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# Anti-calponin, anti-PCNA immunohistochemical study of the parenchymatous cells in mice parotid salivary glands exposed to dental radiograph

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## Abstract

**Background:** Radiation therapy with ionizing radiation is associated with an increased risk of salivary gland cancer and xerostomia due to an abnormally high expression of the regulatory protein calponin. PCNA is a cofactor for DNA polymerase-delta and is involved in cell proliferation.

**Methods**: This study evaluated the anti-calponin and anti-PCNA immunohistochemical reactivity in the parotid salivary glands of mice after frequent exposure to dental X-ray.

**Results**: The results showed that exposure to radiation causes marked differences in PCNA reactivity. Radiation therapy has a significant impact on salivary glands, particularly on myoepithelial cells.

**Conclusion**: The exposure to radiation can cause Dental X-ray exposure increases the risk of developing cancer and other health problems due to damage to DNA and inflammatory destruction and further research is needed to mitigate these effects and improve patient outcomes.

## Introduction

Radiation therapy with ionizing radiation (IR) has been associated with an increased risk of salivary gland cancer and xerostomia [1]. There is a statistically significant link between these two variables. Malignant tumors of the salivary gland are characterized by an abnormally high expression of the regulatory protein calponin [2]. This is because the salivary glands can't work properly without calponin.

The parotid glands, is serous gland and the largest of the main glands in humans, begin to develop towards the beginning of the pregnancy. The glands are made up of serous acini that secrete aqueous saliva, and the main excretory channel is called Stensen's duct [3,4].

Due to their epithelial and contractile features, myoepithelial cells are an integral part of the salivary acinar glands [5].

Calponin is a protein that is notable for its capacity to block actin-activated myosin ATPase. It is a member of a family of actin-binding proteins and has this property in particular [6]. It has been hypothesized that it has something to do with the regulation of the contractions of smooth muscles. It is exacerbated by carbachol or okadaic acid contraction and can be seen on thin filaments that are distributed throughout the cytoplasm of the cell [7]. PCNA, or proliferating cell nuclear antigen, is a crucial component in both the replication and repair of DNA. It is found in proliferating cells. It is created in great numbers by proliferating cells of normal and altered origin, although it is present in normal non-dividing cells and tissues in only trace amounts [8]. During the process of DNA synthesis, it performs the roles of a sliding clamp, a polymerase switch factor, and a recruitment factor [9]. It has been demonstrated that it is necessary for the resynthesis stage of both the nucleotide excision repair (NER) and the base excision repair (BER) in vitro, and the induction of this stage by X-ray irradiation is dose- and time-dependent [10].

PCNA is a monoclonal non-histone nuclear protein antibody that serves as a cofactor for DNA polymerase-delta and is involved in the initiation of cell proliferation [11]. This study evaluated the anti-calponin and anti-PCNA immunohistochemical reactivity in the major salivary glands of mice after frequent exposure to diagnostic doses of dental radiograph. This study examined the anti-calponin immunohistochemical reactivity in salivary gland tissues exposed to variable doses of dental X-ray.

## Methods

This study divided 75 adult male mice into three groups: control group (15 mice), experimental group (EX1, EX2), and control group (EX15). Each group was exposed to dental x-ray at the ventral head region in different intervals.

The animal was held in a fixed position and exposed to dental x-ray, with the EX1 group exposed to 4 times in the same session and the EX2 group exposed to 4 times in 4 sessions. Animals were scarified, decapitated, and inverted T shape incisions were made to expose salivary glands and obtain sample collection figures.

Tissue samples were kept in 10% Na2HPO4 (6gm) neutral buffered formalin PH: 7.H2O, Formalin, and Distilled Water. Infiltrated, paraffin-embedded tissues. Richert-Jung, 2030 MOT Biocut, 5m-thick microtome sagittal sections. Section ribbons were collected on clean glass slides from 40°C hot water. Histology employed hematoxylin and eosin. Eosin counterstains show cell types and histology. Staining requires dewaxing, rehydrating, washing, staining, dehydrating, and mounting. Light microscopes examined frontal cortical histology [12].

Slide baking, deparaffinization, rehydration, hydrogen peroxide block, protein block, primary antibody, secondary antibody reagent, and streptavidine-HRP antibodies were performed overnight in a hot air oven at 60°C [13]. Aperio Image Scope software examined immunohistochemical labeling pictures from Richert Chung light microscope and camera in Histology Department, Medical College / University of Al-Nahrain. This program measures Anti-PCNA and Anti-Calponin 1 antibody marker reaction intensity as "positivity" for quantitative amount of specific color in tissue slice figure (3.12). This system's default input parameter was color intensity degree: brown, orange, yellow, blue, or white for strong positive, positive, weak positive, and negative colors.

## Results

The EX2A experimental group showed no reactivity, while control group showed brown to dark brown reactivity in the whole field with specific areas around ducts and blood vessels. The EX2A group showed no reactivity, while the control group showed high intensity of the reaction spread throughout the area surrounding acini, ducts, connective tissue and blood vessels. Reaction intensified around some acini, while many acini did not. PCNA antibodies reactivity showed a wide distribution in EX2B group, while in control group. The expression of, the intensity of which differ a lot from control group where the reaction observed faint surrounding the acini and area around the ducts, figure (Figure 1).

Calponin reactivity in experimental EX1B group show the same distribution of reaction in control group but to a lesser extent, the control group showed high

intensity around acini, ducts and connective tissue, no involvement of cell cytoplasm was seen, (Figure 2).

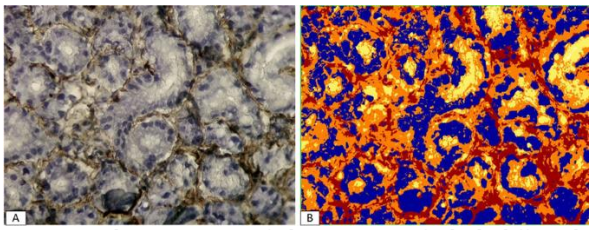

**Figure 1:** The expression of, the intensity of which differ a lot from control group where the reaction.

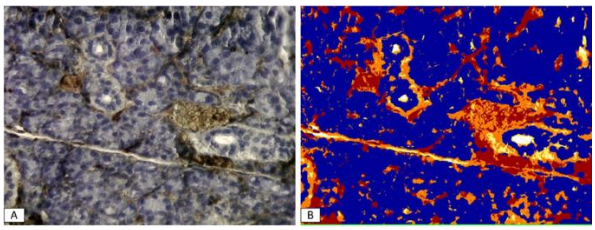

**Figure 2:** the control group showed high intensity around acini, ducts and connective tissue, no involvement of cell cytoplasm was seen.

The mean value of positive pixels for salivary gland tissue immunohistochemical reactivity labelled with Calponin and PCNA was significantly higher than that of EX1A group. (Table 1).

| Salivary gland | Markers | control pixel/$\mu_2$ (n=60) | EX1A pixel/$\mu_2$ (n=60) | p-value |
|---|---|---|---|---|
| Parotid | Calponin | $26.3\times10^4\pm1.1\times10^4$ | $20.9\times10^4\pm0.58\times10^4$ | ≤0.001 |
| | PCNA | $24.8\times10^4\pm0.39\times10^4$ | $14.3\times10^4\pm0.34\times10^4$ | ≤0.001 |

**Table 1:** Calponin and PCNA Expression in Parotid salivary glands EX1A vs control.

| Salivary gland | Markers | control pixel/$\mu_2$ (n=60) | EX1B pixel/$\mu_2$ (n=60) | p-value |
|---|---|---|---|---|
| Parotid | Calponin | $26.3\times10^4\pm1.1\times10^4$ | $8.2\times10^4\pm0.4\times10^4$ | <0.001 |
| | PCNA | $24.8\times10^4\pm0.39\times10^4$ | $16.7\times10^4\pm0.41\times10^4$ | <0.001 |

**Table 2:** Calponin and PCNA Expression in Parotid salivary glands EX1B vs control.

The mean value of positive pixels for salivary gland tissue immunohistochemical reactivity labelled with Calponin and PCNA was significantly higher in the EX1B group (Table 2). The mean value of positive pixels for salivary gland tissue immunohistochemical reactivity labelled with Calponin and PCNA was significantly higher in the control group than EX2A group (Table 3).

| Salivary gland | Markers | control pixel/$\mu_2$ (n=60) | EX2A pixel/$\mu_2$ (n=60) | p-value |
|---|---|---|---|---|
| Parotid | Calponin | $26.3\times10^4\pm1.1\times10^4$ | $5.74\times10^4\pm0.47\times10^4$ | <0.001 |
| | PCNA | $24.8\times10^4\pm0.39\times10^4$ | $4.7\times10^4\pm0.37\times10^4$ | <0.001 |

**Table 3:** Calponin and PCNA Expression in Parotid salivary glands EX2A vs control.

The mean values of positive pixels evaluated by Aperio Image Scope software for salivary gland tissue immunohistochemical reactivity were significantly higher than those of EX2B group (Table 4).

| Salivary gland | Markers | control pixel/$\mu_2$ (n=60) | EX2B pixel/$\mu_2$ (n=60) | p-value |
|---|---|---|---|---|
| Parotid | Calponin | $26.3\times10^4\pm1.1\times10^4$ | $21.2\times10^4\pm0.59\times10^4$ | <0.001 |
| | PCNA | $24.8\times10^4\pm0.39\times10^4$ | $21.0\times10^4\pm0.61\times10^4$ | <0.001 |

**Table 4:** Calponin and PCNA Expression in Parotid salivary glands EX2B vs control.

## Discussion

This research investigated the potential risks associated with dental X-rays and provided evidence-based recommendations for safe use. It found that exposure to radiation causes retardation in the incorporation of iron and a decline in hemoglobin binding to the erythrocyte membrane, agree with [14]. The calponin antibody in EX1A group of parotid glands was mainly seen around acini as intense brownish coloration, thus we agree with [15]. Also, the EX1A group showed marked differences in PCNA reactivity compared to control group, with generalized distribution of the reaction in both acini and ducts, but intense brownish color in the area surrounding the duct and connective tissue. EX1A group parotid gland showed no such picture, agree with [16]. In addition, the control group showed high intensity around acini, ducts and connective tissue, no involvement of cell cytoplasm was seen , and we agree with [17].

The statistical appraisal of these counted values suggested that the number of myoepithelial cells predicted were significantly decreased when comparing the experimental serous parotid and submandibular tissues of the EX1A and EX2A groups with that of the control group. These findings suggest that radiation therapy has a significant impact on the salivary glands, particularly on the myoepithelial cells. Further research is needed to explore potential interventions to mitigate these effects and improve patient outcomes, agree with [18]. The cell kinetics is an important aspect of cell biology, providing insights into the effects of environmental factors on cellular behavior. This study examined the proliferation of acinar and ductal cells in salivary tissues exposed to post-dental radiation injury. Further studies are needed to determine the kinetics of PCNA activation in the parotid gland and assess its potential as a marker for tissue repair. Exposure to radiation can cause Dental X-ray exposure increases the risk of developing cancer and other health problems due to damage to DNA and inflammatory destruction. The expression of Calponin antibody in immunohistochemical reactivity was higher in control group than that of experimental group. The number of myoepithelial cells predicted were significantly decreased when comparing the experimental serous parotid tissues groups with that of the control group. Further research is needed to explore potential interventions to mitigate these effects and improve patient outcomes.

## Author Contributions

Conceptualization : Ali M. Hasan , Hind S. Jasim , The authors were equally contributed in this study.

## Competing Interest

The authors declare that there is no conflict of interest.

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