Original Article / Özgün Araştırma

Evaluation of Saliva Melatonin Levels in Head-neck Radiotherapy Patients

Baş-boyun Radyoterapi Hastalarında Tükürük Melatonin Düzeylerinin Değerlendirilmesi

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Abstract

Objective: Melatonin (MT), which performs many missions such as the activation of the immune system, regulation of the body's circadian rhythm and body temperature, is a powerful antioxidant. This study investigated the impact of radiation on the saliva MT level in individuals with head and neck cancer.

Materials and Methods: Sixteen patients receiving head and neck radiotherapy were included in the study. The MT levels were measured in salivary samples taken from these patients before and after radiotherapy and in salivary samples taken from thirty healthy individuals who had no radiotherapy. Paired t-test and Student's t-test were used in the analysis of the data obtained.

Results: When the saliva MT levels were compared before and after radiotherapy in the patient group, although there was a decrease after radiotherapy, there was not found a statistically significant difference ($p>0.05$). Additionally, although the saliva MT levels before and after radiotherapy were determined to be lower than the control group, there was no significant ($p>0.05$).

Conclusion: Although there was no significant difference, the important decrease in saliva MT levels after radiotherapy in the patient group will enable us to understand more clearly the effect of ionizing radiation on saliva MT level.

Keywords
Head and neck cancer, melatonin, radiotherapy, saliva

Anahtar Kelimeler
Baş ve boyun kanserleri, melatonin, radyoterapi, tükürük

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Öz

Amaç: Bağışıklık sisteminin aktivasyonu, vücudun sirkadiyen ritminin ve vücut ısısının düzenlenmesi gibi birçok görevi yerine getiren melatonin güçlü bir antioksidandır. Bu araştırma baş ve boyun kanserli bireylerde radyasyonun tükürük melatonin seviyesine etkisini araştırmayı amaçladı.

Gereç ve Yöntemler: Çalışmaya baş ve boyun radyoterapisi uygulanan on altı hasta dahil edildi. Bu hastalardan radyoterapi öncesi ve sonrası alınan tükürük örneklerinde ve radyoterapi almamış otuz sağlıklı bireyin tükürük örneklerinde melatonin seviyeleri ölçüldü. Elde edilen verilerin analizinde paired t-testi ve Student's t-testi kullanıldı.

Bulgular: Hasta grubunda radyoterapi öncesi ve sonrası tükürük melatonin seviyeleri karşılaştırıldığında, radyoterapi sonrası azalma görülse de istatistiksel olarak anlamlı bir fark bulunmadı ($p>0,05$). Ayrıca, radyoterapi öncesi ve sonrası tükürük melatonin seviyeleri kontrol grubundan daha düşük bulunmasına rağmen anlamlı değildi ($p>0,05$).

Sonuç: Anlamlı bir fark olmamasına rağmen, hasta grubunda radyoterapi sonrası tükürük melatonin seviyesinde görülen belirgin azalma, iyonize radyasyonun tükürük melatonin seviyesine etkisini daha net anlamamızı sağlayacaktır.

Introduction

Head and neck cancers constitute approximately 3 to 5% of all cancer, and radiotherapy and/or chemotherapy are extensively used in the treatment (1,2). Radiotherapy relies on the ability of radiation to destroy tumor cells and has a considerable place in the treatment of head and neck cancers (3).

Ionizing radiation, with the toxic effect of reactive oxygen species (ROS) that it causes to increase, can cause tissue damage through multiple mechanisms such as cell membrane, DNA, RNA and protein damage, lipid peroxidation and enzyme oxidation (4-7). ROS are free molecules that occur during the conversion of nutrients to energy using oxygen, and it has been reported that these molecules have an important place in carcinogenesis (8). Antioxidant defense systems evolved to control ROS production and to get ahead of the harmful effects of ROS in the body. Antioxidants protect healthy cells against the harmful effects of radiation by various enzymatic (such as glutathione peroxidase, glutathione reductase, and superoxide dismutase) and non-enzymatic [such as glutathione, melatonin (MT), vitamins, selenium, uric acid, beta carotene, and alpha-tocopherol] systems (5,8-11).

In particular, MT is a powerful ROS cleansing antioxidant, which is secreted from foremost the pineal gland (epiphysis), lens, bone marrow, gallbladder, and gastrointestinal tract (12,13). It stimulates the circadian rhythm of the body, arrangement of body temperature, activation of the immune system, cell proliferation of osteoblasts, type I collagen synthesis and bone formation (12-14). It has anti-inflammatory and anti-tumoral effects due to its immunomodulating effect (12). It also prevents oxidative stress by stimulating some antioxidative enzymes or detoxifying free radicals (12,13).

In patients receiving head and neck radiotherapy, it is predicted that may occur changes in the salivary glands as well as changes in the quality and biochemical content of saliva. These biochemical changes are related to the mechanism of radiation's effect in the body, and it is very important in terms of patients' quality of life. The objective of our study was to investigate the essence of the saliva MT level by detecting biochemical changes in saliva before and after radiotherapy in individuals with head and neck cancer.

Materials and Methods

Study Design

In the present study, it was planned to investigate the level of MT in saliva samples that will be collected from head and neck radiotherapy patients admitted to Atatürk University Faculty of Medicine and healthy individuals. After all planning, by taking the informed consent form obeying the Declaration of Helsinki with the decision of Atatürk University Faculty of Dentistry Ethics Committee (decision number: 54, date: 21.06.2017), the saliva samples were taken from the patients who will receive radiotherapy before and after treatment and the healthy individuals. The data obtained as a result of biochemical analysis of saliva samples were compared statistically.

Patients

The patients who will receive head and neck radiotherapy in the Department of Radiation Oncology were referred to the Atatürk University Faculty of Dentistry to eliminate infection foci and to improve oral hygiene before radiotherapy. Sixteen head and neck cancer patients who had not been treated for cancer before and who did not have any periodontal and systemic problems that could lead to changes in saliva content were included in this study. Eight (50%) of patients diagnosed the head-neck cancer were laryngeal cancer, 3 (18.75%) lymphoma, 1 (6.25%) non-Hodgkin lymphoma, 1 (6.25%) nasopharyngeal cancer, 1 (6.25%) lower lip cancer, 1 (6.25%) maxillary sinus tumor, 1 (6.25%) mucoepidermoid carcinoma. The total fraction number of radiotherapy applied to the patients was between 13-36 days, the daily radiation dose was between 1.8-3 Gray (Gy), the total radiation dose was between 30-70 Gy, and no individuals received chemotherapy.

Also, thirty healthy individuals who consulted at Atatürk University Faculty of Dentistry and did not receive radiotherapy were included in the control group.

Saliva Samples

After all individuals in the study rinse their mouths with water in a quiet room, unstimulated saliva samples of these individuals were taken into Eppendorf tubes. In the patient group, saliva samples were taken before radiotherapy and in the second month after the start of radiotherapy. All samples were stored in a refrigerator at -80 °C.

Laboratory Analysis

The MT level of samples were analyzed by Human Enzyme-Linked Immunosorbent Assay Kit for MT, (Catalog No. CEA908Ge, Wuhan USCN Business Co., Ltd., USA) in Atatürk University Faculty of Medicine Biochemistry Laboratories.

Statistical Analysis

The data obtained after biochemical analysis were analyzed by SPSS (SPSS 25.0, SPSS Inc., IBM Corp., Chicago, IL) software program. While paired t-test was used to compare the measurements before and after radiotherapy, Student's t-test was used to compare the measurements before and after radiotherapy with the control group.

Results

The distribution of the control and patient groups by gender is shown in Table 1. While the mean age of the control group was 41.66±16.44, it was 50.68±13.51 in the patient group.

When the saliva MT levels (1252.83±437.8 pg/mL, 1003.04±543.94 pg/mL, respectively) were compared before and after radiotherapy in the patient group, although there was a decrease after radiotherapy, there was not found a statistically significant difference as shown in Table 2. When the saliva MT levels of the patient group were compared with the control group, although mean saliva MT levels before and after radiotherapy (1252.83±437.8 pg/mL, 1003.04±543.94 pg/mL, respectively) were determined to be lower than the control group (1314.5±504.2 pg/mL), these differences were no statistically significant as shown in Table 3 and Table 4.

Table 1. Distribution of the control and patient groups by gender

	Control group		Patient group	
	n	%	n	%
Male	18	60	14	87.5
Female	12	40	2	12.5

Discussion

Head and neck radiotherapy causes not only loss of function in major salivary glands but therewithal changes in the saliva flow ratio, quantity, viscosity, pH, buffering capacity, and biochemical parameters such as some enzyme levels, oxidant-antioxidant balance (15,16). It is believed that these changes in the salivary glands are mediated by radiation-induced free radicals and ROS (17).

The effects of radiotherapy on oxidant and antioxidant mechanisms are still controversial today. Khalil Arjmandi et al. (5), in their study on serum antioxidant/oxidant balance before and after radiotherapy, observed a statistically significant decrease in serum Selenium concentration, total antioxidant status (TAS), Superoxide Dismutase levels and Glutathione Peroxidase activity ($p<0.05$). Shariff et al. (7) also determined lower serum TAS levels before treatment in patients with head and neck malignancy than the control group and found this difference statistically significant. In the same study, they also observed a decrease in serum TAS levels after radiotherapy in the patient group compared to before treatment and associated this with increased oxidative stress. In contrast, Babiuch et al. (18) reported that TAS levels were higher in the patients with premalignant/malignant lesions. It has been suggested that this situation may be related to the

Table 2. Statistical comparisons of salivary melatonin levels before and after radiotherapy in the patient group

	n	Before radiotherapy	After radiotherapy	t	p-value
		Mean ± SD	Mean ± SD		
Melatonin (pg/mL)	16	1252.83±437.8	1003.04±543.94	1.540	0.144*

*Paired t-test, $p>0.05$, SD: Standard deviation

Table 3. Statistical comparison of salivary melatonin levels before radiotherapy and the control group

	Control group		Before radiotherapy		t	p-value
	n	Mean ± SD	n	Mean ± SD		
Melatonin (pg/mL)	30	1314.5±504.2	16	1252.83±437.8	0.413	0.682*

*Student's t-test, $p>0.05$, SD: Standard deviation

Table 4. Statistical comparison of salivary melatonin levels after radiotherapy and the control group

	Control group		After radiotherapy		t	p-value
	n	Mean ± SD	n	Mean ± SD		
Melatonin (pg/mL)	30	1314.5±504.2	16	1003.04±543.94	1.942	0.059*

*Student's t-test, p>0.05, SD: Standard deviation

increased activity of antioxidant defense systems in response to oxidative stress.

Although there are many studies on total antioxidant capacity in the literature, there is a limited number of studies on MT, one of the most important antioxidants. These studies are related either to the relation of MT with type II diabetes (12) and periodontal diseases (13,19,20) or to its therapeutic effect. Whereas, MT is today considered not only as a hormone but also as an important cell protector (17). It is important for oral defense because of its anti-inflammatory effects in the oral cavity and more effective than conventional antioxidants in reducing oxidative stress (21,22). In this context, our study will have an important place in contributing to the determination of the relationship between MT and radiotherapy.

In studies, it is shown that approximately 24-33% of active plasma MT is found in saliva (14,23). Therefore, in the evaluation of MT levels, it is recommended that the saliva samples, which are one of the low-cost and non-invasive methods, have recently been preferred to blood samples (14,24). Our study was planned on saliva samples because of to be more easily accessible and to be considered sufficient in the evaluation of the MT level.

In the present study, although there is no statistically significant difference, the decrease in saliva MT levels after radiotherapy may be attributed to ROS toxicity and oxidative stress resulting from ionizing radiation. Also, mitochondrial and metabolic dysfunctions and mutations in damaged cancer cells significantly increase ROS formation (25). In our study, the low levels of saliva MT before and after radiotherapy compared to the control group may also be related to the cancer development mechanism and tissue damage. In this context, saliva MT measurement can be considered as an assistant parameter in the evaluation of head-neck malignancies.

The limitation of the present study is the inability of patient follow-up for a long time because of time constraints. Patient follow-up is recommended to identify changes and complications that may occur in a long time after radiotherapy. Additionally, the study population remained small due to both the capacity of the oncology unit and the time constraints.

Conclusion

In conclusion, although there is no statistically significant difference in the present study, we can say that the decrease in the saliva MT level in head and neck malignancy patients is exacerbated by the effect of radiation, based on the marginal difference between groups. This too will enable us to understand clearly the effect of ionizing radiation on the saliva MT level. Although it is estimated that some biochemical changes occur in saliva with the effect of radiation, there are not many studies in the literature on MT levels in head and neck cancer patients. The present study will contribute to the data obtained in this respect. Also, a choice of saliva for examining the MT level will be an advantage in terms of both cost and sample collection compared to the commonly used blood tissue.

Ethics

Ethics Committee Approval: After all planning, by taking the informed consent form obeying the Declaration of Helsinki with the decision of Atatürk University Faculty of Dentistry Ethics Committee (decision number: 54, date: 21.06.2017).

Informed Consent: Informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.A., N.A., Concept: G.A., N.A., Design: G.A., N.A., Data Collection or Processing: G.A., Analysis or Interpretation: G.A., N.A., Literature Search: G.A., Writing: G.A., N.A.

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