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Short Term Effect of Gamma-Irradiation on Cytokeratin 17 and Calponin Expression in Submandibular Glands of Rats

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DESPITE the tremendous development in radiotherapy techniques, salivary glands among tissue are unintentionally exposed to radiation during head and neck cancer radiotherapy due to anatomical issue. Therefore, the purpose of the present study is to evaluate the histopathological consequence of gamma radiation (15 Gy) on submandibular glands as well as cytokeratin (CK) 17 and calponin expressions for deeper understanding of the mechanism of radiation –induced salivary gland injury. Rats were divided into control and irradiated groups. Rats' heads in irradiated group were subjected to a single dose of gamma radiation (15Gy). The specimens of submandibular gland were examined for the histological changes and CK 17 and calponin expression. The intensity of CK 17 expression and also the area percent of CK 17 and calponin expression were measured. Data revealed acinar cytoplasmic vacuoles, loss of architecture and shrinkage in irradiated glands. Some areas showed degranulation of granular convoluted tubules and duct degeneration. The intensity of previously mentioned changes was increased by time. The intensity of CK 17 expression rose with time for both ductal and acinar cells while the area percent of expression decreased. The area of calponin expression decreased up to seven days after radiation and then increased at fourteen days. In conclusion, the obtained results about the histological changes as well as intensity and area of CK 17 and calponin expression indicate the deleterious acute effect of single dose (15Gy) of gamma radiation on the submandibular gland parenchyma that may hinder salivary production and secretion.

Keywords: Area percent, Gland injury, Histomorphometry, Immunohistochemical, Ionizing radiation.

Introduction

Radiotherapy has been used effectively in treating most of head and neck cancers either alone or in combination with surgery or chemotherapy. It is used for cancer treatment and also as palliative treatment to relieve symptoms associated with cancer (Mizoe et al., 2012). Radiation induced cell damage through two types of actions occur together. The indirect damage occurred due to ionization of water molecules generating reactive oxygen species and subsequent oxidative stress that damage cells and their function by attacking the DNA of the target molecules, while the direct effect was via ionization of target molecules that absorbed the radiation energy (Di Pietro et al., 2006). The neighboring normal tissues to the tumor are dramatically damaged by the used radiation as a result of unavoidable co-irradiation and formed reactive oxygen species that hurt not only the cancer cells but also the adjacent healthy tissues (Kumar et al., 2011; Vissink et al., 2015). Salivary glands have a high impact on the quality of life and considered among the normal tissues affected by ionizing radiation during cancer management in head and neck region since the ionizing beams in many cases have to pass the salivary glands to reach the tumor (Kałużny et al., 2014; Vissink

et al., 2015). The current radiotherapy protocols provide safeguard for parotid glands while submandibular glands are neglected (Clark et al., 2009). Salivary glands characterized by their highly differentiated cellular state that supposed to make them resistant to radiation however they demonstrated squeaky sensitivity to ionizing radiation (Nagler, 2002). The production of saliva via salivary glands exerts a fundamental role in maintain oral health. Saliva provides lubrication needed for chewing, aids in digestion and enamel integrity. Moreover, it protects the mouth, including its various tissues, with its anti-bacterial, antifungal and anti-inflammatory effect (Chamani et al., 2011). One of the most adverse effects of radiotherapy for neck and head cancer, occurring in up to 90% of patients who undergo this conventional treatment is xerostomia. It is known as dry mouth due to absence or reduction of salivary flow due to salivary gland damage (Lee et al., 2007). Consequently, it may lead to mucositis, oral ulceration, increased incidence of massive dental caries, chewing and swallowing difficulty, loss of taste that end in cachexia (Joshi, 2010).

Due to the importance of maintaining the salivary glands to provide a better comfort and satisfactory life after radiotherapy and as submandibular glands contribute 2/3 of the resting production of saliva, the purpose of this research is to assess the histopathological consequence of gamma-irradiation (15 Gy) on submandibular glands of the rats in addition to cytokeratin 17 and calponin expressions for deeper understanding of the mechanism of radiation-induced salivary gland injury that helps to provide appropriate treatment solutions and preventive measures.

Experimental

The present experimental study was carried out on16 male albino rats (8-12 weeks old and 210 ± 20 g), obtained from the animal care house of the National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority. The experimental protocol follows the European Communities Council guiding principles for the use and care of laboratory animals. All rats were randomly allocated as four rats per cage, kept under similar laboratory conditions (23 ± 2 °C, 12:12 h dark\light cycle, max. humidity 55% with same access to food and water supply.

Rats were divided into two groups. The control group (C) was sham-irradiated (0 Gy) and composed of 4 rats. The irradiated group (R) consisted of twelve rats divided into 3 subgroups (R1, R7 and R14) according to time of sacrifice; 1, 7 and 14 days after radiation exposure. Rats in the irradiated group were exposed to a single dose 15 Gy gamma radiation (^{60}Co), using the Indian Gamma Cell, at a dose rate of 12.77 Gy/min. Before radiation, the rats in groups R1, R7 and R14 were anesthetized by ketamine (50 mg/kg B.W.) IM; they were completely immobilized on a special shield and the tube placement ensures that the rats' whole cranium was in the irradiation field. After the end of the radiation session cycle, rats were returned to the Animal Care Center of the National Center for Radiation Research and Technology. Three rats from each group were sacrificed by over dose anesthesia Ketamine (100 mg/kg B.W.) one, seven and fourteen days after irradiation.

Submandibular glands were dissected, fixed in 10% formalin and inserted in paraffin. 5 μm sections stained with hematoxylin and eosin for histological evaluation and histomorphometric analysis of glandular changes under the light microscope by pathologists. Other sections were stained using mouse anti CK 17 monoclonal antibody and mouse anti calponin monoclonal antibody (Santa Cruz Biotechnology, USA). In brief, sections of 5 μm thickness were obtained and mounted on positively charged glass slides. These slides were dewaxed in xylene, then rehydrated in descending grades of ethanol, washed and inserted in Tris buffered solution for antigen retrieval. Afterwards, slides were incubated in 0.3% hydrogen peroxide solution for 30 minutes to block endogenous peroxidase activity then were inserted in normal goat serum for 30 minutes at room temperature to block non-specific antibody reaction. Following that, incubation over night at 4°C with the primary antibody (CK 17 mouse monoclonal antibody, Santa Cruz Biotechnology, USA and calponin monoclonal antibody, Santa Cruz Biotechnology, USA) took place then, slides were washed by PBS. The sections were incubated with the corresponding biotinylated secondary anti-immunoglobulin followed by avidin-biotin horseradish peroxidase complex. Peroxidase reaction was completed using

Diaminobenzidine (DAB) as chromogen, counterstained by hematoxylin, dehydrated in graded alcohol, cleared by xylene and the cover slips were mounted using purified Canada balsam (Ramos-Vara et al., 2008).

Area percent of cytokeratin 17 and calponin expression were measured in 5 histological fields (×400) randomly captured in each slide with a digitized image analysis system using the software Leica Qwin 500. Then, the intensity of CK 17 staining was assessed semi-quantitively in acini and intercalated, striated and excretory ducts separately and scored as follows: negative staining (0), trace or weak (1), mild (2), moderate (3), and strong staining (4). Slides were examined microscopically by two viewers of the working group separately. Each one recorded the cytokeratin staining intensity of each slide in 5 different histological fields.

Data are expressed as mean ± standard deviation (SD). One-way analysis of variance was performed to compare group differences. Multiple range tests were used when differences among groups were significant. Statistical analysis was conducted using Statgraphics Centurion XIX software, Statpoint Technologies, Inc., 560 Broadview Ave. Warrenton, Virginia 20182. P<0.05 was considered significant and P<0.01 was considered highly significant.

Results

Histopathological examination
The sham-irradiated submandibular gland revealed normal architecture of acinar and ductal cells. The secretory terminal portion consisted mainly of serous acini lined with pyramidal cells with basophilic cytoplasm containing dark granules and basally situated dark stained round nuclei surrounding a narrow lumen in addition to few mucous acini made up of cuboidal cells with clear cytoplasm containing pale mucin and flattened nuclei with wide lumen. Both types of acini demonstrated well defined, regular outline. The duct system compromised of the intercalated, striated, excretory, and main excretory ducts. The intercalated duct lined by single layer of low cuboidal epithelial cells with centrally placed nuclei. The striated ducts were round and lined by single layer of columnar cells with pale cytoplasm striated basally and centrally placed nuclei. The excretory duct

was interlobular large duct; its epithelium consists of various types of columnar cells. The convoluted granular tubule composed of columnar secretory cell containing many secretory granules in its supranuclear cytoplasm and rounded basal nuclei. The submandibular gland was surrounded by a fibrous connective tissue capsule with loose interstitial connective tissue dividing the gland into lobules and containing ducts, blood vessels and nerves (Fig.1, A and 1, B).

Examination of submandibular glands one day after gamma-irradiation exhibited mild loss of acinar outline and slight acinar vacuolization in some area while, other areas showed mild acinar shrinkage. Most granular convoluted tubules were normal while few of them showed degranulation. All intercalated ducts and most of striated ducts were normal while some of striated ducts showed areas of degeneration (Fig.1, C and 1, D). Seven days after irradiation, the submandibular glands revealed loss of acinar outline; acinar vacuoles increased in size and number affecting most of the gland tissues. The granular convoluted tubules showed a decreased secretory content in addition to their diminished number. The intercalated ducts were almost normal while striated ducts showed a sign of degeneration (Fig.1, E and 1, F). After fourteen days, some areas of the submandibular gland manifested with complete loss of acinar outline and architecture, other areas showed sever acinar shrinkage with complete degeneration of some acini. Most of gland tissue showed small sized acini associated with massive vacuolization of large-sized vacuoles. The granular convoluted tubules exhibited marked decrease in number with loss of their granular content. Intercalated ducts appeared normal while striated ducts showed a sign of degeneration with a space surrounding some of them (Fig.1, G and 1, H).

The area and perimeter of acinar vacuoles, serous acini, and intercalated, striated and excretory ducts were measured and presented in Table 1. The obtained data revealed a highly significant difference between the different experimental groups (P<0.01) for acinar vacuoles perimeter and area. Sham-irradiated submandibular glands showed no vacuoles that appeared one day after irradiation and increased in both perimeter and area with time. The vacuoles perimeter and area significantly

increased in irradiated group at all studied dates over the control. With time, the vacuoles perimeter and area significantly increased from day 1 to day 7 and from day 7 to day 14. The area and perimeter of serous acini followed the same trend as vacuoles but in the opposite way. The acinar perimeter and area significantly

decreased in response to irradiation with progressive reduction as time pass. Again, there was a significant difference between different studied dates of irradiation group; however no significance was detected between day 7 and day 14 regarding the perimeter of serous acini.
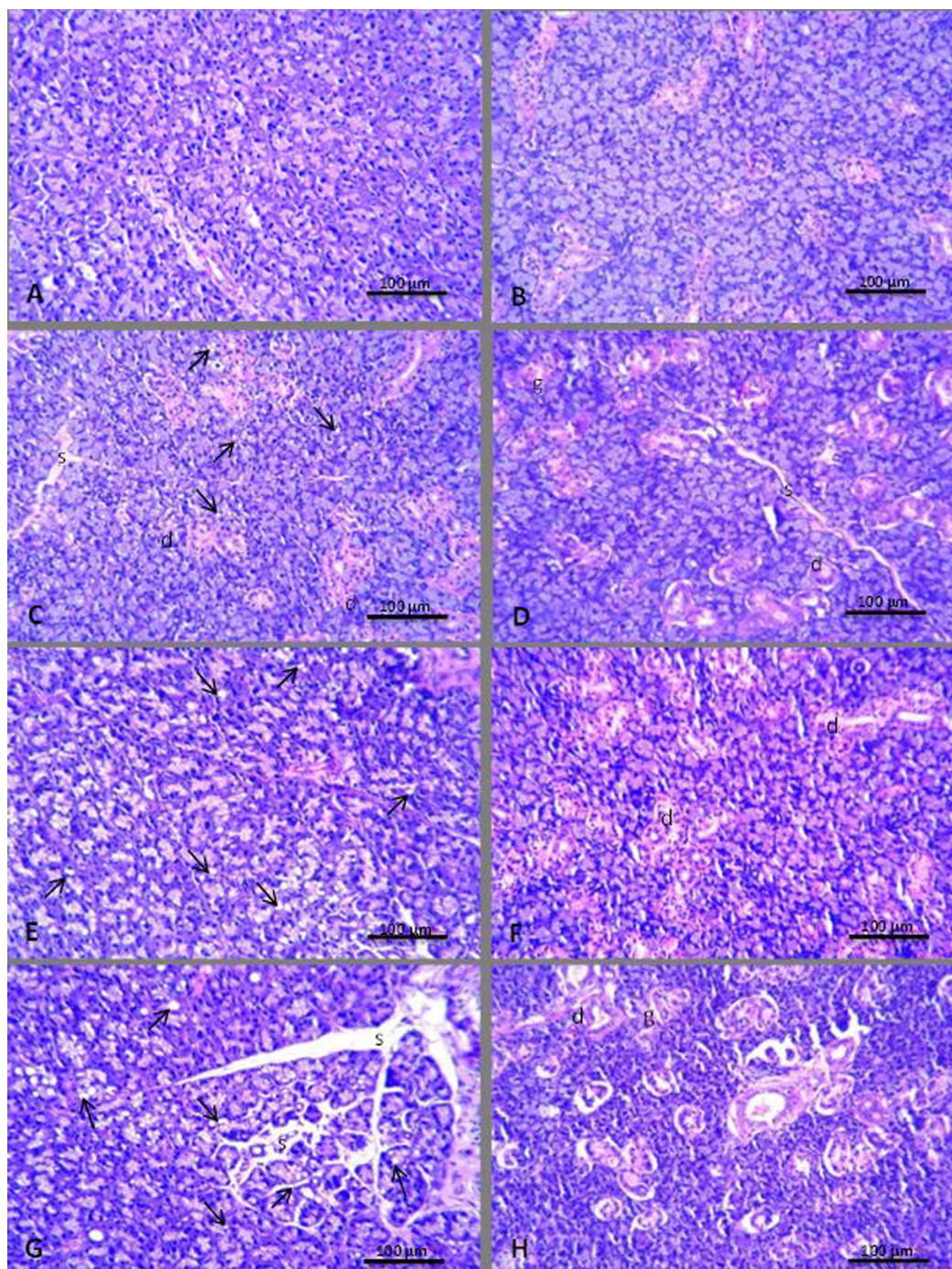

Fig. 1. Photomicrograph of (A & B) sham-irradiated submandibular gland, (C & D), (E & F), and (G & H) 1, 7 and 14 days after irradiation, respectively showing acinar vacuoles (black arrow), acinar shrinkage (s), degranulated granular convoluted tubules (g), degenerated ducts (d) [H. & E.]

TABLE 1. The perimeter (µm) and area (µm²) of acinar vacuoles, serous acini and intercalated, striated and excretory ducts of different experimental groups

	Acinar vacuoles	Serous acini	Intercalated ducts	Striated ducts	Excretory ducts
Groups			The perimeter (µm)		
C	0 ± 0 a	201.99 ± 12.24 a	112.6 ± 8.04 a	256.93 ± 12.43 a	308.70 ± 18.84 a
R 1	36.75 ± 3.93 b	183.85 ± 12.08 b	96.2 ± 7.13 b	207.59 ± 16.03 b	349.61 ± 24.41 b
R 7	49.70 ± 10.73 c	143.31 ± 12.28 c	75.4 ± 7.35 c	174.37 ± 16.73 c	529.74 ± 34.95 c
R 14	60.18 ± 13.26 d	139.20 ± 13.91 c	67.3 ± 5.20 d	169.86 ± 14.36 c	738.83 ± 47.44 d
P value	0.0000	0.0000	0.0000	0.0000	0.0000
			The area (µm²)		
C	0 ± 0 a	2642.48 ± 193.18 a	622.80 ± 32.38 a	3678.58 ± 141.67 a	8056.42 ± 396.67 a
R 1	62.68 ± 7.18 b	2185.26 ± 170.98 b	621.70 ± 28.08 a	3624.75 ± 176.97 a	18672.20 ± 536.57 b
R 7	185.32 ± 19.54 c	1780.80 ± 165.79 c	591.90 ± 34.65 b	2836.40 ± 152.96 b	22168.80 ± 784.10 c
R 14	238.70 ± 27.82 d	1534.79 ± 144.69 d	577.25 ±13.04 b	2616.70 ± 148.73 b	23511.10 ± 854.41 d
P value	0.0000	0.0000	0.0062	0.0000	0.0000

C; control group, R1, R7and R14; irradiated groups 1, 7 and 14 days after irradiation, respectively.

Different letters means significant difference. P < 0.05 means significant and P < 0.01means highly significant.

Intercalated ducts perimeter of irradiated gland significantly decreased with time as compared to sham-irradiated gland. A significant difference was detected among the studied dates in the irradiated group. The area of intercalated ducts decreased upon exposure to radiation however the reduction was significant as compared to sham-irradiated gland after 7 days of irradiation i.e. R7 and R14. In the irradiated group, there was a significant decrease of intercalated ducts area between R1 and R7 while no significant difference could be detected between R7 and R14. The striated ducts of irradiated group (R1, R7 and R14) exhibited a significant decrease of their perimeters with time as compared to sham-irradiated gland. Among the studied dates of irradiated group, a significant difference was estimated between day 1 and day 7 only. The area of striated ducts followed the exact pattern of the area intercalated ducts. The area of striated ducts showed a significant lower value as compared to sham-irradiated gland in R7 and R14 groups. Within the irradiated group, a significant difference between R1 and R7 could be detected. Similar to acinar vacuoles, excretory ducts perimeter and areas increased with time in response to irradiation. There were significant differences between irradiated group at the different studied dates and sham-irradiated glands and among the studied dates in the irradiated group.

Cytokeratin 17 expression

The normal submandibular gland showed a weak to moderate positive cytokeratin 17 expression in the luminal cells of intercalated, striated and excretory ducts while, serous acinar epithelial and basal cells showed a weak positive expression. Some acinar epithelial cells revealed a negative expression. Mucous acinar cells were immune negative (Fig. 2, A). At the first day after irradiation, few serous acini showed a weak positive CK 17 expression on their peripheries while, some showed a negative expression. The intercalated, striated and excretory ducts showed a weak to moderate CK 17 expression (Fig. 2, B). On the seventh day after irradiation, the acini revealed a weak expression for CK 17 on their peripheries. Some intercalated ducts showed a moderate expression for CK 17 while few of them weakly expressed CK 17. Both striated and excretory ducts showed a moderate CK 17 expression (Fig. 2, C). Fourteen days after irradiation, some acini showed a moderate CK 17 expression on periphery while most acini showed a weak expression. Intercalated ducts showed a moderate positive expression however, striated and excretory ducts revealed a moderate to intense positive expression (Fig. 2, D).

The intensity of CK 17 expression in different gland compartment was estimated and presented in Table 2. In general, the CK 17 intensity increased in acinar, intercalated, the striated and excretory duct cells of the irradiated gland at all the studied dates as compared to sham-irradiated gland. The acini and striated ducts gave the same response

where significant increment in CK 17 intensity was detected in R7 and R14 as compared to the sham-irradiated gland. Among the studied dates after radiation, a significant increase was found only between R1 and R14. For the intercalated and excretory ducts, the intensity of CK 17 expression elevated with significance of R14 as compared to the sham-irradiated gland. In addition, there was a significant difference only between R1 and R14 among the different dates of the irradiated group (Table 2).
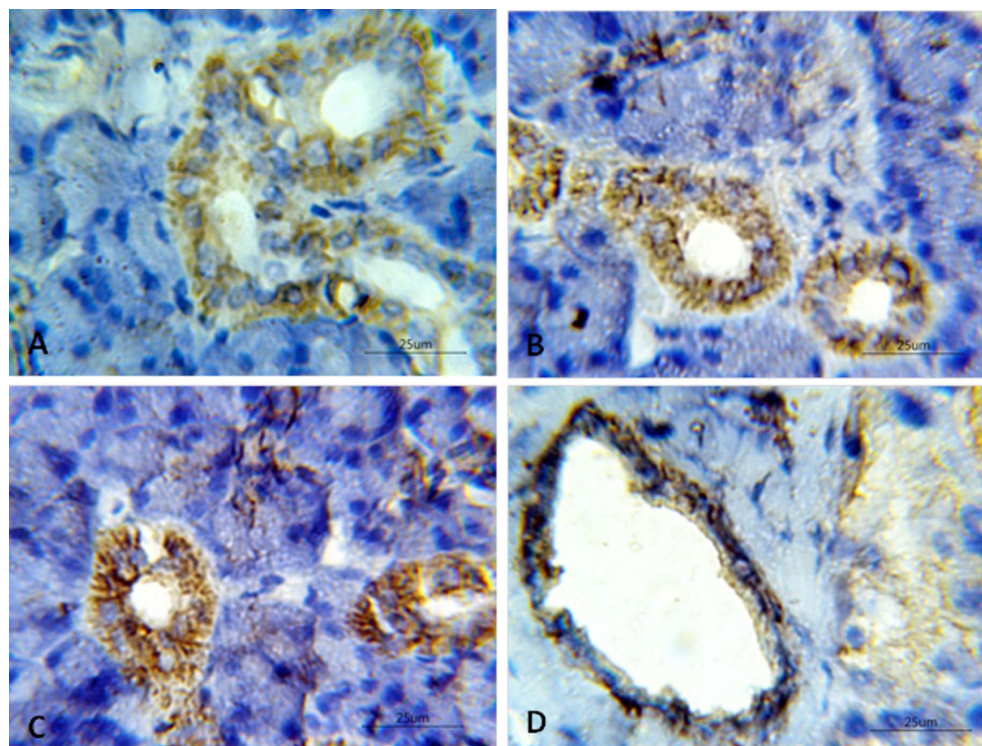

Fig. 2. Photomicrograph of intensity of CK 17 expression in submandibular gland (A) sham-irradiated; weak expression, (B) 1 day after irradiation; moderate expression, (C) 7 days after irradiation; moderate expression, (D) 14 days after irradiation; intense expression

TABLE 2. Intensity of cytokeratin 17 expression in serous acini and intercalated, striated and excretory ducts and area percent of cytokeratin 17 and calponin expressions in the whole gland of different experimental groups

	Serous acini	Intercalated ducts	Striated ducts	Excretory ducts
Groups	Intensity of cytokeratin 17 expression			
C	0.667 ± 0.778 a	1.167 ± 0.389 a	1.333 ± 0.492 a	1.500 ± 0.522 a
R 1	1.000 ± 0.603 ab	1.333 ± 0.492 ab	1.750 ± 0.452 ab	1.583 ± 0.669 ab
R 7	1.417 ± 0.669 bc	1.667 ± 0.985 abc	2.083 ± 0.793 bc	2.000 ± 0.739 abc
R 14	1.667 ± 0.985 c	2.000 ± 0.855 c	2.333 ± 0.778 c	2.417 ± 0.669 c
P value	0.0141	0.0348	0.0031	0.0045
	Area % of cytokeratin 17 expression		Area % of calponin expression	
C	45.046 ± 1.096 a		15.848 ± 1.154 a	
R 1	34.056 ± 1.136 b		12.134 ± 0.879 b	
R 7	22.263 ± 0.850 c		2.682 ± 0.293 d	
R 14	14.583 ± 0.743 d		8.553 ± 0.385 c	
P value	0.0000		0.0000	

C; control group, R1, R7and R14; irradiated groups 1, 7 and 14 days after irradiation, respectively.

Different letters means significant difference. P<0.05 means significant and P<0.01 means highly significant.

In contrast to CK 17 intensity, the area of CK 17 expression was significantly decreased in irradiated gland at all the studied dates in comparison to the sham-irradiated gland. Furthermore, a significant reduction in the area of CK 17 expression was detected among the studied dates of the irradiated gland (Table 2).

Calponin expression

The normal submandibular gland showed positive expression of calponin at the periphery of acini and intercalated ducts (Fig. 3, A). One day after irradiation, many acini showed a positive calponin expression on their peripheries while other showed a negative expression. The intercalated ducts exhibited a positive expression of calponin at the periphery (Fig. 3, B). Seven days after irradiation, few acini and intercalated ducts revealed a positive expression for calponin on their peripheries while most of them were negative (Fig. 3, C). Fourteen days after irradiation, some acini showed a positive calponin expression on periphery. Only few intercalated ducts showed a positive expression of calponin on their peripheries (Fig. 3, D).

The mean area of calponin expression significantly decreased 1 and 7 days after radiation as compared to the sham-irradiated gland. On the fourteenth day after radiation, the area of calponin expression increased, but it was still significantly below the normal value. Among the different studied dates of the irradiated group, the area of calponin expression significantly decreased from day 1 to day 7 and then significantly increased from day 7 to day 14 however, it was significantly less than day 1 after radiation (Table 2).
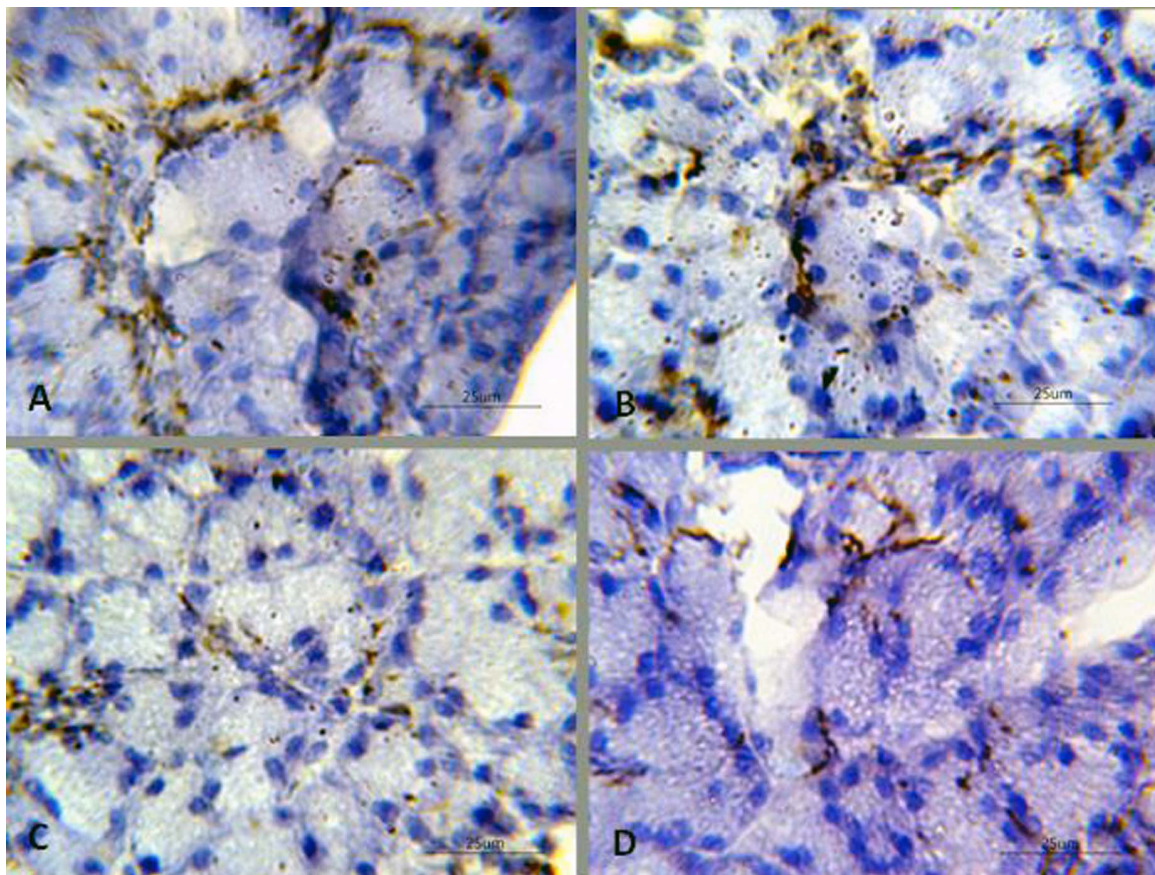

Fig. 3. Photomicrograph of calponin expression in submandibular gland (A) sham-irradiated; many acini showed positive expression , (B) 1 day after irradiation; many acini showed positive expression, (C) 7 days after irradiation; most acini showed negative expression, (D), 14 days after irradiation; few acini showed positive expression

Discussion

Cancer of head and neck is considered to be the seventh widespread cancer worldwide with more than 800000 newly cases being diagnosed per the year (Global Burden of Disease Cancer Collaboration, 2017). Radiotherapy has a major role in different cancer treatment protocols. It may be the definitive treatment with or without chemotherapy or adjuvant therapy after surgery and in case of local failure after surgery. Moreover, radiotherapy offers an advantageous solution for maintaining organs as well as their functions. Improvement of overall survival and reduction of local recurrences implied intensification of radiotherapy regimens and addition of concomitant chemotherapy which has been accompanied by increased toxicity (Bourhis et al., 2006; Pignon et al., 2009). The recently used radiotherapy techniques are based on precise shaping of radiation beam to be as much as possible near to the target volume to give an opportunity for administration of high sufficient doses of radiation to tumor volume (Ghosh-Laskar et al., 2016) and in the same time obviate critical surrounding normal tissue and minimize associated toxicity (Jellema et al., 2007). In the case of head and neck cancer, the major salivary glands including parotid, submandibular and sublingual as well as minor salivary glands widely distributed over the oral mucosa are inadvertently exposed. However, parotid glands are spared in modern intensity modulated radiotherapy, but submandibular glands are generally overlooked (Clark et al., 2009). Thus, the present study is conducted to assess the acute effect of single high dose (15 Gy) of gamma – irradiation (De la Cal et al., 2012) on the submandibular gland histological structure and expression of cytokeratin 17 and calponin for better understand of pathogenesis of associated gland toxicity to allow the development of suitable prophylactic and treatment measures.

Gamma-irradiation (15 Gy) resulted in serous acinar vacuolization, loss of outline and mild shrinkage while the mucous acini were normal. The granular convoluted tubules showed a mild degranulation. The mentioned alterations increased by time in intensity and involved more of the glandular tissue. Some granular convoluted tubules showed loss of granules while others exhibited degeneration. Fourteen days after irradiation, there was a complete loss of acinar outline and architecture with areas of obvious acinar shrinkage and a complete degeneration of some acini. The duct system showed more resistance to radiation where intercalated and excretory ducts appeared more or less normal. Some striated ducts showed signs of degeneration with hollow space around them. According to the performed histomorphometric analysis, the serous acini decreased in perimeter and size and such reduction increased as time pass. In the same time, the vacuoles perimeter and size increased by time and sspread over a larger area of the gland tissue. Intercalated and striated showed significant continuous decrease in perimeter and size in response to radiation on the other hand, excretory ducts exhibited increased perimeter and size. The obtained results went parallel with those obtained by Kim et al. (2016) and Vissink et al. (1991) who reported the development of cytoplasmic acinar vacuolization and degranulation of granular convoluted tubule 4 days after irradiation. The early changes of submandibular gland in response to single 15 Gy irradiation was studied by El-Direny et al. (2009) where they reported acinar cytoplasmic vacuolization and increased inter acini distances 7 days after irradiation. The acinar cytoplasmic vacuoles are a manifestation of the destructive effect of radiation on the cell membrane resulting in cell irregularities and reduction of amylase secretion. The sensitivity of serous acini to radiation over the mucous one was attributed to the proteolytic and metallic transmission enzymes found in serous secretion granules. Such metallic transmission materials enhanced oxidative stress, destroying the membranes of the serous granule followed by proteolytic enzymes infiltration and cytoplasm damage ending with autolysis and cellular death (Abok et al., 1984; Coppes et al., 2002).

Cytokeratins (CKs) designate a family of intermediate filament proteins expressed on cells of epithelial origin in a tissue-specific manner (Hassan et al., 2020) and related to state of epithelial cells differentiation (Gustafsson et al., 1998). CKs participate in preservation of cellular architecture, tissue integrity and enhance the intracellular materials transport. Cytokeratin 17 characterized by cytoplasmic expression in all duct cells of normal salivary gland while acinar cells exhibited a weak expression (Burns et al., 1988). The strong CK 17 expression in duct cells over the acinar cells was attributed to the cellular degree of differentiation where the acini are highly differentiated than other parenchymal cells

(Hassan et al., 2020). Normal acinar cells showed either diffuse CK 17 expression when the gland was in resting status or intensified expression concise to the basal cell part that referred to active ionic exchange during the secretory state. This intensified expression had an essential role in strengthening the acinar cell part facing myoepithelial cells to augment the squeezing power which results in saliva rushing through the ducts. All compartments of the duct system showed a mild CK 17 expression confined to the apical portion of the cells (Hassan et al., 2020). The obtained results demonstrated a significant increment of CK 17 expression appeared in serous acinar and ductal cells of gamma-irradiated submandibular glands which continues to increase over time. Moreover, mucous acinar cells revealed a negative CK 17 expression in the whole glandular tissue. Similar results were found in diabetic and duct ligated parotid glands where CK 17 expression increased in both ductal and acinar cells as compared to the control (Hassan & Alqahtani, 2022). Another study by Hassan et al. (2020) on the effect of fractionated irradiation (5Gy X 5) on submandibular gland, they found that the serous acinar cell exhibited a mild to moderate diffuse CK 17 expression while mucous acinar cells showed a negative expression. The striated and intercalated ducts exhibited similar intensity of CK 17 expression that was moderate to strong. They also demonstrated similar patterns of expression either the luminal part showed a strong expression with a mild expression in the basal part or a uniform diffuse over the cell. The excretory expressed CK 17 strongly in patterns similar to those of striated and intercalated ducts (Hassan et al., 2020).

In contrast to CK 17 intensity, the measured area% of total CK 17 expression in irradiated glands was significantly decreased which kept decreasing by time. This may be due to the negative CK 17 expression by degenerated acini and striated ducts. The current results were on the contrary to those of Hassan et al. (2020) who found a mild CK 17 expression in degenerated acinar and ductal cells. The difference could be attributed to the severity of acinar and ductal degeneration. The hypo salivation manifested among early changes occurred after radiation could be attributed to the luminal expression of CK 17 in both acinar and duct cells that interfere with parenchymal function and saliva adjustment, respectively (Hassan et al., 2020). They added that

luminal distribution pattern represents an early stage of radiation damage, whereas the diffuse pattern was the advanced one.

The present results regarding calponin expression in the periphery of both acinar and intercalated ducts of normal salivary glands were in agreement with those of Abdulrahman et al. (2019). One day after radiation, the area of calponin expression significantly decreased as compared to the sham-irradiated gland. This reduction increased seven days after radiation and then increased at 14 days after radiation. The early reduction in the calponin expression was more related to the loss of myoepithelial around the acini. Similar results were obtained by Hakim et al. (2004) who reported a significant loss of smooth muscle actin alpha stained myoepithelial cells of parotid glands one day after 15 Gy, irradiation. Alpha- smooth muscle actin reaction was limited to few areas of blood vessels and acini while those around the intercalated and striated ducts were similar to the normal. These results were supported by myofilaments focal condensation and rarefaction in myoepithelial cells of irradiated glands (Hakim et al., 2002). The reduction of calponin expression in irradiated gland could explain the radiation induced xerostomia in terms of deteriorated excretion process.

Conclusion

The acute negative effect of gamma radiation at a single dose of 15 Gy on the submandibular glands could be confirmed by the different histological changes we have. In addition, the changes that occurred on the cytokeratin 17 expressions and calponin in different parts of the gland could give an explanation for the decreased output and secretion of saliva, which results in early xerostomia.

Disclosure of interest: The authors report no conflicts of interests.

References

Abdulrahman, S.S., Mohammad, D.N., Hamied, M.A.S., Abdulqadir, M.O. (2019) Immunohistochemical evaluation of salivary gland tumors differentiation and proliferation by using calponin and telomerase. *Saudi Dental Journal,* **31**(1), 105-114. doi: 10.1016/j.sdentj.2018.11.007.

Abok, K., Brunk, U., Jung, B., Ericsson, J. (1984) Morphologic and histochemical studies on the differing radiosensitivity of ductular and acinar cells of the rat submandibular gland. *Virchows Archiv. B, Cell Pathology Including Molecular Pathology*, **45**(4), 443-460. doi: 10.1007/BF02889885.

Bourhis, J., Overgaard, J., Audry, H., Ang, K.K., Saunders, M., Bernier, J., et al. (2006) Hyperfractionated or accelerated radiotherapy in head and neck cancer: a meta-analysis. *Lancet*, **368**(9538), 843-854. doi: 10.1016/S0140-6736(06)69121-6.

Burns, B.F., Dardick, I., Parks, W.R. (1988) Intermediate filament expression in normal parotid glands and pleomorphic adenomas. *Virchows Archiv A, Pathological Anatomy and Histopathology*, **413**(2), 103–112. doi: 10.1007/BF00749671.

Chamani, G., Zarei, M.R., Mehrabani, M. and Taghiabadi, Y. (2011) Evaluation of effects of *Zingiber officinale* on salivation in rats. *Acta Medica Iranica,* **49**(6), 336- 340.

Clark, C.H., Miles, E.A., Urbano, M.T., Bhide, S.A., Bidmead, A.M., Harrington, K.J., Nutting, C.M. (2009) Pre-trial quality assurance processes for an intensity-modulated radiation therapy (IMRT) trial: PARSPORT, a UK multicentre Phase III trial comparing conventional radiotherapy and parotid-sparing IMRT for locally advanced head and neck cancer. *British Journal of Radiology*, **82**(979), 585–594. doi: 10.1259/bjr/31966505.

Coppes, R.P., Vissink, A., Konings, A.W.T. (2002) Comparison of radiosensitivity of rat parotid and submandibular glands after different radiation schedules. *Radiotherapy and Oncology*, **63**(3), 321-328. doi: 10.1016/s0167-8140(02)00129-9.

De la Cal, C., Fernández-Solari, J., Mohn, C.E., Prestifilippo, J.P., Pugnaloni, A., Medina, V.A., Elverdin, J.C. (2012) Radiation produces irreversible chronic dysfunction in the submandibular glands of the rat. *The Open Dentistry Journal*, **6**, 8-13. doi: 10.2174/1874210601206010008.

Di Pietro, C., Piro, S., Tabbi, G., Ragusa, M., Di Pietro, V., Zimmitti,V., Cuda,F., et al. (2006) Cellular and molecular effects of protons: apoptosis induction and potential implications for cancer therapy. *Apoptosis*, **11**(1), 57–66. doi: 10.1007/s10495-005-3346-1.

El-Direny, E.A., Shalaby, N.M. and Afif, O.K. (2009) Effect of irradiation on secretory cells of submandibular gland of adult male albino rat. Histological and immunohistochemical study. *Egyptian Journal of Histology*, **32**(2), 324-332.

Ghosh-Laskar, S., Yathiraj PH, Dutta D, Rangarajan, V., Purandare, N., Gupta, T., Budrukkar, A., Murthy, V., Kannan, S., Agarwal, J.P. (2016) Prospective randomized controlled trial to compare 3-dimensional conformal radiotherapy to intensity-modulated radiotherapy in head and neck squamous cell carcinoma: Long-term results. *Head Neck*, **38**(1), E1481-1487. doi: 10.1002/hed.24263.

Global Burden of Disease Cancer Collaboration (2017) Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. JAMA Oncol; 3:524-48.

Gustafsson, H., Aalto, Y., Franzén, L., Thornell, L.E., Henriksson, R. (1998) Effects of fractionated irradiation on the cytoskeleton and basal lamina in parotid glands—an immunohistochemical study. *Acta Oncologica,* **37**(1), 33–40. doi: 10.1080/028418698423159.

Hakim, S.G., Kosmehl, H., Lauer, I., Nadrowitz, R., Wedel, T., Sieg, P. (2002) The role of myoepithelial cells in the short-term radiogenic impairment of salivary glands. An immunohistochemical, ultrastructural and scintigraphic study. *Anticancer Research*, **22**(6C), 4121-4128.

Hakim, S.G., Jacobsen, H.C., Hermes, D., Kosmehl, H., Lauer, I., Nadrowitz, R., Sieg, P. (2004) Early immunohistochemical and functional markers indicating radiation damage of the parotid gland. *Clinical Oral Investigations*, **8**(1), 30-35. doi: 10.1007/s00784-003-0249-9.

Hassan, S.S., Alqahtani, M.S. (2022) Comparative study of cytokeratin immunostaining of parotid gland parenchyma in normal, diabetic, and excretory duct ligation of Mongrel dogs. *European Journal of Dentistry*, Article published online: 21-6-2022. doi: 10.1055/s-0042-1744372.

Hassan, S.S., Attia, M.A., Attia, A.M., Nofal, R.A., Fathi, A. (2020) Distribution of cytokeratin 17 in the parenchymal elements of rat's submandibular

glands subjected to fractionated radiotherapy. *European Journal of Dentistry*, **14**(03), 440-447. doi: 10.1055/s-0040-1713705.

Jellema, A.P., Slotman, B.J., Doornaert, P., Leemans, C.R., Langendijk, J.A. (2007) Impact of radiation-induced xerostomia on quality of life after primary radiotherapy among patients with head and neck cancer. *International Journal of Radiation Oncology - Biology - Physics*, **69**(3), 751-760. doi: 10.1016/j.ijrobp.2007.04.021.

Joshi, V.K. (2010) Dental treatment planning and management for the mouth cancer patient. *Oral Oncology,* **46**(6), 475-479. doi: 10.1016/j.oraloncology.2010.03.010.

Kałużny, J., Wierzbicka, M., Nogala, H., Milecki, P., Kopeć, T. (2014) Radiotherapy induced xerostomia: mechanisms, diagnostics, prevention and treatment—evidence based up to2013. *Otolaryngologia Polska*, **68**(1), 1-14. doi: 10.1016/j.otpol.2013.09.002.

Kim, J.H., Kim, K.M., Jung, M.H., Jung, J.H., Kang, K.M., Jeong, B.K., Kim, J.P., Park, J.J., Woo, S.H. (2016) Protective effects of alpha lipoic acid on radiation-induced salivary gland injury in rats. *Oncotarget*, **7**(20), 29143-29153. doi: 10.18632/oncotarget.8661.

Kumar, S., Ram, S., Navazesh, M. (2011) Salivary gland and associated complications in head and neck cancer therapy. *Journal of the California Dental Association*, **39**(9), 639–647.

Lee, N., Puri, D.R., Blanco, A.I., Chao, K.S. (2007) Intensity-modulated radiation therapy in head and neck cancers: an update. *Head Neck*, **29**(4), 387–400. doi: 10.1002/hed.20332.

Mizoe, J.E., Hasegawa A, Jingu K, Takagi, R., Bessyo, H., Morikawa, T., et al. (2012) Results of carbon ion radiotherapy for head and neck cancer. *Radiotherapy and Oncology*, **103**(1), 32–37. doi: 10.1016/j.radonc.2011.12.013.

Nagler, R.M. (2002) The enigmatic mechanism of irradiation-induced damage to the major salivary glands. *Oral Diseases*, **8**(3), 141-146. doi: 10.1034/j.1601-0825.2002.02838.x.

Pignon, J.P., le Maître, A., Maillard, E., Bourhiset, J. (2009) Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. *Radiotherapy and Oncology*, **92**(1), 4-14. doi: 10.1016/j.radonc.2009.04.014.

Ramos-Vara, J.A., Kiupel, M., Baszler, T., Bliven, L., Brodersen, B., Chelack, B., et al. (2008) Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. *Journal of Veterinary Diagnostic Investigation,* **20**(4), 393-413. doi: 10.1177/104063870802000401.

Vissink, A., Kalicharan, D., S-Gravenmade, E.J., Jongebloed, W.L., Ligeon, E.E., Nieuwenhuis, P., Konings, A.W.T. (1991) Acute irradiation effects on morphology and function of rat submandibular glands. *Journal of Oral Pathology & Medicine,* **20**(9), 449-456. doi: 10.1111/j.1600-0714.1991.tb00437.x.

Vissink, A., van Luijk, P., Langendijk, J., Coppes, R. (2015) Current ideas to reduce or salvage radiation damage to salivary glands. *Oral Diseases*, **21**(1), e1-10. doi: 10.1111/odi.12222.