Investigation of the efficacy of systemic N-Acetyl Cysteine therapy preventing nasal mucositis following radiotherapy*

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Abstract

Background: Radiotherapy (RT) is one of the main methods used in the treatment of head and neck cancers but may cause mucosal side effects in the tumor area and surrounding structures. These include nasal mucosal disorders and chronic rhinosinusitis due to disruption of the mucociliary system. This situation seriously affects the quality of life of the patients and there is no accepted effective method for its treatment yet. In our study, we aimed to examine the side effects of RT on the nasal mucosa and mucociliary system and to investigate histopathologically and immunohistochemically the effectiveness of N-acetyl cysteine (NAC) in preventing these side effects of RT.

Methodology: The study was carried out with 30 female Sprague Dawley rats devided in three groups. No intervention was made in the control group. On the second day of the experiment, 30 Gy radiotherapy was applied to the head area in the RT group. NAC was administered intraperitoneally at a dose of 1 g/kg/day for 14 days from the first day of the study to the RT+ NAC group. On the second day, 30 Gy of radiotherapy was applied to the head area 1 hour after the NAC application. On the 14th day, 1 hour after NAC was applied to the RT+NAC group, all animals were sacrificed. The nasal mucosa samples were stained with hematoxylin-eosin, and the intensity and extent of staining sentan in the nasopharyngeal tissue samples were evaluated by immunohistochemical staining using anti-SNTN antibody.

Results: The loss of cilia in the nasal tissue was lower in the RT+NAC group than in the RT group. The intensity and extent of staining in the nasopharyngeal tissue of Sentan was higher in the RT+NAC group than in the RT group. Mucosal neutrophil and mononuclear inflammatory cell infiltration in the nasal tissue, vascular dilatation, hyperemia and hemorrhage, erosion and shedding of the mucosal epithelium, mucosal ulceration were found to be similar in the RT+NAC group and the control group. It was milder in the RT+NAC group than in the RT group, but not statistically significant.

Conclusions: Radiotherapy caused pathological changes in the nasal mucosa, caused loss of cilia and a decrease in the level of Sentan, the cilia apical protein. The results of our study showed that NAC treatment can reduce the side effects of RT on the nasal mucosa. It also showed that NAC was effective in preventing the loss of cilia, which is the building block of the mucociliary system, and improving the expression of Sentan.

Key words: antioxidant, cilia, mucositis, N-Acetyl Cysteine, radiotherapy, Sentan

Introduction

Head and neck cancers are the 6th most common cancer in the world [(1)]. In the treatment of early-stage cancers, a single treatment method is usually applied in the form of surgery or radiotherapy. In advanced stage cancers, multimodal treatment methods such as adjuvant-neoadjuvant radiotherapy or chemo-radiotherapy are applied together with surgical treatment [(2)]. Radiotherapy has an important role in the treatment of head and neck cancers. It is a clinical treatment method that causes changes in the DNA of cancer cells. Ionizing radiation affects the cell in two ways. It is a direct effect that causes DNA damage by making chemical or biological changes in the cell, and an indirect effect that occurs by the deterioration of the structure of nucleic acids, proteins and lipids by means of reactive oxygen radicals formed as a result of hydrolysis of water. Depending on the location of the tumor, it usually causes some mucosal side

effects in the tumor area and surrounding structures [3].
Nasal side effects such as nasal dryness, itching, thickening of the nasal mucosa and paranasal sinus mucosa, rhinorrhea, nasal obstruction, rhinosinusitis, edema, erosion, ulceration, adhesion, fibrosis, stenosis, and increase in nasopharyngeal secretions due to proximity to the irradiated area in patients undergoing radiotherapy for head and neck cancer can be seen [4-6]. Radiation causes destruction of cilia in mucosal epithelial cells in the sinonasal region, resulting in deterioration in mucociliary clearance and sinus drainage. This increases the risk of developing chronic rhinosinusitis [7, 8]. Nasal mucositis and chronic rhinosinusitis are the most common side effects of RT. This situation seriously impairs the quality of life of patients, and there are very few studies examining the effect of radiotherapy on the nasal mucosa and mucosal ciliary system [8-11].
Medical treatment such as nasal irrigation, nasal steroids, topical nigella sativa oil, mesenchymal stem cell application, endoscopic sinus surgery and some conservative surgical approaches have been tried for the nasal/paranasal side effects of irradiation, but there is no definite way to prevent the harmful effects of radiotherapy on the nasal mucosa and ciliary structures. There is no accepted effective treatment procedure [4, 12-14].
N-acetylcysteine (NAC) is an agent used especially as a mucolytic and as an antidote in acetaminophen intoxications. It is an acetylated derivative of the amino acid L-cysteine. It is an aminothiol and a precursor to glutathione. Glutathione in vivo; It is an endogenous antioxidant. It plays a role in detoxification of reactive oxygen radicals, detoxification of xenobiotics, storage and transport of cysteine, regulation of cell proliferation, and regulation of leukotriene and prostaglandin synthesis. In addition, it has been reported that NAC inhibits reactive oxygen radical-induced apoptosis by reducing caspase-3 expression. In addition, it has been shown to inhibit local inflammatory and fibrotic response by decreasing interleukin-1b, interleukin-8, TNF-α, NF-κB and TGF-β levels, which are also involved in the pathogenesis of mucositis [15-18]. By emphasizing the anti-inflammatory and antioxidant properties of NAC, the protective effects of radiation on the intestinal system, salivary gland and heart have been demonstrated in previous studies [19-21]. In this experimental study, we aimed to comprehensively examine the harmful effects of RT on the nasal mucosa and mucociliary system and to examine the effectiveness of NAC in preventing these side effects of RT histopathologically and immunohistochemically.

Materials and methods

Our study received ethical approval numbered 2021/66 from Acıbadem Mehmet Ali Aydınlar University Animal Experiments Local Ethics Committee. It was supported by the University of Health Sciences Scientific Research Projects Coordinatorship with the decision with project number 2022/010 dated 07.02.2022. It was performed on a total of 30 female Sprague Dawley rats, with an average weight of 292-312 grams, adult, 10 rats in each group, to randomised to 3 groups. 1st group (Control group): the rats in this group did not undergo any surgical or experimental procedure. 2nd Group (RT group): the experimental animals in this group were given 30 Gy radiotherapy in a single fraction on the head area on the second day of the experiment. 3rd Group (RT+ NAC group): NAC (Asist 300 mg / 3 mL ampoule, Bilim Pharmaceuticals, Turkey) was administered intraperitoneally at a dose of 1 g/kg/day for 14 days from the first day of the study to the experimental animals in this group. On the second day, 1 hour after NAC application, a single dose of 30 Gy radiotherapy was applied to the head areas. On the 14th day, the experimental animals in all three groups were sacrificed 1 hour after the NAC application to the RT+NAC group.

Irradiation

In the literature, single-dose X-irradiation was applied at various doses in studies dealing with mucosal damage induced by ionizing radiation. In studies, radiation-induced mucositis was observed in a single dose in the range of 20-40 Gy [22]. In our study, a total of 30 Gy external ionizing radiation was applied in a single fraction. On the 2nd day of the experiment, 1 hour after NAC administration to the RT+NAC group, rats in the RT group and RT+NAC group were treated with 50 mg/kg ketamine (Ketalar, Pfizer, Istanbul, Turkey) and 5 mg/kg intraperitoneally administered to cause mucosal damage by radiation. It was placed in the prone position inside a specially constructed apparatus under xylazine (Rompun, Bayer, Istanbul, Turkey) anesthesia. Rats were irradiated in a Siemens Oncor Impression plus (Siemens Medical Solutions USA, Inc) linear accelerator device with a field size of 5x35 cm using SSD technique at 0° gantry angle and 6 MV energy.

Euthanasia

After irradiation, three animals were excluded from the experiment because two rats from the RT group died unexpectedly at week 1 and one rat from the RT+NAC group at day 10. Watanabe et al. Mucositis findings after RT 8 - 14. observed that the maximum per day [22]. The rats in all groups were euthanized on the 14th day of the experiment by decapitation after intraperitoneal (100 mg/kg) pentobarbital injection. Nasal mucosa samples were cut in the coronal plane as 2 mm sections, and nasopharyngeal samples were obtained using punch. All nasal and nasopharyngeal specimens were fixed in 10% buffered formaldehyde for 24 hours.

Histomorphological and immunohistochemical evaluation

Slides obtained from nasal tissues were prepared by staining with hematoxylin-eosin in an automatic staining closure device (Sakura Tissue Tek Film). Hematoxylin-eosin-stained slides of nasal sections were evaluated and scored separately by the

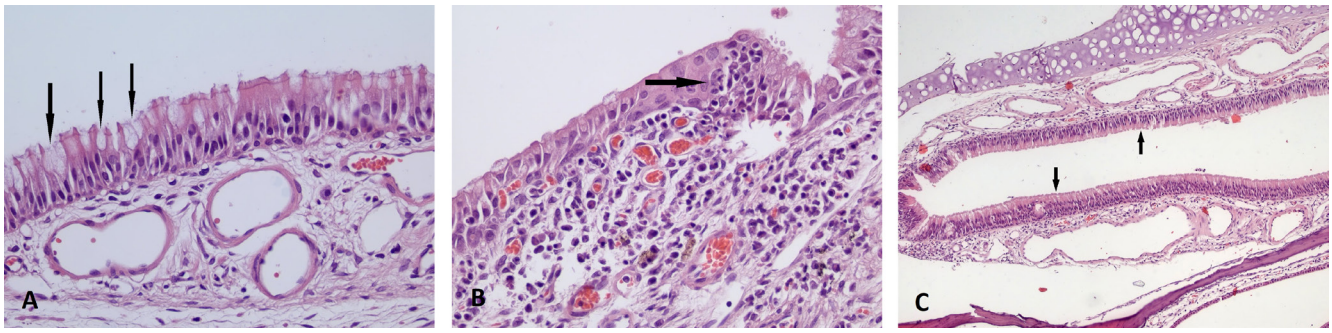
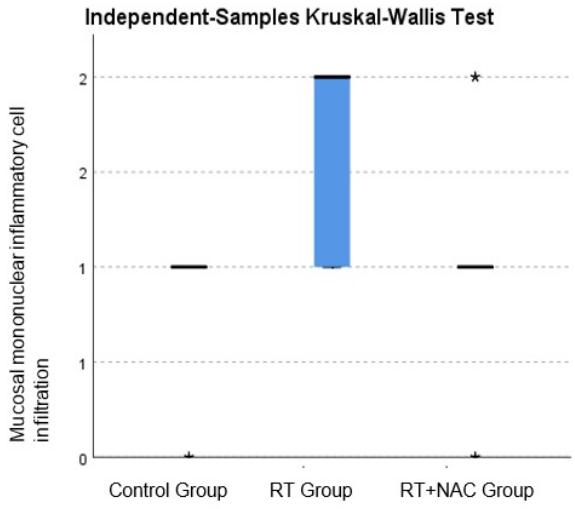


Figure 1. Nasal mucosa lined with regular stratified ciliated epithelium containing goblet cells (arrow) in the control group (A). Intense mononuclear cell infiltration in the nasal mucosa in the RT group, intraepithelial lymphocytes (arrow) (B). Nasal mucosa lined by ciliated stratified epithelium (arrow) with mild mononuclear cell infiltration in the RT+NAC group (C).



Graph 1. Mucosal mononuclear inflammatory cell infiltration.

pathologist in terms of mucosal mononuclear inflammatory cell infiltration, mucosal neutrophil infiltration, vascular dilatation, hyperemia and hemorrhage, erosion and shedding of the mucosal epithelium, mucosal ulceration, and loss of cilia.

For the demonstration of Sentan, the cilia apical protein, positively charged slide biopsies from nasopharyngeal tissues were placed in an automatic immunohistochemistry device (Leica Bond Max) for immunohistochemistry staining. Anti-SNTN antibody (Anti-SNTN antibody, HPA058399, ATLAS Bromma, Sweden) was used at 1:500 dilution in immunohistochemical staining. After immunohistochemical staining, brown chromogen staining intensity and extent of epithelial cilia in nasopharyngeal tissues were scored.

All evaluations were made in Olympus CX41RF (Japan) trinocular light microscope with x40, x100, x200 and x400 lenses and microphotographs were taken with an adapted digital camera (Olympus EP50).

Statistical analysis

IBM® SPSS® 26 (SPSS Inc., Chicago, IL, USA) program was used. Kruskal-Wallis analysis was used for comparisons between more than two groups in order to detect statistically significant associations in terms of histopathological and immunohistochemical findings. In addition, Bonferroni correction test was applied for pairwise comparisons. Cases with a p-value below 0.05 were considered statistically significant.

Results

Nasal mucosal evaluations

There was a significant difference between the groups in terms of mucosal mononuclear inflammatory cell infiltration (p=0.043, P < 0.05). Mucosal mononuclear inflammatory cell infiltration was found to be significantly higher in the RT group compared to the control group (p=0.016, P < 0.05), and there was no difference between the control group and the RT+NAC group (p=1,000, P > 0.05). In addition, although mucosal mononuclear inflammatory cell infiltration was found to be milder in the RT+NAC group than in the RT group, the difference was not statistically significant (p=0.129, P > 0.05) (Table 1-2, Figure 1, Graph 1).

There was a significant difference between the groups in terms of mucosal neutrophil infiltration (p=0.044, P < 0.05). Mucosal neutrophil infiltration was found to be significantly higher in the RT group compared to the control group (p=0.010, P< 0.05), and there was no difference between the control group and the RT+NAC group (p=0.1000, P > 0.05). In addition, although mucosal neutrophil infiltration was found to be milder in the RT+NAC group than in the RT group, the difference was not statistically significant (p=0.111, P > 0.05) (Table 1-2, Figure 2, Graph 2)

There was a significant difference between the groups in terms of vascular dilatation in the nasal mucosa (p=0.008, P < 0.05). Vascular dilatation was found to be significantly higher in the RT group compared to the control group (p=0.006, P< 0.05), and there was no difference between the control group and the RT+NAC group (p=0.588, P > 0.05). In addition, although vascular dilatation was found to be milder in the RT+NAC group than in the RT group, the difference was not statistically significant

Table 1. Distribution of pathological parameters in nasal mucosa between groups.

Variables	Grade	Groups			
		Control Group	RT Group	RT+NAC Group	Total
		n (%)			
Mucosal mononuclear inflammatory cell infiltration	1 - No	2(20)	0(0)	2(22,2)	4(14,8)
	2 - Mild	8(80,0)	3(37,5)	5(55,6)	16(59,3)
	3 - Severe	0(0)	5(62,5)	2(22,2)	7(25,9)
Mucosal Neutrophil infiltration	1 - No	6(60,0)	0(0)	4(44,4)	10(37,0)
	2 - Mild	4(40,0)	5(62,5)	4(44,4)	13(48,1)
	3 - Severe	0(0)	3(37,5)	1(11,1)	4(14,8)
Vascular Dilation	1 - No	1(10,0)	0(0)	1(11,1)	2(7,4)
	2 - Mild	8(80,0)	1(12,5)	4(44,4)	13(48,1)
	3 - Severe	1(10,0)	7(87,5)	4(44,4)	12(44,4)
Hyperemia and hemorrhage	1 - No	0(0)	0(0)	1(11,1)	1(3,7)
	2 - Mild	6(60,0)	2(25,0)	4(44,4)	12(44,4)
	3 - Severe	4(40,0)	6(75,0)	4(44,4)	14(51,9)
Erosion and shedding of the mucosal epithelium	1 - No	10(100)	2(25,0)	5(55,6)	17(63,0)
	2 - Mild	0(0)	2(25,0)	3(33,3)	5(18,5)
	3 - Severe	0(0)	4(50,0)	1(11,1)	5(18,5)
Mucosal Ulceration	1 - No	10(100)	5(62,5)	7(77,8)	22(81,5)
	2 - Mild	0(0)	2(25,0)	2(22,2)	4(14,8)
	3 - Severe	0(0)	1(12,5)	0(0)	1(3,7)
Loss of Cilia	1 - No / Focal loss	10(100)	3(37,5)	8(88,9)	21(77,8)
	2 - Diffuse loss	0(0)	5(62,5)	1(11,1)	6(22,2)

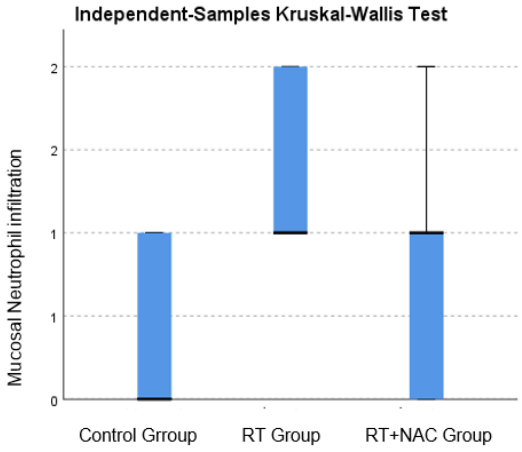
Table 2. Evaluation of pathological parameters in the nasal mucosa.

Variables	G1		G2		G3		p	G1 - G3	G1 - G2	G2 - G3
	M	Min-Max	M	Min-Max	M	Min-Max				
Mucosal mononuclear inflammatory cell infiltration	1	0-1	2	1-2	1	0-2	**0,018**	1,000	**0,016**	0,129
Mucosal Neutrophil infiltration	0	0-1	1	1-2	1	0-2	**0,011**	1,000	**0,010**	0,111
Vascular Dilation	1	1-2	2	1-3	1	0-2	**0,008**	0,588	**0,006**	0,215
Hyperemia and hemorrhage	0	0-0	2	1-2	1	0-2	0,292	0,926	0,149	0,184
Erosion and shedding of the mucosal epithelium	0	0-0	2	0-2	0	0-2	**0,004**	0,247	**0,003**	0,328
Mucosal Ulceration	0	0-0	0	0-2	0	0-1	0,121	0,125	**0,040**	0,433
Loss of Cilia	1	1-1	2	1-2	1	1-2	**0,005**	1,000	**0,006**	**0,038**

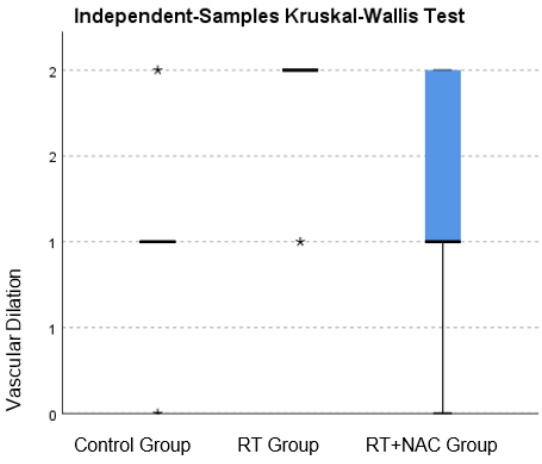
Kruskal-Wallis analysis and Bonferroni correction were applied. p<0.05 is significant. M; Median. G1; Control Group, G2; RT Group, G3; RT+NAC Group

(p=0.215, P > 0.05) (Table 1-2, Figure 3, Graph 3).

There was a significant difference between the groups in terms of epithelial erosion and shedding in the nasal mucosa (p=0.004, P< 0.05). Erosion and shedding of the mucosal epithelium were significantly higher in the RT group than in the control group (p=0.003, P < 0.05), and there was no difference between the control group and the RT+NAC group (p=0.247, P > 0.05). In addition, erosion and shedding of the mucosal epithelium were found to be milder in the RT+NAC group than in the RT group, but the difference was not statistically significant (p=0.328, P >



Graph 2. Mucosal neutrophil infiltration.



Graph 3. Vascular dilation.

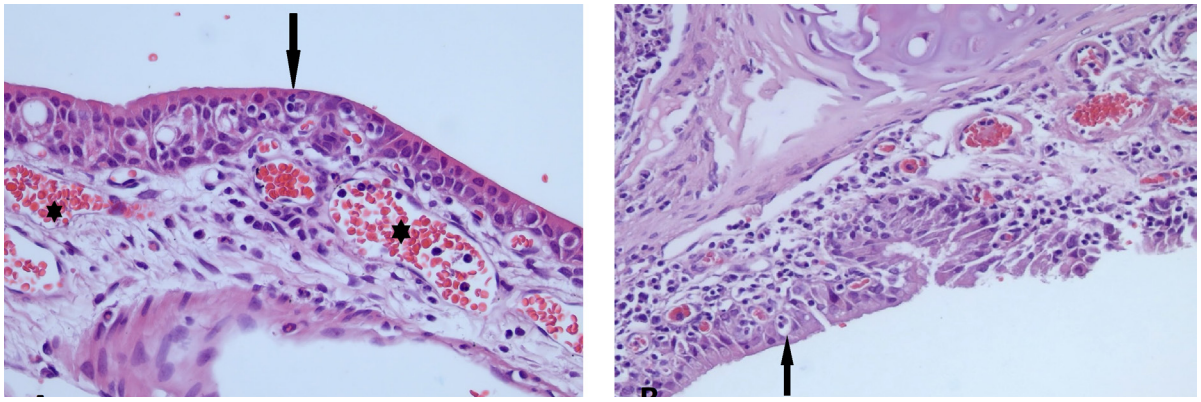


Figure 2. Intraepithelial neutrophils in the nasal mucosa (arrow) and hyperemia (star) in the vessels in the RT group (A). Rare intraepithelial neutrophil (arrow) cell appearance in the nasal mucosa in the RT+NAC group (B).

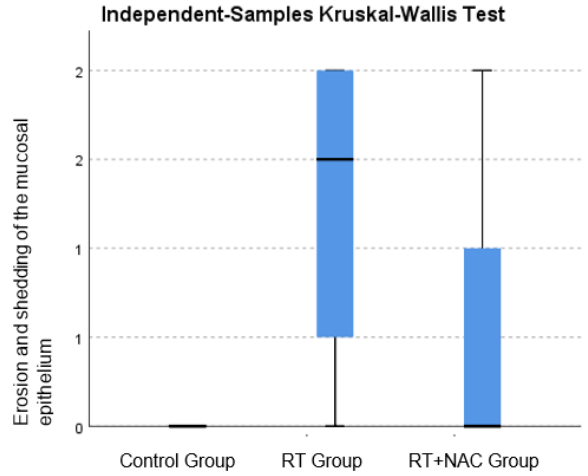
0.05) (Table 1-2, Graph 4).
There was a significant difference between the groups in terms of loss of cilia in the nasal mucosa epithelium (p=0.005, P < 0.05). It was determined that cilia loss was significantly higher in the RT group compared to the control group (p=0.006, P< 0.05), and there was no difference between the control group and the RT+NAC group (p=1.000, P > 0.05). In addition, cilia loss was found to be significantly lower in the RT+NAC group than in the RT group (p=0.038, P< 0.05) (Table 1-2, Figure 4, Graph 5).
Mucosal ulceration, mucosal hyperemia and hemorrhage were found to be more severe in the RT group, but the difference between the groups was not statistically significant (p:0.121, p:0.292, P > 0.05)(Table 1-2, Graph 6-7).

Evaluation of the nasopharynx

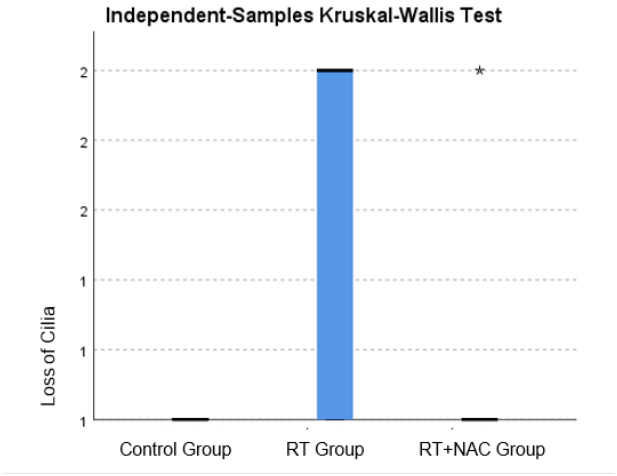
There was a significant difference between the three experimental groups in terms of the extent of sentan staining in the nasopharynx (p=0.002, P < 0.05). In subgroup comparison;
The extent of sentan staining in the RT group was found to be significantly lower than in the control group (p=0.002, P < 0.05), and there was no difference between the control group and the RT+NAC group (p=1.000, P > 0.05). In addition, the extent of sentan staining in the RT+NAC group was found to be significantly higher than in the RT group (p=0.024, P < 0.05) (Table 3-4, Figure 5, Graph 8).
There was a significant difference between the three groups in terms of intensity of staining sentan in the nasopharynx (p < 0.0001). In the subgroup comparison, it was found that the intensity of staining sentan in the RT group was significantly lower than the control group (p<0.0001), and there was no difference between the control group and the RT+NAC group (p=1.000, P > 0.05). In addition, intensity of staining sentan was found to be significantly higher in the RT+NAC group than in the RT group (p=0.001, P < 0.05) (Table 3-4, Figure 6, Graph 9)

Discussion

Radiotherapy is an important and curative method for the treatment of head and neck cancers such as oral cavity, oropharynx, larynx, hypopharynx, nasopharyngeal cancer, nasal and paranasal sinus tumors [2].



Graph 4. Erosion and shedding of the mucosal epithelium.



Graph 5. Loss of cilia.

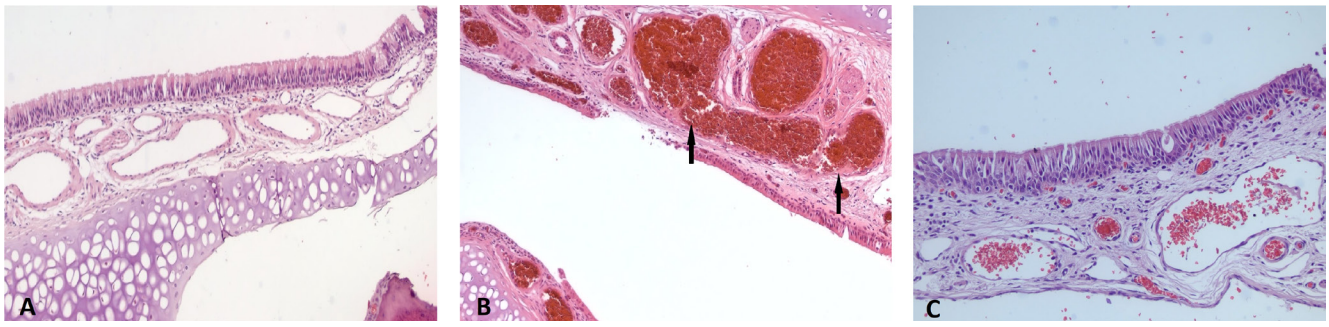


Figure 3. Normal nasal mucosa and vascular structures in the control group (A). Vascular dilatation and severe hyperemia in the nasal mucosa in the RT group (B). Mild vascular dilatation and hyperemia of the nasal mucosa in the RT+NAC group (C).

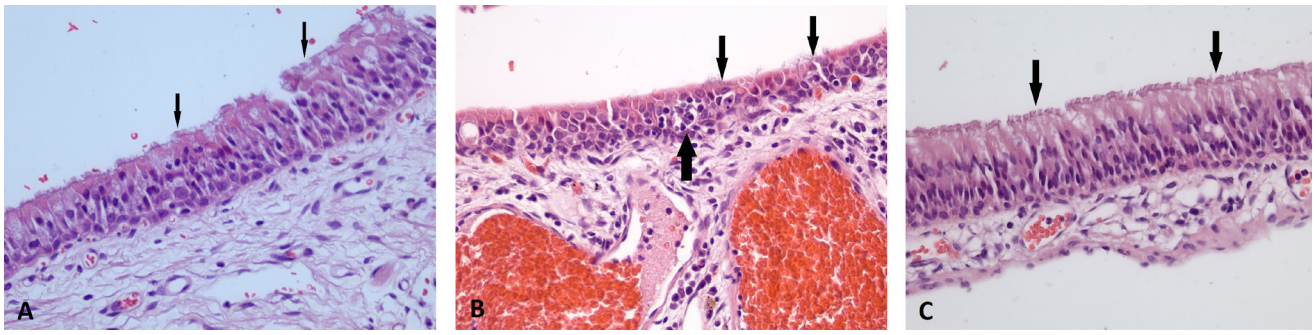
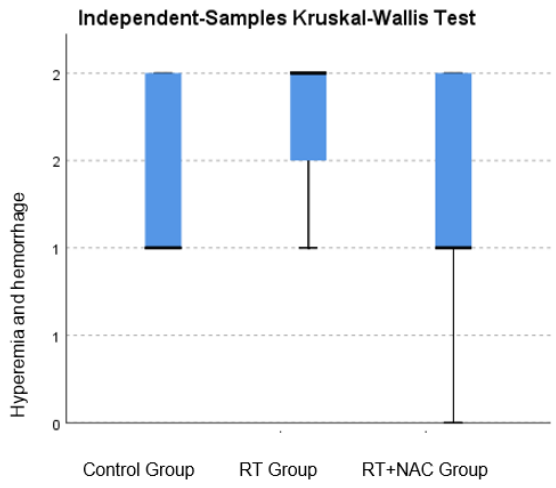


Figure 4. Nasal ciliated columnar epithelium (arrow) in the control group (A). Diffuse cilia loss (B) in the RT group, except for the limited area (thin arrow) in the mucosa containing intraepithelial lymphocytes (thick arrow). Columnar epithelium (arrow) showing cilia continuity in the nasal mucosa in the RT+NAC group (C).

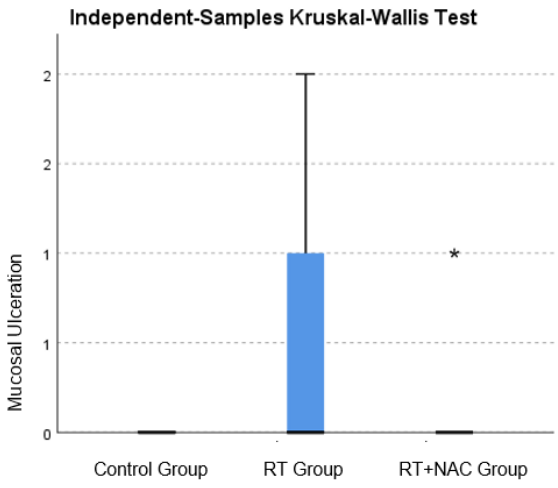
Reactive oxygen species are by-products of oxygen metabolism. Normally, there is a delicate balance between ROS production in the body and tissue concentrations of antioxidants. ROT generation is a known consequence of ionizing radiation therapy. Oxidative stress caused by the imbalance between oxidant and antioxidant states plays an important role in the pathogenesis of radiation-induced side effects [12,21,23].

Mucositis is one of the most common complications of RT [9].

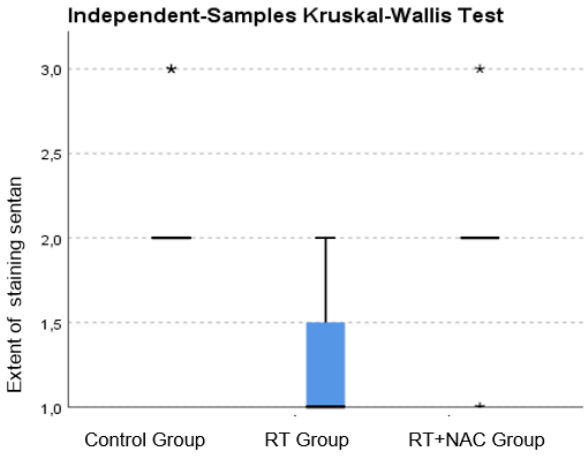
Radiation causes mucositis by causing a progressive process that starts with local tissue damage caused by cytokines such as TNF-α, interleukin-1 and interleukin-6 [24]. However, it causes destruction of cilia in mucosal epithelial cells in the sinonasal region, resulting in impaired mucociliary clearance and an increased risk of developing chronic rhinosinusitis, which is one of the most common side effects [7,8]. Huang et al. found sinus mucosal disease in at least two-thirds of patients receiving RT for



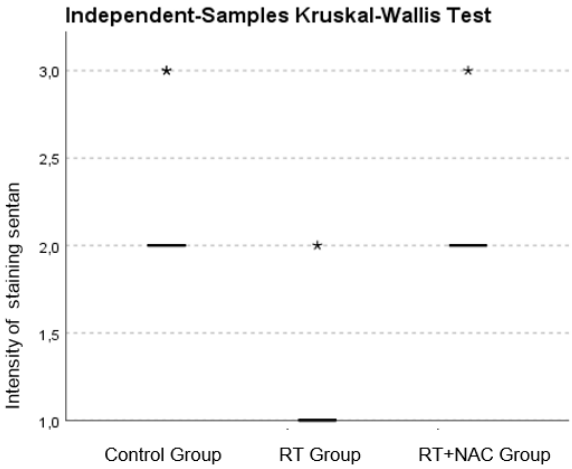
Graph 6. Hyperemia and hemorrhage.



Graph 7. Mucosal ulceration.



Graph 8. Extent of staining sentan.



Graph 9. Intensity of staining sentan.

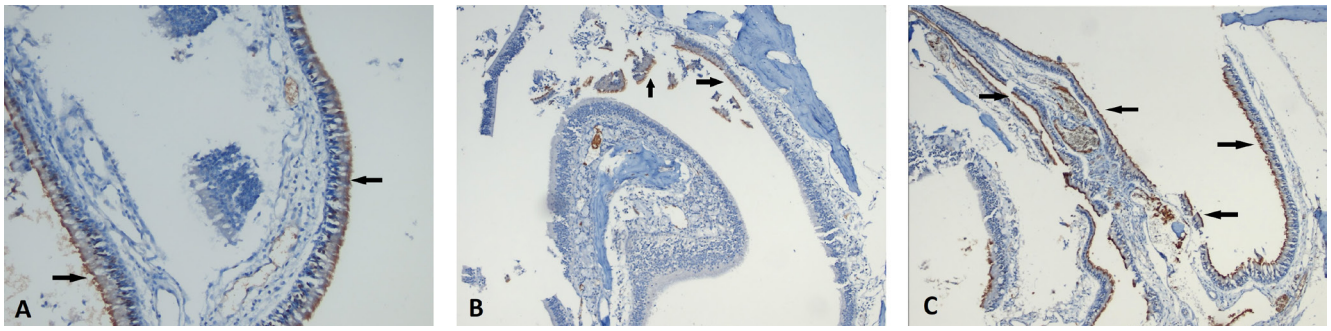


Figure 5. Widespread sentan positivity in the nasopharynx in the control group (arrow). Immunohistochemistry x100 (A). Sentan positivity in the focal area of the ciliated epithelium in the nasopharynx in the RT group (arrow). Immunohistochemistry x100 (B). Widespread and very strong Sentan positivity (arrow) along the ciliated epithelium in the nasopharynx in the RT+NAC group. Immunohistochemistry x100 (C).

nasopharyngeal carcinoma [25]. The treatment of rhinosinusitis, which is a common problem after RT, is controversial. Endoscopic sinus surgery can be performed for excision of the sinechial bands, drainage and ventilation of the sinuses, and correction of choanal stenosis. Some improvement in nasal symptoms has been reported in patients with surgical treatment, but the long-term effect of surgery remains unclear, although there is a risk of poor wound healing and intraoperative bleeding. There is no

Table 3. Distribution of Sentane evaluations in the nasopharynx between groups

Variables	Grade	Groups			
		Control Group	RT Group	RT+NAC Group	Total
		n (%)			
Extent of staining sentan	Low rate of staining < 10%	0(0)	6(75,0)	1(11,1)	7(25,9)
	Moderate rate of staining 11-50%	8(80,0)	2(25,0)	7(77,8)	17(63,0)
	High rate of Staining > 50%	2(20,0)	0(0)	1(11,1)	3(11,1)
Intensity of staining sentan	Low intensity of staining	0(0)	7(87,5)	0(0)	7(25,9)
	Moderate of intensity staining	8(80,0)	1(12,5)	8(88,9)	17(63,0)
	Severe intensity of staining	2(20,0)	0(0)	1(11,1)	3(11,1)

Table 4. Evaluation of Sentan level in the nasopharynx between groups.

Variables	G1		G2		G3		p	G1 - G3	G1 - G2	G2 - G3
	M	Min-Max	M	Min-Max	M	Min-Max				
Extent of staining sentan	2	2-3	1	1-2	2	1-3	**0,002**	1	**0,002**	**0,024**
Intensity of staining sentan	2	2-3	1	1-2	2	2-3	**<0,0001**	1	**<0,0001**	**0,001**

Kruskal-Wallis analysis and Bonferroni correction were applied. p<0.05 is significant.M; Median. G1; Control Group, G2; RT Group, G3; RT+NAC Group.

accepted effective medical or surgical treatment [4,13,26].

N-acetylcysteine, the precursor of glutathione, is an agent used especially as a mucolytic and as an antidote in acetaminophen intoxications. It has a role in the detoxification of reactive oxygen radicals through glutathione, the detoxification of electrophilic xenobiotics, the storage and transport of cysteine, the regulation of cell proliferation, and the regulation of leukotriene and prostaglandin synthesis [15]. In addition, NAC inhibits ROS-induced apoptosis by decreasing caspase-3 expression and thus has a protective effect against free radical damage, and also reduces local inflammatory and TGF-β levels by reducing interleukin-1b, interleukin-8, TNF-α, NF-kB and TGF-β levels. has been shown to inhibit the fibrotic response [16,17]. In our study, we investigated the effectiveness of systemic NAC application in the treatment of nasal mucositis after radiotherapy, taking into account the anti-inflammatory, antioxidant and antifibrotic properties of NAC.

Riva et al. evaluated the late effects of irradiation in patients receiving RT. They found that neutrophilic infiltration was significantly higher in the nasal cytological evaluation of patients exposed to radiation compared to the healthy group [7,27]. In the study of Çanakçı et al. also found that inflammatory cell infiltration was higher in the RT group than in the control group in their study in which they investigated the effectiveness of topically applied black seed oil for nasal mucositis caused by radiation [12]. In our study, similar to the studies in the literature, nasal mucosal mononuclear cell and neutrophil infiltration were found to be higher in the RT group compared to the control group. NAC treatment group and control group were found to be similar. It was observed that NAC treatment could be effective in reducing inflammation in the nasal mucosa.

In the study in which topical nigella sativa was applied, superficial erosion in the nasal mucosa was found to be high in the RT group and low in the treatment group, but no difference was found between the groups in terms of vascular dilatation. In our study, we found that the nasal mucosal epithelial erosion was higher in the RT group compared to the control group, and that the treatment group and the control group were similar. However, unlike the previous study, we found vascular dilatation in the RT group to be significantly higher than in the control group, and similar in the RT+NAC group and the control group in our study [12]. According to these data, it was seen that NAC could be effective in reducing nasal mucosal erosion and vascular dilatation.

We also evaluated nasal mucosal hyperemia and hemorrhage and mucosal ulceration in order to approach the subject more comprehensively and to evaluate nasal mucositis better. Although mucosal ulceration, mucosal hyperemia and hemorrhage were found to be more severe in the RT group, the difference between the study groups was not statistically significant.

The respiratory tract is lined by pseudostratified ciliated cylindrical epithelium. Cilia are the building blocks of the MCC mechanism. The MCC mechanism has an important role in the clearance and defense of the upper respiratory tract. This mechanism filters inhaled particles through the nasal mucosa layer, ensuring that excess mucus and inhaled foreign materials

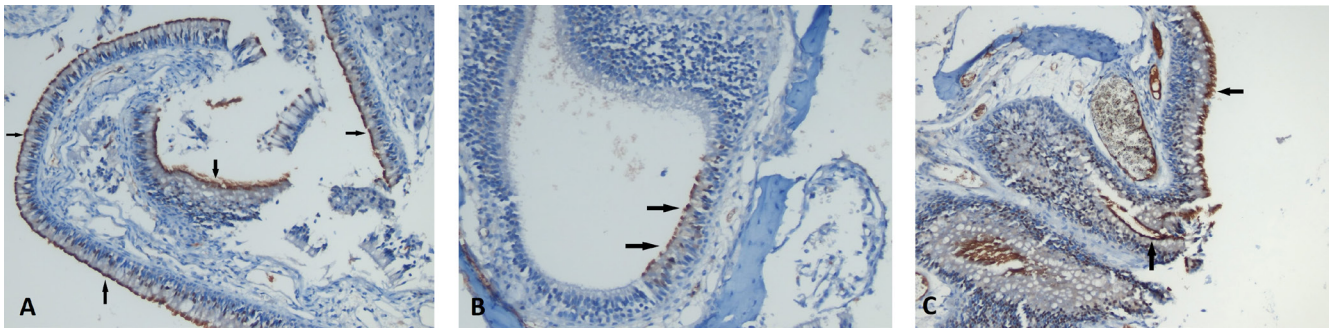


Figure 6. Severe Sentane positivity (arrow) in the ciliated epithelium of the nasopharynx in the control group. Immunohistochemistry x100 (A). Weak Sentane positivity in the ciliated epithelium in the nasopharynx in the RT group (arrow). Immunohistochemistry x100 (B). Very strong (dense) Sentane positivity (arrow) in the ciliated epithelium of the nasopharynx in the RT+NAC group. Immunohistochemistry x100 (C).

are cleared from the airways. Nasal MCC system injury plays an important role in nasal complications after RT [9,10,28]. Rhinosinusitis after radiation is believed to result from impaired mucociliary function. Disruption of mucociliary function causes deterioration of sinus drainage, making it easier for bacteria to colonize the sinuses. Studies have shown that mucociliary clearance is significantly impaired after radiotherapy applied to the head and neck region [8,10,11,28]. Kamel et al. found that after RT in patients with nasopharyngeal cancer, MCC progressively worsened over time up to 6 months, then stabilized and became permanent [9]. In another clinical study, it was emphasized that epithelial damage such as nasal mucosal crusting, loss of cilia, and ciliary dysmorphism persisted for a long time in patients undergoing RT for nasopharyngeal cancer [10]. In previous studies, it was stated that radiation therapy causes ciliary loss and dysmorphism. Lou et al. demonstrated in their study that ciliary loss, ciliary dysmorphism, and impaired mucociliary function persisted in the infundibular epithelium even many years after RT [4,9-11]. In our study, similar to the literature, RT was found to cause cilia loss. However, it was observed that NAC treatment significantly inhibited cilia loss. Since MCC starts in the nasal mucosa and continues towards the nasopharynx, the cilia structure in the nasopharyngeal mucosa plays an active role in this mechanism. Cilia are composed of 9+2 axoneme structures, consisting of 9 pairs of peripheral microtubules arranged around 2 central microtubules. Peripheral microtubule pairs each consist of A and B tubules. In the extreme apical part of the cilium, the B subunit and other structures are absent, and only one tubule is present [29]. It is located in the distal type region of the sentan motile cilia. By binding to phosphatidylserine, it connects the cell membrane with peripheral single microtubules (A-tubules) in the constricted distal part of the cilia. It has been found to make the distal part of the cilia narrow and stiff to provide better airway clearance [30,31]. It has been reported in the literature that the syntan protein is highly expressed in normal nasopharyngeal cilia [32]. Based on this information, we evaluated the cilia structure in the nasopharynx using immunohistochemical methods and examined the extent and intensity of sentan, the cilia apical protein.

There are very few studies on syntan in the literature. In one study, it was stated that syntan was decreased in nasopharyngeal cancer, but more studies are needed to establish its exact relationship with oncogenesis [32]. In another study, it was emphasized that syntan was expressed in a decreased manner in the ciliary structures of the nasal epithelium after exposure to trichloroacetic acid [33]. We also examined immunohistochemically the change in the extent and intensity of the staining of the cilia in the nasopharynx epithelium as a result of radiation exposure. We found that the extent and intensity of syntane staining was significantly reduced in the RT group. We found that the extent and intensity of syntane staining was similarly high in the control group and the group receiving NAC treatment. These findings showed that systemic NAC therapy is effective in preventing cilia loss in the nasopharynx due to radiotherapy. When we looked at the results of our research, we saw that NAC treatment prevented radiation-induced ciliary loss in the epithelium and protected cilia apical proteins from radiation. In the light of these findings, we predict that NAC can prevent the harmful effects of radiation on the mucociliary system and improve mucociliary clearance. In addition, we suggest investigating the role of syntane in other acquired and congenital ciliary diseases and investigating whether treatments that can increase the level of syntane can be beneficial in combating these diseases. Our research is an experimental study that comprehensively examines the effects of radiation on the nasal mucosa. In addition, it is the first study to immunohistochemically examine the apical protein of epithelial cilia, which is an important building block of the mucociliary system, due to radiation exposure. In addition, it is the first study in which NAC therapy was applied to reverse the harmful effects of radiotherapy on the nasal mucosa. Our study needs to be supported by clinical studies in which electron microscopic examination is carried out and its effects on humans are investigated.

Conclusion

Sinonasal mucosal disorders caused by radiotherapy and chronic rhinosinusitis caused by deterioration in the mucociliary system seriously affect the quality of life of patients, and there is no accepted effective method for its treatment. It is known that cell death, production of reactive oxygen species, changes in gene expression and expression of both proinflammatory and profibrotic cytokines are involved in the pathogenesis of normal tissue damage after RT. Considering the antioxidant, anti-inflammatory and antifibrotic effects of NAC, our findings show that NAC treatment can be effective in reversing the harmful effects of RT on the nasal mucosa. Our study is the first to investigate the effects of NAC in the treatment of radiation-induced nasal mucositis. It is also the first study to perform immunohistochemical evaluation on nasal mucositis caused by radiation. Our in vivo study will guide future clinical studies on this topic. Our study needs to be supported by clinical studies with large groups of subjects.

Authorship contribution

NKÇ contributed to every stage of the study. SKÖ contributed to the planning and writing of the study. İP contributed to the realization of the experiment. AS contributed to the pathological examination of the samples. GY and EA contributed to the administration of radiotherapy to rats. HSD contributed to the writing of the study.

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Conflict of interest

The authors of this article have no conflicts of interest regarding the study.

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