR.MUBABOL.REC.1399.437). Written informed consent was obtained from all the participants, and the patients were informed that the study protocol was not an invasive procedure. Micronuclei and cellular changes were evaluated bilaterally before and 12 days after the MDCT and CBCT examinations.

Sample Size

The sample size was calculated using the estimated means of the groups and test power, considering any significant differences between the groups. The following equation was used to determine the sample size.[19]

$$N = \frac{(r+1)\left(Z_{\alpha/2} + Z_{1-\beta}\right)^2 \sigma^2}{rd^2}$$

Where $Z_{\alpha/2}$ is the normal deviation at a level of significance ($Z_{\alpha/2}$ is 1.96 for 5% level of significance and 2.58 for 1% level of significance), and $Z_{1-\beta}$ is the normal deviation at 1-β% power with β% of type II error (1.28 at 90% power in this study). Moreover, r=n1/n2 denotes the ratio of the sample size required for the two groups, which is considered one in this study. The standard deviation and the difference in the ratio of the two groups are represented by σ and d, respectively. These values were obtained by conducting a pilot study. After conducting a pilot study on three patients, an 18% difference between the toxic effect (frequency of micronucleus formation) of CBCT and MDCT, as well as the standard deviation of 16% were considered, and the sample size of 17 patients was obtained.

We considered 30 patients for each group to obtain higher statistically significant results. In a similar study comparing the cytotoxic effect of CBCT in comparison with dental radiography, 24 patients were considered in each group.[20]

Participants

The present study included 60 adult patients. Thirty patients, who were referred to the Department of Oral and Maxillofacial Radiology (Babol University of Medical Sciences, Babol, Iran), were selected for CBCT examination including 13 men and 17 women aged 21-50 years (34.37±7.72). In addition, 30 patients, who were referred to the Department of Radiology and Medical Imaging Center of Shahid Beheshti Hospital (Babol, Iran), were selected for MDCT examination, including 13 men and 17 women ranging in age from 23-48 years (34.97±6.83). All patients were referred for CBCT and MDCT dental examinations based on their physicians' prescriptions for dental implants. Table 1 summarizes the demograohic characteristics of the patients.

Exclusion Criteria

The patients should not have a history of tobacco or alcohol consumption. In addition, they should not have used mouthwash in the preceding three days. Besides, they should not have used immunosuppressants, or cytotoxic medications in the previous year before examination. The patients with systemic diseases, such as leukemia, lymphoma, rheumatoid disorders, diabetes mellitus, megaloblastic anemia, coronavirus infection, and a history of head and neck radiotherapy were also excluded. The participants should not have any clinically apparent mucosal diseases, including mucosal inflammation, periodontal diseases (localized or generalized), and local irritating factors such as partial and total prostheses. Furthermore, they should not have done any radiographic examination in the previous three months. The participants with menopause, pregnancy, and lack of physical ability were also excluded. It should be highlighted that none of the included patients were employed by any form of radiation institution to minimize false-positive results. Following the selection of patients based on the aforementioned criteria, the study procedure was explained verbally, and written informed consent was obtained from all the participants. Demographic information including age, sex, as well as the complete case history and clinical findings of the patients were collected. Besides, the clinical examination was performed with a sterile mouth mirror and probe under artificial lighting. These procedures were performed by a single observer.

Scanning Protocol

CBCT examinations were performed in high-resolution conditions using X MIND (ACTEON Olgiate Olona Italy), and the following scan parameters: For men, FOV: 8×11cm^2, tube voltage: 90 kVp, and tube current: 8 mA. For women, the tube voltage and current values were 85 kVp and 8 mA, respectively. In the present study, the difference in kVp (5 kV) between men and women was related to the slightly larger bony structures in men.

MDCT examinations were performed utilizing a SIEMENS scanner (16 slices Somatom Sensation, Siemens, Germany) in accordance with the hospital's standard clinical protocol. The patients were scanned in the supine position, and the scan parameters were 110 kVp tube voltage, 35 mA tube current, and a pitch factor of 1.

Collection of Buccal Mucosal Cells

An experienced dentist collected exfoliated buccal cells of the left and right sides of cheek mucosa bilaterally both pre-exposure and 12 days following CBCT or MDCT examinations. Before each cytological smear preparation, the patient rinsed his/her mouth with water, and a gauze pad was applied to the designated area to remove debris from the mucosa. Exfoliated cytological smear was obtained by scraping and rotating a cytobrush on the cheek mucosa 10-15 times with moderate and constant pressure. While scraping, there should be no bleeding in the region. The collected cells were smeared on a sterile glass slide. Then, the cells were fixed immediately with two puffs of a 95% ethanol fixative spray (Patofix, Padtanteb, Iran). The smears were stained by Papanicolaou (PAP)

Table 1: Demographic information of patients

Variable		Multidetector computed tomography (MDCT)	Cone beam computed tomography (CBCT)	P value
Age	Range (years)	23-48	21-50	0.49
	Mean±SD (years)	34.97±6.83	34.37±7.72	0.49
Sex	Women, n (%)	16 (53.3%)	17 (56.7%)	0.46
	Men, n (%)	14 (46.7%)	13 (43.3%)	0.46

SD: standard deviation; An independent-sample *t* test was used to compare the patients' age, and Chi square statistical analysis was used to compare the patients' sex.

within a maximum of three days.[21] Before and 12 days after the MCDT or CBCT examination, two slides were prepared for each patient, and in total, 120 slides were prepared for all the patients.

Genotoxicity and Cytotoxicity Analyses

An experienced oral and maxillofacial pathologist who was blinded to demographic information and the time of the cell sample collection, analyzed the smears. A light microscope (Olympus Corporation, Tokyo, Japan) with x400 magnification was used for evaluation. The cytological characteristics of the first 2000 cells were examined for micronucleus and cytological alterations (pyknosis, karyorrhexis, and karyolysis) in the absence of hemorrhage, necrosis, and exudate. These samples were evaluated based on Tolbert's criteria.[22] The criteria for abnormality of micronucleus formation were as follows: 1) rounded shape with a smooth perimeter; 2) having an additional nucleus that was less than one-third the diameter of the related nucleus, but large enough to identify shape and color; 3) Folgen-positive (i.e., pink under bright field illumination); 4) staining intensity comparable to that of the associated original nucleus; 5) texture similar to that of the associated original nucleus; 6) identical focal plane as a nucleus; and 7) lack of overlap with or bridge to the original nucleus. Micronucleated, binucleation, the broken egg phenomenon, pycnosis, condensed chromatin, karyorrhexis, and karyolysis must all be present in the observed micronucleus samples. The certainty of cell micronucleated could vary depending on the number of the aforementioned criteria involved. Cells that met all of the aforementioned micronucleated criteria were considered high certainty. The cells that were slightly deficient in micronucleated criteria 4, 5, or 6, while meeting all of the other criteria were classified as medium certainty. The total micronucleus count included micronucleated cells with medium or high certainty.

Pyknosis abnormality criteria included high density and evenly stained nuclei caused by chromatin condensation and nucleus shrinkage. Pyknotic nuclei fragmentation and degeneration met the criteria for karyorrhexis; whereas the total disintegration of chromatin, which gave karyolitic cells a ghost-like appearance, met the criteria for karyolysis.

Statistical Analysis

Data were analyzed using SPSS software, version 18.0 (IBM, USA). The Kolmogorov-Smirnov (K-S) test was used to evaluate the normality of the data distributions for the quantitative parameters with a 95% confidence interval. To compare variables between the samples collected before and after CBCT/MDCT examinations, paired sample *t* test was used as a parametric statistical test, and the Wilcoxon and Mann-Whitney tests were used as non-parametric statistical tests. The differences between the two methods (CBCT and MDCT), as well as between men and women were determined using an independent-sample *t* test, and Mann-Whitney tests. $P<0.05$ was considered statistically significant.

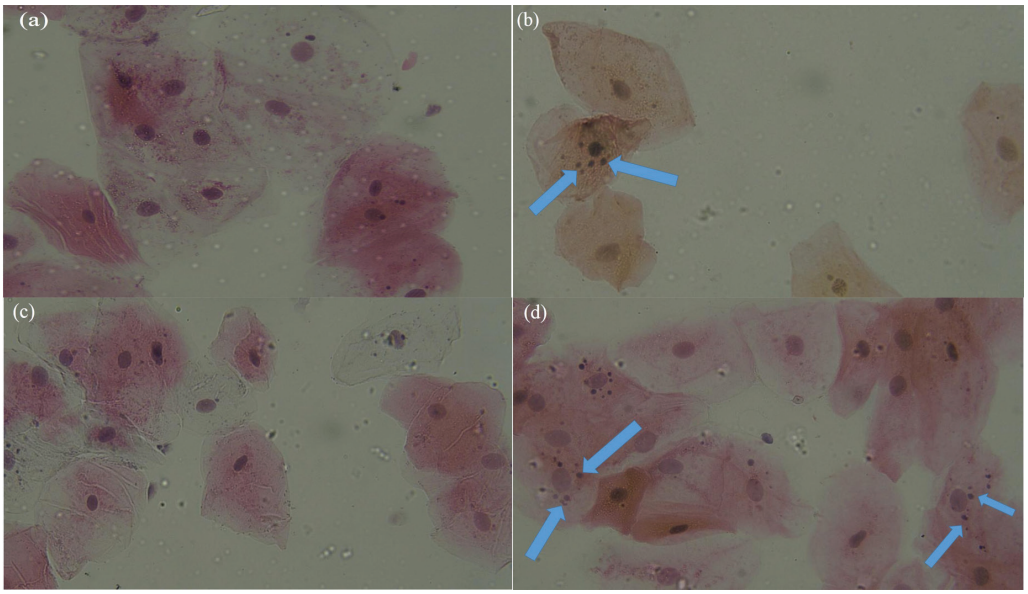

Figure 1: Microscopic imaging of Papanicolaou-stained smears before (a) and after (b) the multidetector computed tomography (MDCT). Figures (c) and (d) show similar images before and after the cone beam computed tomography (CBCT) examinations, respectively. Blue arrows indicate sample cells with multiple micronuclei.

Results

Table 1 shows the demographic information of patients who participated in the study. The statistical analysis showed no significant differences in sex and age between the patients in the CBCT and MDCT studied groups.

Figure 1 shows the microscopic images of smears stained by PAP before and after MDCT and CBCT examinations for one patient. In addition, sample images of the other nuclear changes such as pyknosis, karyolysis, and karyorrhexis are shown in figure 2. The findings of micronuclei assay and cytotoxic changes, pre-exposure and 12 days after CBCT or MDCT examinations are presented in table 2. The frequencies were compared before and after performing CBCT or MDCT examinations. The paired sample *t* test indicated that after CBCT and MDCT examinations, micronucleus assay parameters, including micronucleus frequency (per 2000 cells), the mean frequency of micronuclei (in each cell), and cytotoxic changes, were significantly higher (P≤0.001).

Furthermore, the relevant statistical analysis (sample *t* test) showed that these post-exposure parameters obtained from patients differed significantly between MDCT and CBCT examinations, indicating that MDCT had higher toxic effects than CBCT (P<0.001). Figure 3 shows the statistical differences between

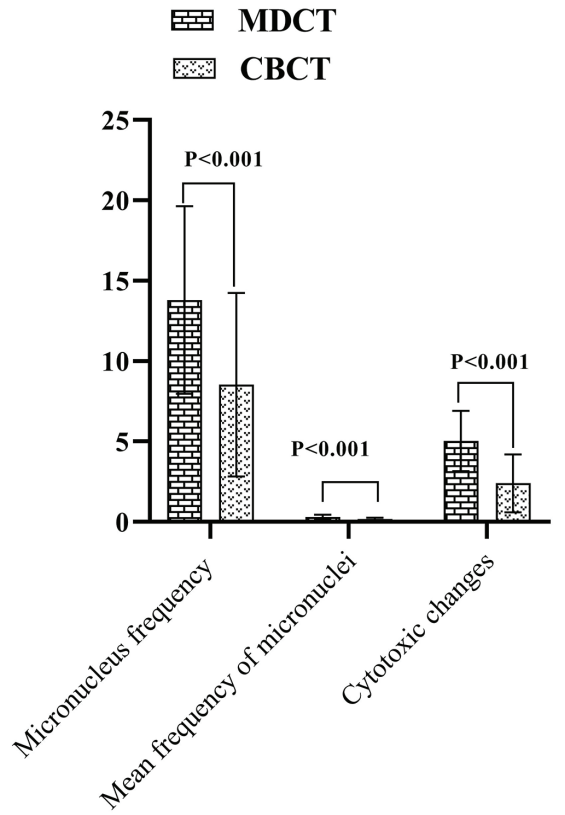

Figure 3: The differences between multidetector computed tomography (MDCT) and cone beam computed tomography (CBCT) examinations for micronuclei and other cytotoxic changes before and after exposure are shown. P obtained from the sample *t* test is presented for comparing the results between the groups. P<0.05 was considered statistically significant.

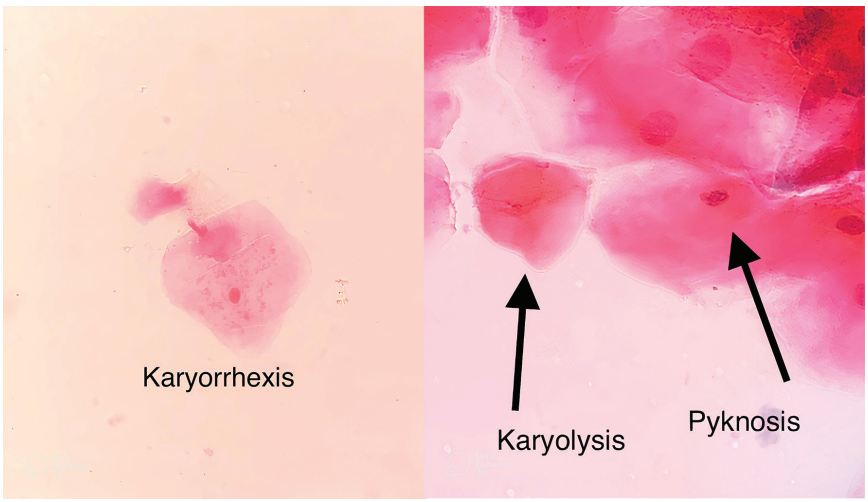

Figure 2: Sample of photomicrographs of cells that exhibits karyorrhexis, karyolysis, and pyknosis.

Table 2: The parameters related to micronuclei and other cytotoxic changes pre- and post-exposure to multidetector computed tomography (MDCT) and cone beam computed tomography (CBCT)

Variable	Multidetector computed tomography (MDCT)			Cone beam computed tomography (CBCT)		
	Post-exposure	Pre-exposure	P value	Post-exposure	Pre-exposure	P value
Micronucleus frequency	46.70±6.10	32.90±5.33	0.001	42.7±11.46	34.17±9.17	0.001
Mean frequency of micronuclei	0.80±0.10	0.49±0.09	<0.001	0.65±0.14	0.48±0.10	0.001
Cytotoxic changes	9.90±2.27	4.87±1.57	<0.001	7.07±2.24	4.67±2.64	<0.001

Paired sample *t* test was used to compare the results between the groups. P<0.05 was considered statistically significant.

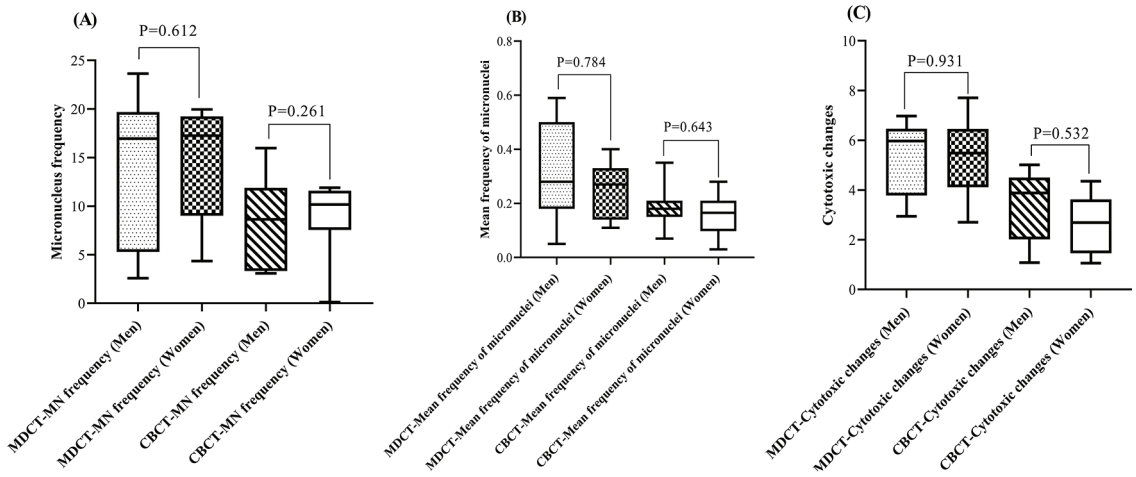

Figure 4: The comparison of micronucleus frequency (A), mean frequency of micronuclei (B), and cytotoxic changes (C) between men and women after multidetector computed tomography (MDCT) and cone beam computed tomography (CBCT) examinations is illustrated. The central horizontal line in the box represents the median of the samples, and the box edges (hinges) represent the first and third quartiles. The whiskers show the range of observed values. P values obtained from the Mann-Whitney test are presented for comparing the results between the groups. P<0.05 was considered statistically significant.

different modalities for values obtained pre- and post-exposure from the patients. As indicated, the pre-exposure values did not differ between the patients undergoing CBCT and MDCT examinations.

According to the findings of the Mann-Whitney test, the differences in micronuclei and other cytotoxic parameters between men and women in the two dental imaging methods (MDCT and CBCT) were not statistically significant (figure 4).

Discussion

The current study evaluated micronucleus and cytotoxicity changes caused by CBCT and MDCT scans on the exfoliated buccal mucosa of adult patients. Although the toxic effects of CBCT in buccal mucosa have been reported in previous studies,[15-18] these effects on patients undergoing dental MDCT have not been reported. Furthermore, we have focused on evaluating the differences between these two imaging techniques to determine which one can induce more severe cytotoxic changes. To the best of our knowledge, this study was the first study comparing cellular changes between CBCT and MDCT modalities. Besides, previous studies were usually conducted on smaller sample sizes.[14-16, 23] However, we attempted to include a larger number of patients (60 patients and 120 samples) to detect small changes with more statistical confidence.

In previous studies, peripheral lymphocytes were typically used to evaluate genotoxic and cytotoxic changes utilizing micronucleus assay.[6, 14, 24, 25] Evaluating exfoliated buccal epithelial cells for micronucleus assay in the current work is of particular interest due to their simple gathering procedure, cost-effectiveness, non-aggressive, painless, fast, and repeatable examination. In addition, the micronucleus results obtained from these cells were found to have high correlations with the lymphocyte assay results.[14, 25] Additionally, most oral epithelium, such as buccal mucosa, includes non-keratinized stratified squamous cells, which uptake the stain easily. Consequently, a more precise evaluation of cellular changes could be achieved.[26, 27] Other nuclear anomalies such as pyknosis, karyolysis, and karyorrhexis could be performed along with micronucleus counting; since they could occur during cellular death due to DNA damage.[28]

Micronucleus formations occurred in the epithelium basal cells as a result of the genotoxic effects of diagnostic radiations. The time it took for epithelial cells to come up to the surface and exfoliate was between 7-16 days.[1, 6, 17, 23] In this regard, in the present study, the basal cell collections were performed after 12 days of CBCT/MDCT examinations. It should be noted that several factors, including age, oral hygiene, viruses, smoking, and immune system conditions, might affect buccal mucosa biomonitoring investigations that examined cellular changes in a patient.[14] In this study, we tried to control these disturbing factors by including only healthy adults with good oral hygiene in the age range of 20-50 years old.

In the present study, the pre-exposure micronuclei counts in the buccal mucosa cells were 32.90-34.17 (per 2000 cells). The relevant differences in counts from previous studies[29, 30] could be due to the population characteristics and methodological aspects such as differences

in the sites, collection of the cells, various staining procedures, fixing techniques, number of cells counted, and scoring criteria for micronuclei.

Several studies reported the toxic effects of CBCT in buccal mucosa cells.[15-18] Although Fonte and others[18] indicated that CBCT could induce genotoxicity and cytotoxicity effects in adults' oral cells, Yang and colleagues[17] demonstrated a significant increase in only cytotoxic changes rather than micronucleus outcomes. In the present study, we found that the percentage of cells with micronucleus significantly increased 12 days after CBCT and MDCT scans, which was consistent with the findings of Fonte and others.[18] After the CBCT examination, Basha and Essawy found significantly higher micronucleus frequency values.[15] The differences in demographic characteristics, evaluation methods, and radiation parameters might cause probable controversies among the findings of previous studies.

A few studies carried out the micronucleus evaluation of lymphocytes and buccal mucosa for patients undergoing head and neck MDCT examinations.[23, 31] However, no research were conducted on the micronucleus evaluation of buccal mucosa cells in individuals undergoing dental MDCT scans. Palla and colleagues compared counts of cells with micronucleus, nuclear bud formations, and bi-nucleated cells in different head and neck areas before and after MDCT scans.[23] Following all of the head and neck MDCT scans, they found that nuclear changes were statistically significant. The present study indicated that nuclear changes after facial bone MDCT scans were statistically significant. The differences between pre- and post-exposure variables were statistically higher in MDCT examinations than in CBCT, which could be attributed to the larger field of view, higher tube current, CBCT conical exposure geometry, and longer MDCT exposure periods. Additionally, the majority of CBCT devices used pulsed emission rather than continuous emission, which reduced the exposure time.[12]

A recent literature review study reported a higher frequency of cytotoxicity in all the previous studies (22 studies) on dental radiographs (panoramic, lateral, or intraoral).[13] Only 10 studies found an increase in micronucleus frequency. These controversial findings demonstrated the challenges in assessing the cytotoxic effects of low radiation doses. Arora and colleagues showed that the micronucleus changed significantly both before and after panoramic radiography.[32] However, the majority of the studies found statistically significant cytotoxic effects without a change in micronucleus counts.[25, 33] In line with previous studies, the present study indicated significant cytotoxic changes after CBCT/MDCT scans including an average of all pyknotic, karyohectic, and karyolytic cells.[14, 15, 20] However, we also found significant changes in micronucleus counts. This controversy might be related to higher radiation doses in CBCT and MDCT techniques than panoramic or intraoral radiography examinations.[9, 10, 30]

We attempted to select an equal number of men and women with comparable age ranges for both MDCT and CBCT methods. To minimize any epidemiological disparities in radiation sensitivity, the patients were also selected from a single province (Mazandaran, Iran). In both the MDCT and CBCT groups, our findings indicated no significant differences in micronucleus scores and other cellular changes between men and women. Similar to our findings, previous studies found no correlation between sex for micronucleus-containing cells and other cellular changes. However, age might be a variable associated with the induction of genetic material damage.[34, 35]

The present study had several limitations. We investigated the toxic effects of MDCT and CBCT on the buccal mucosa of adults. However, there were other dental radiography modalities whose effects on the epithelial cells were not evaluated or compared in this study. Furthermore, this study did not include younger patients or children who are more concerned about radiation side effects than adults. Furthermore, other radiobiological tests, such as comet tail assay or Gamma H2ax, were not conducted. Thus, it is recommended to investigate these radiobiological tests by conducting multicenter studies with larger sample sizes and longer durations in the future.

Conclusion

The findings of the present study suggested that chromosomal damage and cytotoxicity caused by CBCT and MDCT exams in the buccal mucosa were independent of sex. MDCT demonstrated higher cytotoxic and genotoxic effects than CBCT (9.4%, 23.1%, and 40.0 % higher values in the micronucleus frequency, mean frequency of micronuclei, and other cytotoxic changes, respectively). Due to lower radiation risks, CBCT rather than MDCT can be recommended for dental examinations.

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Authors' Contribution

E.M: Study conception, design, acquisition of data, and drafting. Z.J, H.Gh, M.SM, M.N, K.EG, SA.SM: Study design, data analysis, and drafting. All authors read and approved the final manuscript and are responsible for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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