Effects of X-ray from Dental Radiographic Devices on Changes Of Methylation Level in Human Thyroid Cells

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Abstract

To evaluate the level of DNA methylation in human thyroid cells after X-ray exposure from dental radiographic units. Human thyroid squamous cell carcinoma cells line (SW 579) were irradiated 0 and 4 doses of intra-oral periapical radiography (5.1 mGy). 17 times of cone-beam computed tomographic scans (682 mGy) were used as a positive control for a high-level of low-dose radiation (<1 Gy). DNA methylation was investigated by methylation assay on day 4 and 7 after the exposure. No methylation change was observed on day 4 and 7 after low-dose of dental irradiation, while hypermethylation was observed in the positive group. The high-level of low-dose dental radiation (positive control) may increase the methylation level on the 4th and 7th day after irradiation. The response of low dose radiation varied depending on individual factors. The high-level of lose-dose dental radiation may affect DNA methylation; however, this multiple scan setting has not implemented in a real clinical situation. In clinical practice, the ALARA principle (As Low As Reasonably Achievable) should always apply.

Keywords: *Dental, DNA Methylation, Radiation, Thyroid cells*

1. Introduction

Since, the discovery of X-ray in 1895 by Wilhelm Conrad Röntgen, radiography has been widely used as a diagnostic tool in the medical field. Dental radiographs are necessary for proper diagnosis and treatment planning in dentistry. Two-dimensional conventional radiographs, digital or film-based, are comprised of intraoral and extraoral radiographic techniques. In recent years, cone-beam computed tomography (CBCT) has become more popular as it can offer 3D images with a generally lower radiation dose than that of multi-slice computed tomography (MSCT) (Pauwels et al., 2012).

It is known that high-dose radiation (>1Gy) can cause a deleterious effect on cellular organisms, for example, induction of DNA damage leading to mutation and cell death (Otomo et al., 2004). Dental radiography is however considered as a low-level radiation imaging technique (Napier, 1999). Cellular responses following low dose irradiation have been widely debated (Feinendegen, Pollycove, and Sondhaus, 2004). Many studies have shown the detrimental effects of low dose irradiation (Feinendegen, Pollycove, and Sondhaus, 2004; Saha et al., 2014; Katsura et al., 2016). Nonetheless, the biological effects of low-dose irradiation from dental radiography on cellular responses remain unclear (Haghdoost et al., 2006). Previous studies have reported that low-dose irradiation from intraoral radiography may pose an effect on oral tissues and bone cells (Goldberg et al., 2004; Cerqueira et al., 2008; Silva et al., 2008; da Fonte et al., 2018). A recent clinical study showed that panoramic radiography could induce chromosomal damage to oral mucosal cells 10 days post-irradiation (Cerqueira et al., 2008). It has been reported that CBCT scans may produce a cytotoxic effect and cause DNA damage (da Fonte et al., 2018).

Thyroid cells are sensitive to radiation carcinogenesis. Moreover, thyroid glands in children are more sensitive to radiation than adults (Weisenberger et al., 2005; Wilson, Power, and Molloy, 2007). Thus, radiation exposure to the thyroid gland in children should be more considered (Weisenberger et al., 2005; Wilson et al., 2007). It was found that radiation could induce changes in gene expression in the transplanted human thyroid tissue (Rodríguez-Rodero et al., 2013). Previous studies showed that radiation more than 0.5 Gy could increase the risk of thyroid cancer in Japanese bomb survivors (Wahba, Lehman, and Tofilon, 2017) and significantly elevated risk of developing thyroid cancer following low-dose radiation exposure had been found in childhood (Brenner et al., 2003).

Epigenetic is the study of mitotic changes in gene expression by DNA sequence alteration (Weinhold, 2006). All cells in an organism contain the same genetic information, but not every gene

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simultaneously express by all cell types (Brenner and Hall, 2007). A major epigenetic mechanism involves direct chemical modification to the DNA called DNA methylation (Nomura and Barbas, 2007), which is a major epigenetic modification by adding of a methyl group to the 5-position of cytosine. This epigenetic mark has the power to turn the expression of genes on or off (Ezaki, Takeichi, and Yoshimoto, 1991). DNA methylation is one of the most studied epigenetic modifications since it has an important influence on normal cell function as it plays an important role in transcription regulation process. The defects occurred in this process may lead to diseases including cancer (Ron et al., 1989).

Methylation site was usually found repeated sequences and transposable elements, such as ALU element and Long-interspersed nuclear element, LINE-1 (Wilson et al., 2007). ALU and LINE-1 were correlated with total genomic methylation content (Weisenberger et al., 2005). The alteration of ALU and LINE-1 methylation level associated with over gene expression that increased the risk of cancer (Rodríguez-Rodero et al., 2013).

A few studies showed that DNA methylation could be affected by radiation exposure. The degree of these effects varied depending on radiation dose, duration of exposure and frequency (Avery, MAcLEOD and McCarty, 1995; Bennett, Mester, and Eng, 2010). A previous study showed that chronic low-dose radiation (LDR) exposure resulted in significant loss of DNA methylation in mice (Moore, Le, and Fan, 2013). No study has evaluated the effect of low-dose dental X-ray on the methylation level in human thyroid cells. Therefore, the aim of this study was to determine the effects of X-ray from dental radiographic devices on the methylation level in human thyroid cells.

2. Objectives

The objective of the study was to evaluate the level of DNA methylation in human thyroid cells after X-ray exposure from dental radiographic units.

3. Materials and Methods

Cell culture

Thyroid squamous cell carcinoma cells line, SW 579 cell line, obtained from ATCC®, USA, were cultured in complete RPMI 1640 medium containing 10% fetal bovine serum (FBS) with anti-mycotic 100U/ml at 37º in 95% air 5% CO_2 for 4-5 days. The cells were maintained in culture unit confluence and seeded in 5 well plates at a density of 40,000 cells per well.

Radiation exposures

Cell cultures were placed in a Poly (methyl methacrylate) (PMMA) cylindrical phantom with a plastic bottle filled with water to simulate typical levels of X-ray attenuation and scatter radiation in a human head. An X-ray measuring device, Unfors Mult-O-Meter (Unfors Instruments AB, Billdal, Sweden) was used to measure the radiation dose during exposure.

Intraoral exposures

Culture cells were exposed to intra-oral periapical radiography by CS2200 Intraoral X-ray System (Carestream Dental, Rochester, NY, USA), using a cylindrical head with a focal spot to skin distance of 20 cm. An exposure setting of 70 kVp, 7 mA, 0.347s had used. The culture examples were irradiated 4 times equal to 5.1 mGy of radiation dose.

Positive control

A radiation dose of approximately 682 mGy was used as a positive control for a high-level of low-dose radiation (<1 Gy). Tissue culturing plates were placed on the scanning platform without the PMMA cylindrical phantom. The samples were exposed using 3D Accuitomo 170 (J. Morita Corp., Kyoto, Japan). Parameter settings were set at FOV 140x100 mm, Hi-Fi mode, 90 kVp, 8 mA, exposure time 30.8 seconds. The scan was repeated 17 times to reach the expected dose. The same X-ray measuring detector was placed as close to the samples as possible to monitor the radiation dose (Figure 1).

Negative control

Tissue culturing plates were placed outside of the incubator without exposing to any radiation.

Figure 1 A setting for positive control exposures

DNA extraction

DNA was extracted from the culture samples on Day 4 and 7 after the irradiation, by DNeasy® Blood & Tissue kit (Qiagen, USA). Culture cells were treated with the proprietary DNA isolation buffer. DNA was recovered in quantities of 10-20 µl by a specially designed F-Spin column. DNA was then ready for downstream applications.

Methylation measurements

Methylated DNA quantification test was carried to define global 5-methylcytosine (5-mC) levels using, Methylated DNA kit (Epigentek, USA). An ELISA-based assay was applied to explore the quality of DNA methylation in cells. DNA from cell extraction was combined with 5-mC antibodies in an ELISA plate and the concentration of substrate was recorded. DNA was bound to strip wells that were specifically treated to have a high DNA affinity. The methylated fraction of DNA was detected using capture and detection antibody and then quantified fluorometrically by reading the relative fluorescence units (RFU) with a fluorescence spectrophotometer. The amount of methylated DNA was proportional to the fluorescence intensity measured.

LINE-1 methylation analysis was carried out after the cultured cells were extracted DNA by DNeasy® Blood & Tissue kit (Qiagen, USA). The DNA was purified and incubated in 0.33 M NaOH at 25ºC for 5 min, ethanol-precipitated, then washed with 70% ethanol and re-suspended in 20 µl TE buffer. A 2 µl sample of bisulfate DNA was subjected to 35 cycles of PCR with two primers at an annealing temperature of 53ºC. The amplicons were digested in 30 µl reaction volumes with 2U of *Taql* or 8U of *Tasl* in 1x*Taql* buffer (MBI Fermentas) at 65ºC overnight and then electrophoresed in 8% non-denaturing polyacrylamide gels. The LINE-1 methylation level was calculated as a percentage (Patchsung et al., 2012).

ALU methylation analysis was quantitated by pyrosequencing with the following primers and conditions: 20µM forward primer 5'GGT GGT TTA MGT TTG TAA TTT TAG TAT TT-3' and 20 µM reverse biotinylated primer 5'-ATT TCA CCA TAT TAA CCA AAC TAA TC-3'. The purified, single-stranded biotinylated PCR products acted as a template for annealing of the first pyrosequencing primer to detect CpG in the first 3 positions of the ALU amplicon and of the second pyrosequencing primer to detect CpG in the last 4 positions of the ALU amplicon (0.3 µM final concentration). The degree of methylation was evaluated at CpG methylation site (Patchsung et al., 2012).

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 22 (IBM, Armonk, NY). Differences of the LINE-1 and ALU methylation among negative control, positive control and low-dose radiation on day 4 and day 7 after irradiation were tested using the Kruskal-Wallis H test. A *p*-value <0.05 was considered statistically significant.

4. Results and Discussion

On day 4 and day 7 after irradiation, no change of the methylation level was observed in all tested groups investigated by LINE-1 and ALU methylation as shown in figure 2 and figure 3. Results revealed that radiation from a dental X-ray device could affect the gene expression by increasing the methylation level on positive control (682 mGy) (Figure 2, 3).

LINE-1 methylation analysis in human thyroid cells showed an increase of methylation level after positive control dose (682 mGy) on both day 4 and 7 (Table 1). Table 1 showed LINE-1 methylation of negative control, positive control and the low-dose group on day 4 and day 7 in triple time. Percentage of LINE-1 methylation in negative control, positive control and low-dose group were 58.45%, 60.49%, 59.21% on day 4 and 59.49%, 63.50%, 59.19% on day 7, respectively. The results demonstrated that the LINE-1 and ALU methylation of low-dose from dental radiation were not significantly different as shown in figure 2 and 3.

The average results of % ALU Methylation on negative control, positive control, and the low-dose were 41.01%, 42.34%, 47.52% on day 4 and 45.76%, 46.68%, 49.74% on day 7, respectively (Figure 3). On day 7, an increase of % ALU methylation was observed in all groups. However, no statistically significant differences were found.

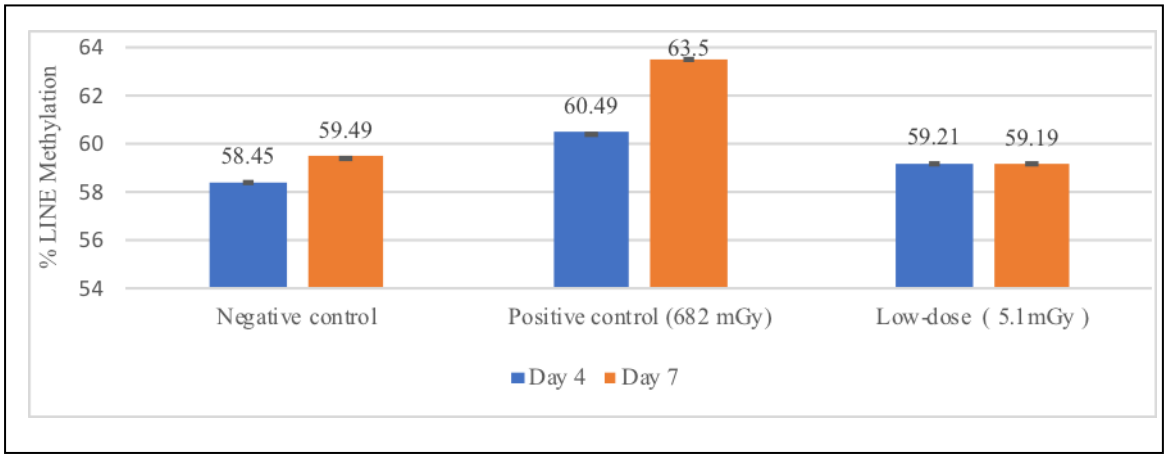

Figure 2 The percentage of LINE-1 methylation assay on day 4 and day 7 after radiation

Table 1 The percentage of LINE-1 methylation in triple time on day 4 and day 7 after dental irradiation

		1st time	2nd time	3rd time	Average	SD
Day 4	Negative control	59.00%	58.24%	58.13%	58.45%	0.00474
	Positive control (682 mGy)	59.19%	59.57%	62.72%	60.49%	0.01938
	Low-dose (5.1 mGy)	58.55%	57.31%	61.79%	59.21%	0.02313
Day 7	Negative control	59.85%	58.84%	59.77%	59.49%	0.00561
	Positive control (682 mGy)	61.54%	62.78%	66.19%	63.50%	0.02408
	Low-dose (5.1 mGy)	59.50%	60.33%	57.75%	59.19%	0.01317

SD, standard deviation

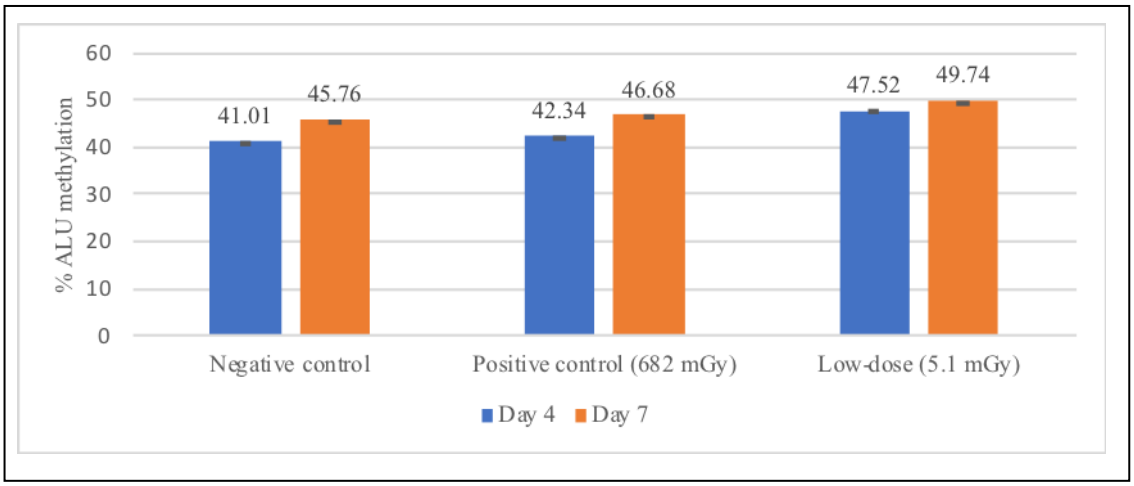

Figure 3 The percentage of ALU methylation on day 4 and day 7 after dental irradiation

Table 2 The percentage of ALU methylation in triple time on day 4 and day 7 after dental irradiation

		1st time	2nd time	3rd time	Average	SD
Day 4	Negative control	42.07%	38.95%	42.36%	41.01%	0.02098
	Positive control (682 mGy)	41.64%	43.28%	42.09%	42.34%	0.00847
	Low-dose (5.1 mGy)	48.78%	48.36%	45.43%	47.52%	0.01825
Day 7	Negative control	46.16%	45.70%	45.43%	45.76%	0.0039
	Positive control (682 mGy)	47.10%	44.63%	48.68%	46.68%	0.0188
	Low-dose (5.1 mGy)	50.61%	49.36%	49.25%	49.74%	0.00755

SD, standard deviation

After irradiation, the levels of the global methylation were increased in all groups on day 7. The global methylation levels were 1.1 times (positive control) and 1.3 times (low-dose) when compared the results to the negative control (Figure 4). This finding coincided with the increase of ALU methylation level.

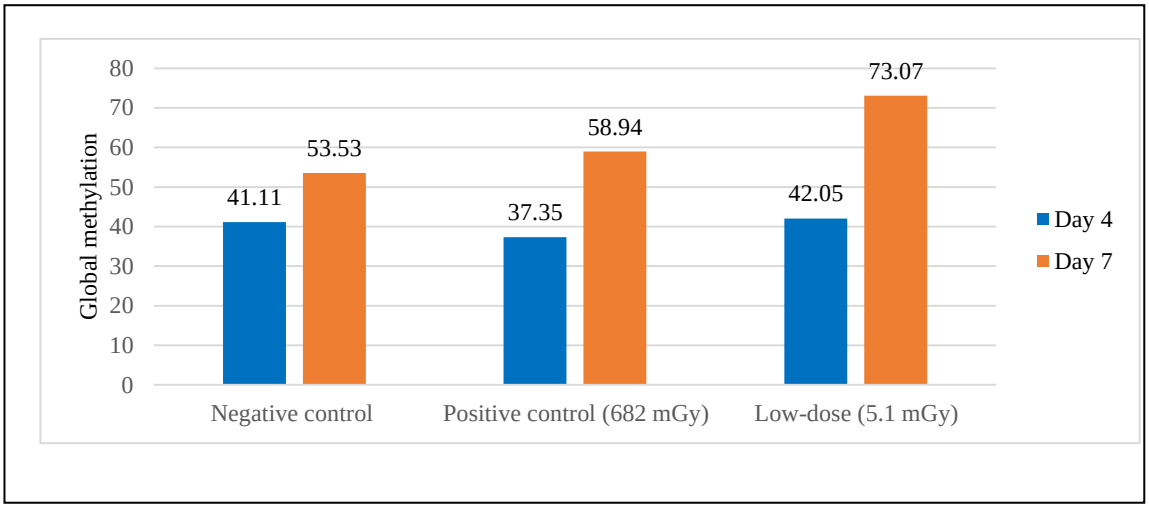

Figure 4 Global methylation on day 4 and day 7 after dental irradiation

The present study evaluated the effects of X-ray from dental radiographic units on human thyroid squamous cell carcinoma cell line. The results presented that low-dose dental radiation did not alter cell expression as regulated by methylation assay. However, the higher-level of low-dose radiation represented as the positive control, which is still within the low-dose range (<1 Gy), could induce hypermethylation. LINE-1 methylation level was increased after the high-level of low dose dental radiation in our study.

A few studies have reported the effects of radiation on DNA methylation (Koturbash et al., 2007; Port et al., 2007; Maierhofer et al., 2017). Koturbash et al., 2007 revealed hypomethylation of LINE-1 after high dose irradiation (20 Gy) in rat spleen tissue. Our results have shown that Global and ALU methylation levels were increased after the high-level of low-dose dental radiation on day 7. The reason for the increased ALU methylation level might be that ALU methylation was found in a large portion methylation site within the genome which in repeat sequence (Weisenberger et al., 2005). Similarly, with the global methylation analysis was found in direct large quantitation of genomic (Nomura and Barbas, 2007).

Maierhofer et al., 2017 revealed that the 2 and 4 Gy of radiation could reduce global DNA methylation but slightly increased ALU methylation level in human fetal fibroblast cells. Many cancer research studies have found evidence that DNA hypomethylation could induce activation of an oncogene. On the other hand, hypermethylation could induce silencing of tumor-suppressor genes (Portela and Esteller, 2010). There was evidence that radiation could either increase or decrease the level of DNA methylation depending on the group of genes (Port et al., 2007).

Thyroid cells were sensitive to radiation carcinogenesis (Brenner and Hall, 2007). Several studies have demonstrated that high-dose radiation could change gene expression in human thyroid epithelial cell line (Bang et al., 2016; Wang et al., 2016). Nomura et al., 2008 found a large dose of X-ray and γ- ray at a high dose rate significantly induced mutation in tumor-related genes, *p53* and *c-kit*, in thyroid cells. The degree of these effects varied depending on radiation dose, duration of exposure and frequency (Brenner et al., 2003; Wahba et al., 2017).

Although the present study offered a promising finding of the effect of dental radiation on the human thyroid cells, one limitation of the study was the type of thyroid cell sample. This study used the human thyroid squamous cell carcinoma cells line (SW 579 from ATCC®), thus these cells are not primary cells derived from normal individuals. Therefore, their responses to radiation might not be able to represent the response of normal thyroid cells. Future studies should evaluate different types of cell lines and possibly continue to explore the effect of radiation in *in vivo* experiment.

5. Conclusion

In conclusion, low-dose radiation from dental radiographic unit did not alter the DNA methylation level of human thyroid squamous cell carcinoma cell line. The high-level of low-dose dental radiation may affect DNA methylation; however, this multiple scans setting has not implemented in a real clinical situation. In clinical practice, the ALARA principle (As Low As Reasonably Achievable) should always be applied.

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