The Genotoxic and Cytotoxic Effects of CT Scan on Buccal Epithelial Cells

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Abstract

Background: Diagnostic radiation is reported to cause significant damage in buccal cells, while the same effects after natural cell turn over cycle were not checked for in previous studies. The buccal cells were studied in patients exposed to computed tomography (CT) scans for evaluating the cells with micronuclei and cytotoxic changes, namely, pyknotic cells, karyorrhectic cells and karyolytic cells. The pre-exposure counts were compared with postexposure counts on 10 and 20 days corresponding to first and second cell turnover cycles. Aim: The aim of this study is to estimate the counts of micronucleus and cytotoxic changes in buccal cells post-exposure to CT scans and report on variance of the same with first and second buccal cell turnover cycles. Materials and Methods: This is an observational study, wherein the buccal smears of patients undergoing CT scans were made before and after CT scan exposures as needed. Papanicolaou (PAP) staining and analysis were performed as per standard criteria for micronuclear and cytotoxic changes, respectively. Statistical test used was paired t-tests. Results: The micronuclear counts revealed 0.4% positive cells before exposure and 1.4% positive cells post 10 days and 20 days of exposure were significant (P < 0.005). The cytotoxic changes showed around 2.5% positive cells before and 5.7% positive cells 10 days after CT exposure (P < 0.005). The cytotoxic cell values from baseline to 20th day were not significant (P < 0.25). Conclusion: CT scans have caused genotoxic effects notable after two cell turnover cycles but the cytotoxic changes have significantly decreased naturally after 2nd cell turnover as per our study.

Keywords: Buccal cells, CT scans, cytotoxicity, karyolysis, micronucleus, pyknosis, radiation

Introduction

Computerized tomography (CT) is a commonly used modality in medical practice for diagnostic and interventional imaging of various parts of the body.¹ The head and neck scans are routinely indicated for suspected skull and spine fractures, also as screening protocol for brain in head trauma cases. The CT scans have a high effective radiation dose and are cautioned due to risk of association to cancers and teratogenic changes.² DNA damage and cytotoxicity noted in buccal cells or peripheral blood lymphocytes are reported to occur due to a number of reasons including radiation exposure. Studies have shown that micronuclear assays can estimate these changes on DNA and cytoplasm corresponding to signs of carcinogenesis.³ Micronucleus originates from a chromosome fragment that lags behind during anaphase of cell division and serves as a simple method in estimating DNA damage.⁴,⁵ The markers of cell death or cytotoxicity, that is, pyknosis, karyorrhexis, and karyolysis depicting cells, also have a role in depicting the dose-dependent radiation damage.⁶ Diagnostic radiation from low-dose dental panoramic radiation has been reported to cause similar effects.⁷,⁸ The buccal cells have a turnover of 7–10 days,⁹ so the basal cells (radiosensitive and pluripotent cells) that underwent radiation abuse would take that time and could be collected by gentle scraping of

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How to cite this article: Palla S, Rangdhol V, Uma AN, Devy SA, Shekar V. The genotoxic and cytotoxic effects of CT scan on buccal epithelial cells. J Cytol 2020;37:189-92.

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Submitted: 10-Sep-2019; Revised: 01-Jul-2020; Accepted: 29-Jul-2020; Published: 16-Sep-2020

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surface mucosa. However, the question of micronucleated or cytotoxic cells being lost in same turnover cycle remains unanswered. The study was planned considering this and to evaluate any significant effects of CT scans after first and second buccal cell turnover cycles. Also, the CT scans have higher dose than diagnostic dental radiation and the reported increased utility in past two decades.[2] The standard criteria by Tolbert and Thomas was used in micronucleus and cytotoxic cell scoring, respectively.[5,6]

**Materials and Methods**

**Sample size determination:** The sample of 35 per group was obtained using the formula sample size = \( \frac{2 \times SD}{\alpha} \times Z_\alpha \times Z_\beta \), where the standard deviation (SD = 0.86) was taken from the previous study.[3] The \( Z_\alpha \) is predetermined as 0.05, \( Z_\beta \) is obtained from the \( Z \) table. In the study with patient follow-up, an estimated attrition of 10% was added to get final sample size of 40. The study was approved form institutional ethical committee, Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth, (Deemed to be University), Pondicherry on 21/01/2015.

**Inclusion criteria:** Patients undergoing any CT scan in head and neck region (CT brain/facial bones/paranasal sinuses) were only considered to standardize the exposure parameters [Table 1]. These scans also focus radiation on buccal cells.

**Exclusion criteria:** All notable cause of oral genotoxicity was excluded. Those including who had habits (tobacco, alcohol), who had detectable potentially malignant or malignant lesions, with known genetic disorders, having pervious exposure to radiation. Patients on long-term antibiotics, chemotherapeutics, uncooperative, or unwilling to participate in study were excluded as well. Recruitment method for sample selection was consecutive systematic sampling, where each subject indicated for CT was considered if applicable to criteria. The study was approved by Institutional Ethical Committee (ref no: IGDISIEC2015NDP03PGSPOMR) and was conducted after obtaining written patient’s consent in both English and local language.

The study was performed as per the STROBE guidelines and Proforma was designed following the standards from the Human micronucleus project (HUMN 2001; http://www.humn.org)[7] wherein a baseline collection prior to exposure to CT scan (equipment used was Make GE, model: Optima 660 120 slice scanner) and second collections done after 10 and 20 days of exposure. Patients were asked to gargle their mouth with water and then buccal smear from patients on right and left buccal mucosa were made by gentle scraping using a wooden spatula and evenly spread to glass slide (microslides, manufactured by Blue Star Ltd., Mumbai, India). The glass slides were stored in appropriate media having absolute alcohol (2% isopropanol, manufactured by Emplura) solution. Then, these slides were dried and fixed under papanicolaou (PAP) stain. The PAP reagent kit (Nice Chemicals Private Ltd., Kerala, India) with PAP Eosin Azure (EA-36) stain and PAP solution—OG 6—were used as per standard protocols for staining all the smears. The stained smears were examined under light microscope (UMDOB 3 model, Olympus research and clinical system solutions, Tokyo, Japan) by two observers at 100× magnifications. The cells were observed as per standards (Tolbert’s criteria (1992) for micronuclear abnormalities and as per Thomas et al.[6] for cytotoxic changes) by a senior oral pathologist and a cytogenetic expert (PhD in medical genetics), who were blinded form each other.

**Results**

A data analyst considered all readings with reference to Bland–Altman test. The test showed that the bias (difference) between the two observers was close to zero (−0.05) and the values lied between the lower and upper line of agreement with a correlation \( r = 0.87 \) (\( P < 0.01 \)), indicating a high agreement between the two observers. All the comparisons before and after exposure (10th or 20th day) were evaluated by paired \( t \)-test by using the Statistical Package for Social Sciences (Version 20 SPSS Inc., Chicago, IL, USA) software.

DNA damage (micronuclear count) comparisons: The counts revealed 0.4% positive cells before exposure and 1.4% positive cells post 10 days of exposure which was highly statistically significant (\( P < 0.005 \)) [Table 2]. The counts from baseline to 20th day also were significant (\( P < 0.005 \)). See Figures 1 and 2 for micronucleated cells.

**Cytotoxic changes:** The pyknotic cells before exposure were around 0.68% while post-exposure values were 2.24% (\( P < 0.001 \)). The karyorrhectic cells (fragmented nuclei cells) when were 0.39% positive before exposure and 1.16% positive after exposure, which was statistically significant (\( P < 0.002 \)). The karyolytic (enucleated cells) showed cells significant after exposure (3.0% positive cells; \( P < 0.001 \)) while 1.4% before

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**Table 1:** Exposure parameter standards for head and neck CT scans

| CT view                       | Exposure parameters | Voltage (kVp) | Current (mA) | Time(s) | Effective dose (mSv) |
|------------------------------|---------------------|---------------|--------------|---------|----------------------|
| CT in head and neck region*  |                      | 80-100        | 100-120      | 1       | 2                    |

*CT scans (taken for brain/paranasal sinuses/facial bones) having above parameters were only included.

**Table 2:** Comparison of micronucleus counts and cell changes before and 10 days after head and neck CT scans

| Parameter               | Variable    | Mean±SD       | \( P \) |
|-------------------------|-------------|---------------|--------|
| DNA damage              |             | 4.58±1.8      | 0.001  |
| (micronucleus)          | Pre-exposure| 14.65±4.9     |        |
| Cytotoxic changes        |             | 25.40±5.87    | 0.001  |
| (PYK + KRH + KRL)       | Postexposure| 57.25±5.09    |        |
exposure. The cumulative cell death changes are mean of the all three stages mentioned from condensed nucleus (PKY), to fragmentation (KRH) and dissolution (KRL) of nucleus. The results showed around 2.5% positive cells before and 5.7% positive cells 10 days after exposure, which was statistically significant ($P < 0.005$). The cumulative values from baseline to 20th day were not significant ($P < 0.25$) (see Table 3). Figure 2 shows cytotoxic cell changes along with some cells with micronucleus.

**DISCUSSION**

Hall and Brenner[1] have reported in an epidemiological survey that more CT scans are being taken past two decades, which has raised concern in terms of cancer induction. CT is an integral component of the oral surgeon’s diagnostic armamentarium while evaluating for complex facial fractures, temporomandibular joint, paranasal sinuses, and dental implants.[7] The predicted risk of 20–36 thyroid malignancies per 1 million CT scans and a mean lifetime cancer risk of 0.04–0.09% per CT scan were reported in literature.[8,9] The estimated cancer risk of CT scans in exposed and exposed groups had a significant difference of 24% as per literature.[10]

Arora et al.[3] have shown in case of panoramic radiography the micronuclei varied significantly before exposure (1.4211 ± 0.86/1000 cells) and after exposure (1.5000 ± 0.83/1000 cells). Similar results were shown by other studies.[11-13] Paradoxically, Cerqueira et al.[12] and Kumari et al.[14] have shown statistically significant DNA damage in terms of cytotoxic signs but not micronucleus counts. However, these findings were significantly noted in the current study for the CT scan doses [Table 2].

Holland et al.[12] have reported baseline frequencies for micronucleated cells in the buccal cells within the 0.5–2.5 micronuclei/1000 cells in healthy individuals. The micronucleus score obtained in the current study; the pre-exposure values were having a mean ± standard deviation = 4.58 ± 1.8, which was consistent with controls who were not exposed to any genotoxins in Indian population.[15,16]

The cumulative cell death changes are mean of the all three stages mentioned above, that is, pyknotic (PYK), karyohectic (KRH), and karyolytic (KL) cells, which were all together, compared to pre- and post-exposures. This is commonly employed in other studies.[17,18] The results of the current study showed significant cytotoxic changes after exposure (CT radiography) which is consistent with previous studies (panoramic radiography).[14,17,18]

A study,[18] which compared the cytotoxic changes evaluated for cone beam computed tomography (CBCT), showed mean ± standard deviation of 8.70 ± 2.45 (pre-exposure) and 15.75 ± 3.95 (pre-exposure) while ours showed 25.40 ± 5.87 (pre-exposure) and 57.25 ± 5.9 (pre-exposure). Justification protocol (As Low As Reasonably Achievable) should be followed while prescribing for a CT scan. CBCT and dental radiographs have shown cumulative cell changes to be statistically significant after CBCT exposure,[18] but the damage from CT scans is very high as evident from the current study. The CBCT has 10–12 times less dose than CT scan and that CBCT can be used to CT scans in dentistry.[19] An Indian survey to evaluate the primary method of radiographic investigation for planning dental implants reported that CT scan was the common method when a 3D view was desired.[20]

A topical vasoconstrictor mouthwash having phenylephrine has provided 100% protection from effects of therapeutic

| Table 3: Comparison of micronucleus counts and cell changes before and 20 days after head and neck CT scans |
|---------------------------------------------------------------|
| **Parameter** | **Variable** | **Mean ± SD** | **P** |
|----------------|-------------|---------------|------|
| DNA damage     | Pre-exposure| 4.58 ± 1.8    | 0.001|
| (micronucleus) | Postexposure| 7.75 ± 2.92   |      |
| Cytotoxic changes | Pre-exposure| 25.25 ± 4.86  | 0.132|
| (PYK + KRH + KRL) | Postexposure| 25.60 ± 4.94  |      |

**Figure 1:** Cell with micronucleus—100 × view (H&E staining)

**Figure 2:** Cell with karyorrhexis (marked in arrow), other cells also show micronuclei—40 × view (H&E staining)
in rodent models,\textsuperscript{[21]} which can be used as a CT scan radioprotection method. Latex impregnated shields made of bismuth which are used to cover the eye/thyroid have resulted in dose reductions up to 40–67\%\textsuperscript{[22]} which can be employed here. The β-carotenes and retinoids increase the cell turnover rates and thus eliminate any mutated cells.\textsuperscript{[23]} Regular biomonitoring is recommended for risk groups like technicians, radiologists, patients with multiple CT exposures, and other occupational risk groups.

The advantage of the study is the use of simple, noninvasive method that can sensitively detect DNA damage. Also, evaluating changes after two cell-turn overs to check for persisting damaged cells is novel. The drawbacks of the study are a small sample-sized, heterogeneous group of subjects needing a head and neck CT scan. The study can be improvised by adopting cytochrome-blocked micronuclear assay and by scoring the nucleoplasmic bridges in nuclear buds containing cells.

The goal of the study was to address the effect of diagnostic radiation due to exposure from head and neck CT scans on the oral epithelium. CT scans have caused genotoxic effects, significantly noted even after 20 days of exposure (2\textsuperscript{nd} cell turnover). The cytotoxic changes detected after 10 days (1\textsuperscript{st} cell turnover) have significantly decreased after 20 days (2\textsuperscript{nd} cell turnover). Such persistent micronuclear cells, if detected on regular biomonitoring methods to revert genotoxicity, can be planned.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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