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(RESEARCH ARTICLE)

Assessment of radiation effects on apoptosis in parotid gland acinar cells during radiographic examination

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Abstract

Radiographic examination plays a crucial role in dentistry for establishing diagnoses and developing treatment plans. Dental X-rays utilize ionizing radiation, which can induce ionization in exposed tissues. The salivary glands, particularly the parotid gland composed of serous acinar cells, are commonly affected during exposure. These parotid acinar cells are highly sensitive to radiation, making them susceptible to DNA damage and triggering programmed cell death (apoptosis). This in vivo study was conducted using male rats as animal models. Eighteen rats, each weighing between 200–250 grams, were divided into three groups: a control group, a group exposed to a periapical radiation dose, and a group exposed to a panoramic radiation dose targeted at the parotid region for approximately one second. Parotid gland tissues were collected on the third day following radiation exposure and prepared for histological examination using hematoxylin-eosin staining. The apoptotic index was evaluated under a light microscope at 400x magnification by two independent observers. A statistically significant increase in apoptotic index was observed in the irradiated groups compared to the control group ($p < 0.05$). Thus, radiation exposure during dental radiographic procedures leads to an elevated rate of apoptosis in the parotid acinar cells of male Wistar rats.

Keywords: Acinar cells; Parotid gland; Apoptosis; Radiographic examination

1. Introduction

Dental caries remains the most prevalent oral health issue, affecting approximately 90% of the Indonesian population, with the highest incidence observed in the first molars [1]. To assess the severity and extent of caries, dentists frequently utilize radiographic imaging as a complementary diagnostic tool. Radiographic evaluations are crucial not only for establishing an accurate diagnosis but also for formulating treatment strategies and monitoring therapeutic outcomes [2]. During dental imaging procedures, radiation exposure inevitably affects surrounding facial regions, particularly the cheek area where the parotid glands are located. Dental radiography employs X-rays, which are a form of ionizing radiation known to potentially cause biological harm. When ionizing radiation interacts with body tissues, it can disrupt cellular integrity either through direct interaction with macromolecules like DNA, RNA, or proteins, or indirectly by generating reactive free radicals via interactions with water molecules [3].

The indirect pathway, through the formation of these radicals, leads to oxidative stress that damages cellular components, especially in tissues with high water content such as salivary glands. In dental settings, radiation exposure commonly involves the lips, cheeks, and chin—regions where the parotid glands, the largest salivary glands, are situated. These glands are primarily composed of serous acinar cells, which secrete watery saliva. Due to their high

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water and trace metal content, serous acinar cells are particularly vulnerable to ionizing radiation, making them prone to DNA fragmentation and apoptosis [3, 4].

Even minimal doses of radiation can trigger such cellular responses. Notably, more than 70% of dental procedures involving radiography or radiotherapy expose the cheek region, increasing the risk to the parotid glands [5, 6]. Compared to other salivary glands, the parotid gland contains the highest proportion of serous acinar cells, further underscoring its radiosensitivity [7, 8].

Dental diagnostic imaging generally involves low-dose radiation, typically ranging between 0.1 and 10 millisieverts (mSv) [9]. Among the common techniques used are periapical and panoramic radiographs, both of which fall within this low-dose category. At the Faculty of Dentistry, University of Jember, for example, the periapical radiograph typically delivers a dose of around 0.16 mSv, while panoramic imaging uses approximately 0.78 mSv [10, 11].

Earlier studies have shown that even low-dose radiation can lead to increased rates of apoptosis and necrosis, with effects observed at doses as low as 0.08 mSv. One such study reported significant cellular changes by day 10 post-radiation in oral mucosa [9]. Another investigation focusing on salivary glands found that the peak of acinar cell apoptosis occurred around the third day following exposure, with signs of cellular regeneration appearing by the sixth and tenth days. This suggests that early observations post-radiation provide a more accurate assessment of apoptotic activity. Most of these studies utilized higher radiation doses and targeted the submandibular gland [7, 12]. The aim of this study was to investigate how X-ray exposure during radiographic examination affects apoptotic changes in parotid gland acinar cells.

2. Material and methods

This study was approved by the Research Ethics Committee of the Faculty of Dentistry, University of Jember. A laboratory-based experimental design with a post-test only control group was employed. The research was conducted from January to March 2021 at three locations: the Pharmacology Laboratory (Biomedical Department), the Radiology Unit of the Dental and Oral Hospital (RSGM), and the Anatomical Pathology Unit of Dr. Soebandi Regional Hospital, all located in Jember, Indonesia.

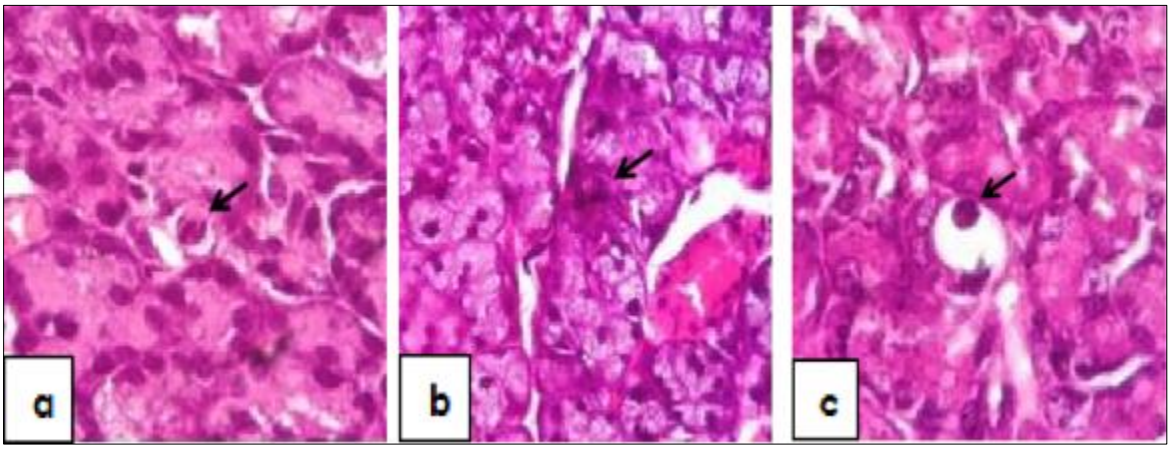

Figure 1 Histological appearance of apoptotic bodies in parotid gland acinar cells of male wistar rats with HE staining and 400x magnification (arrows). Control group (a), treatment I (b), and treatment II (c). In the visual field, it can be seen that there is formation of tightly rounded or pyknotic (a, c) and fragmented nuclei or karyorrhexis (b)

Eighteen healthy male Wistar rats (200–250 g, aged 2–3 months) were used as experimental subjects. The animals were randomly assigned into three groups: a control group, a periapical radiation exposure group (0.16 mSv), and a panoramic radiation exposure group (0.78 mSv). The rats were immobilized using a specialized fixation device resembling a dental chair designed for rodents. Radiation was precisely targeted at the lateral cervical region near the ears, corresponding to the parotid gland area [8]. Seventy-two hours post-exposure, parotid gland tissues were harvested for histological analysis. Samples were stained with hematoxylin-eosin (HE) to facilitate cellular evaluation.

Apoptotic activity in the acinar cells was assessed via the apoptotic index method, using a light microscope at 400x magnification [13]. Two blinded anatomical pathologists independently identified apoptotic bodies—characterized by

condensed, fragmented, basophilic nuclei with perinuclear halos—in three predetermined fields per sample. From each field, the highest observer value was selected. Final data were calculated as the average apoptotic index per sample and per group [14, 15]. The compiled data were tested for normality using the Shapiro-Wilk test and for variance homogeneity using Levene's test. If both assumptions were satisfied, a one-way ANOVA was used for further statistical analysis.

3. Results and discussion

Table 1 The results of calculating the average apoptotic index of parotid gland acinar cells from each group (number of apoptotic cells/100 cells)

	Control	**Periapical dose (P_I)**	**Panoramic dose (P_{II})**
Sample 1	0,33	0,33	0,67
Sample 2	0,33	0,67	1
Sample 3	0,33	0	0,33
Sample 4	0,67	0,67	0,67
Sample 5	0	0,33	1
Sample 6	0	0,67	0,67
Mean	0,2767	0,4450	0,7233
SD	0,2515	0,2744	0,2515

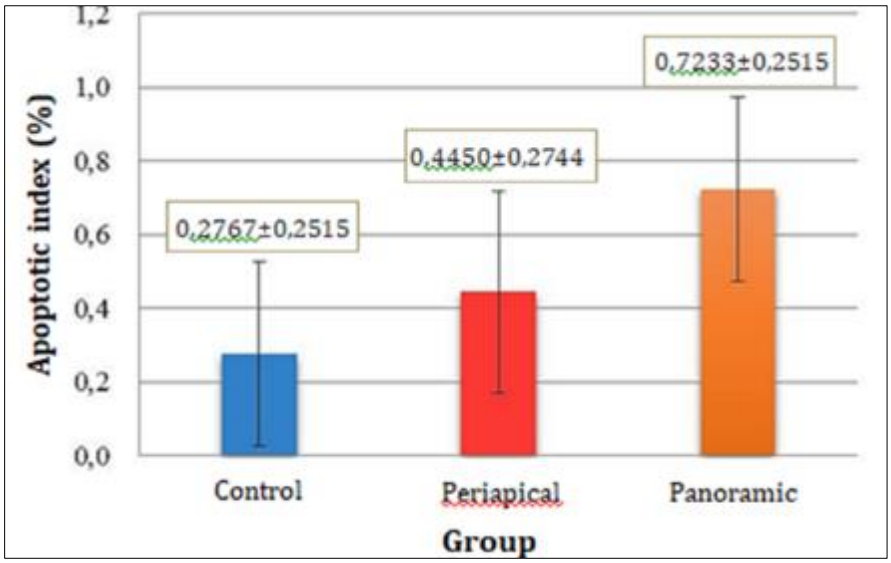

Figure 2 The average number of apoptotic index of each group. The apoptotic index is expressed in percentage of cells (from 100 cells). The standard deviation is indicated by *error bars*

The apoptotic index observed in each experimental group is summarized in Figure 1 and Table 1. Figure 2 illustrates an increase in apoptosis of parotid gland acinar cells following exposure to x-ray radiation at both periapical and panoramic dose levels. To assess the distribution of the data, the Shapiro-Wilk test was conducted. The resulting p-values were 0.211 for the control group, 0.092 for treatment group I (periapical dose), and 0.211 for treatment group II (panoramic dose). Since all p-values exceeded 0.05, the data were considered to be normally distributed.

Subsequently, the homogeneity of variance across the groups was analyzed using Levene's test, which yielded a significance value of 0.848. This result indicates that the data variances were homogeneous. Based on these findings, a one-way analysis of variance (ANOVA) was conducted to determine differences in the apoptotic index among the three groups. The ANOVA revealed a statistically significant difference, with a p-value of 0.029. To further identify which

specific group comparisons were responsible for the observed significance, a post hoc analysis using the Least Significant Difference (LSD) test was performed. A summary of the LSD test outcomes is presented in Table 2.

The results of the LSD post hoc test further revealed a statistically significant difference in the apoptotic index between the control group and treatment group II (panoramic dose), with a p-value of 0.009 ($p < 0.05$). This indicates that exposure to panoramic x-ray radiation significantly increased the level of apoptosis in parotid gland acinar cells compared to the unexposed control group.

Table 2 The results of LSD apoptosis index test in three groups

	Control	**Periapical Dose (PI)**	**Panoramic Dose (PII)**
Control	- 0,279	0,279	0,009*
Periapical Dose (PI) Panoramic Dose (PII)	0,009*	- 0,083	-0,083

Description: * : There is a significant difference (p > 0,05)

Periapical and panoramic radiographs are commonly used in dental practice, offering visual representations of dental tissues either in localized or comprehensive views, all while employing relatively low radiation doses [16]. However, despite their minimal intensity, these exposures are not entirely without biological consequences. The degree of side effects that may occur is influenced by several variables, particularly the magnitude of the radiation dose [6]. Even at low levels, ionizing radiation can trigger biological effects on exposed cells and tissues, manifesting either as stochastic (long-term) or deterministic (short-term) responses [17].

In this study, periapical and panoramic radiographs delivered doses of 0.16 mSv and 0.78 mSv respectively. These values were sourced from a Siemens-Heliodent dental X-ray unit, commonly used at the Radiology Department of the Dental and Oral Hospital, University of Jember. Since radiation dose correlates directly with exposure duration, the 0.16 mSv dose corresponds to an exposure time of approximately 0.180 seconds, while the 0.78 mSv dose aligns with 0.920 seconds [10].

Statistical analysis using One-Way ANOVA revealed a significant rise in the apoptotic index following exposure to these low doses. Further analysis using the LSD post hoc test confirmed a significant difference in apoptotic rates between the control group and the group receiving panoramic radiation. One potential explanation for the increased cell apoptosis is that the exposure field in dental radiography frequently includes major salivary glands—particularly the parotid glands, which are predominantly composed of serous acinar cells known for their heightened radiosensitivity. This sensitivity is attributed to their high intracellular water content, which enhances their reactivity to ionizing radiation [5, 7].

Ionizing radiation has the ability to split water molecules into hydrogen (H●) and hydroxyl (OH●) free radicals, both of which are highly reactive [17]. Although free radicals exist naturally in the body, the additional ones formed through radiation exposure, especially superoxide anions (O_2^-), may interact with hydrogen to produce hydrogen peroxide (H_2O_2). These reactions generate highly oxidative compounds that compromise cellular structures, including membrane lipids and antioxidant systems, ultimately leading to cell damage and death [18, 19].

Previous studies have reported that even low radiation doses (starting from 0.08 mSv) can lead to apoptosis, primarily due to DNA fragmentation. This process is often mediated by the tumor suppressor protein p53, which is activated in response to DNA damage [20]. p53 initiates cell cycle arrest by modulating the activity of cyclin-dependent kinases (CDKs) and regulating the p21 protein, preventing the cell from proceeding to DNA replication in the G1-S transition [21]. If repair mechanisms fail, the cell is directed toward apoptosis [9].

Moreover, p53 influences apoptotic pathways by modulating members of the Bcl-2 protein family [22]. One such pro-apoptotic protein, Bax, acts in opposition to Bcl-2 and promotes the release of cytochrome-c from the mitochondria [23]. This molecule then interacts with Apaf-1 and caspase-9 to form an apoptosome complex, which subsequently activates caspase-3, the main executor of apoptosis [24]. The involvement of p63 further enhances apoptotic signaling, particularly in response to prolonged cell cycle arrest [25].

Empirical findings also suggest that the third day post-radiation exposure is the optimal time point for detecting maximal apoptotic activity in acinar cells, as later days show signs of tissue recovery [7, 12]. Although TUNEL staining

is more sensitive in detecting apoptosis, hematoxylin-eosin (HE) staining remains a reliable and widely used method, capable of identifying approximately two-thirds of apoptotic cells [14, 26].

Another study demonstrated that even at high radiation doses, apoptosis in rat salivary gland acinar cells did not exceed 6%. In the present study, exposure to low-dose radiation resulted in apoptotic rates of less than 1% of the parotid gland's acinar cells. This finding supports the hypothesis that even minimal radiation exposure during dental imaging procedures has measurable biological effects [6].

In summary, based on the findings of this research and supporting literature, it is evident that radiographic exposure, even at low doses, can increase apoptosis in the acinar cells of the parotid gland.

4. Conclusion

Based on the findings, it can be concluded that exposure to periapical and panoramic radiation doses leads to an increase in apoptosis of acinar cells in the parotid glands of male Wistar rats. Nonetheless, further investigation is required to determine the duration and mechanism of recovery of these salivary gland cells following radiation-induced damage.

This study underscores the importance of using dental radiographic imaging strictly for justified diagnostic needs. It also highlights the need to enhance protective measures for both patients and operators during radiographic procedures. Moreover, strict adherence to proper radiographic techniques is essential to avoid unnecessary repeat exposures due to procedural errors.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Statement of ethical approval

This research was approved and supported by the Research Ethics Committee of the Faculty of Dentistry, University of Jember (No. 019/UN25.8/KEPK/DL/2021).

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