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Salivary tumor necrosis factor-α and interleukin-6 in patients with head and neck cancer before and after radiotherapy

Mohammed H. Alburgaiba [1], Fawaz D. Al-Aswad [2], Haider N. Salh[3]
[1]B.D.S. MSc. Oral medicine, PhD. student in college of Dentistry-University of Baghdad/ Assistant lecturer in college of dentistry-University of Alkafeel.
[2] B.D.S. MSc. PhD. Oral medicine, MFDS. RCPS Glasgow, Professor in college of Dentistry-University of Baghdad.
[3] Haider N. Salh MBChB. DM. RT, Clinical oncologist in Middle Euphrates oncology center

ABSTRACT
Head and neck cancer (HNC) means a heterogeneous group of cancers in multiple sites of head and neck structures, including oral and nasal cavity, paranasal sinuses, larynx, pharynx and salivary glands. Radiotherapy (RT) is one of the fundamental treatment options for malignancy and widely used as HNC treatment strategy. Salivary cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) have wide biological activities like regulating inflammatory and immunological events. Aim of the study is to assess the level of salivary TNF-α and IL-6 in HNC patients before and after two techniques of head and neck radiotherapy. The study included thirty healthy individuals as control group and two patients' groups with HNC: the first group consist of 30 patients treated by three-dimensional conformal radiation therapy (3DCRT) and the second group also consist of the same patient's number but treated by intensity-modulated radiation therapy (IMRT). The level of both cytokines are significantly higher in patients before RT than in control group.

The study shows significant elevation in levels of salivary TNF-α and IL-6 after finishing RT. The salivary IL-6 and TNF-α are high in patients with HNC than in healthy individuals, so they can be used as biomarkers for early detection of HNC.

Keywords: Head and neck cancer; radiotherapy; saliva; tumor necrosis factor-α; interleukin-6

Correspondance:
Mohammed H. Alburgaiba
college of Dentistry,
University of Baghdad,
Baghdad,
Email id : Alburgaiba12@gmail.com

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INTRODUCTION

Cancer is a wide term describes the illness that outcomes when abnormal cell changes lead to the uncontrolled cells division and growth. (1)

Head and neck cancer (HNC) means a heterogeneous group of cancers in multiple sites of head and neck structures (2) , including oral and nasal cavity, paranasal sinuses, larynx, pharynx and salivary glands. (3, 4)

Rischin et al., (5) and Miller et al., (4) reported that more than ninety percent of HNC cases are head and neck squamous cell carcinoma(HNSCC) which is epithelial in origin.

Saliva

Saliva is a clear, mucoserous fluid produced in the mouth by three pairs of major and about 450-800 minor salivary glands. The term whole saliva is mean the mixture consist of saliva, mucosal surface transudations, gingival crevicular fluid, desquamated epithelium, expectorated respiratory secretions, bacteria, viruses and fungi. (6) Whole saliva determines the environment of the oral structures. (7)

The saliva can be used as a biomarker fluid since it contains more than 2000 proteins, about 20 – 30% of them shared with serum, according to the above mentioned fact the benefit of using saliva as diagnostic tool because it can be obtained repeatedly and noninvasively. (8, 9)

Cytokines

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine with wide biological activities. It has cytostatic and cytotoxic effects on some cancerous cells, furthermore TNF-α influences many functions in most of cells like, differentiation, and/or the function of virtually every cell type investigated. Moreover, TNF-α is thought to be part of an integral network of interactive signals that orchestrate inflammatory and immunological events. (10, 11)

Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of biological functions in immunity, inflammatory response, hematopoiesis and also oncogenesis (12), IL-6 also regulates regenerative, metabolic and neural processes (13) , the level of salivary IL-6 levels is likely reflect the activity of oral immunity. (14)

Radiotherapy

Radiotherapy (RT), also known as radiation therapy, is a treatment option based on the use of high energy rays or radioactive materials, to damage cancerous cells and to stop cellular growth and division. (15)

According to the above fact RT is one of the fundamental treatment options for malignancy and widely used as cancer treatment strategy, more than half of patients with solid malignancy will receive radiation as part of their treatment plan. (16)

The three dimensional conformal radiation therapy (3DCRT) planning depends on computed tomography imaging which facilitates accurate localization of the tumor shape and size so minimize radiation dose about 50% to surroundings normal tissues, this aim to decrease late hazards. (17, 18)

Intensity-modulated radiation therapy(IMRT) is an enhanced model of high-precision RT that used linear accelerator controlled by computer to deliver very precise radiation doses to the tumor growth or to specific areas inside the tumor tissue and now it's widely used to treat HNC. (19) This specific radiation dose distributions which acquired by IMRT could enhance the rates of tumor control, especially those cancers in the nasopharynx and sinonasal structures since it permits the delivery of high radiation doses to the lesion which is in intimate relation to critical organs and tissues without exceeding the limit of normal tissue tolerance. (20)

MATERIALS AND METHOD

The study included two patients groups with HNSCC: the first group consist of 30 patients treated by 3DCRT: and the second

group also consist of the same patient's number but treated by IMRT.

The total 60 patients were 34 males and 26 females, aged between 19-81 years with mean± standard deviation(SD) was (56.3±14.5) years, diagnosed with HNSCC and treated by their oncologist as shown in(figure 1) at middle Euphrates oncology center/ Najaf-Iraq for the period between April 2018 to June 2019 as shown in table 1.

The treatment period for was continued for all patients between five to seven weeks scheduled as five days every week and the daily fraction was (1.8 - 2.2) gray. The mean dose of whole radiation for the first group treated by 3DCRT was (66.1±5.2) gray, and the radiation dosage was (64.9±5.3) gray for the second patients group treated by IMRT.

The control group was 30 persons (17 males and 13 females), they were looking healthy without any signs or symptoms of systemic diseases, as can as possible match the patients groups in gender and age, with mean ±SD of age was (54.3±14.4) years.

The patients instructed to tilt their heads forward and spit into graduated plastic jar for 10 minutes. The unstimulated saliva was collected from patients at two timing, the first was a half hour before the first session of radiotherapy and the second timing was half hour after the last session of RT, all samples was collected at morning to minimize daily variation, also the patients instructed to don't eat, drink or use any gum one hour before sample collection. (21, 22, 23)

Table 1: display age, gender and tumor site of patients.

Variable		Value		
Patients groups		First group of patients *	Second group of patients **	Total
Gender	Male	18	16	34
	Female	12	14	26
Age in years	Mean± Standard deviation	54.8± 16.2	57.7± 12.8	56.3± 14.5
Tumor location	Oral cavity	9	7	16
	Oropharynx	6	4	10
	Nasopharynx	5	9	14
	Larynx	4	3	7
	Hypopharynx	3	1	4
	Nasal cavity	1	3	4
	Paranasal sinuses	2	3	5

*= Patients treated by 3DCRT, **= Patients treated by IMRT

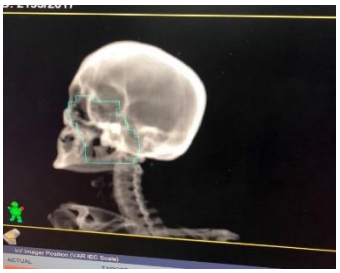

Figure 1: patient with HNSCC receiving radiotherapy.

The saliva stored temporarily in ice box, then centrifuged at 4000 round per minute for ten minutes, after that the supernatant was aspirated and stored at -30 C until the time of analysis by enzyme-linked immunosorbent assay (ELISA).

All patients were informed about the purpose and goal of this study, hazards and benefits and timing of procedures and after their acceptance in participation, they asked to sign in informed consent.

RESULTS

The table(2) demonstrates salivary TNF-α level (mean ±SD) in pg/ml for control subjects (5.4±1.9), and patients pre-radiotherapy (6.3±2.2), which is significantly higher than control group with (P-value=0.04).

In the first patients group, the (mean ±SD) of salivary TNF-α after 3DCRT is (10±4.5) which shows highly significant increase when compared to its level(6.3±2.5) before the starting of first session of radiotherapy. Similar results in the second group of patients, also there is highly significant increase in TNF-α after IMRT (10.5±5) in comparison to its original level (6.3±1.9) before starting the treatment with (P-value=0.001).

There is no difference statically between patients treated by 3DCRT and second group treated by IMRT (P-value=0.69).

Table 2: presents the mean ± SD of salivary TNF-α with p-value in patient pre and post radiotherapy.

Marker	Groups	Number	Mean ± SD	Range	P-value
Salivary TNF-α (pg/ml)	Control	30	5.4±1.9	2.9-9.9	0.04 S
	Total patients pre-radiotherapy	60	6.3±2.2	3.2-13.8	
	Patients pre-3DCRT	30	6.3±2.5	3.2-13.8	0.001 HS
	Patients post 3DCRT	30	10±4.5	5.3-20	
	Patients pre-IMRT	30	6.3±1.9	4.8-13.5	0.001 HS
	Patients post-IMRT	30	10.5±5	5.5-23.1	
	Patients post-3DCRT	30	10±4.5	5.3-20	0.69 NS
	Patients post-IMRT	30	10.5±5	5.5-23.1	

S=Significant, HS=highly significant, NS=Non-significant.

The level of salivary IL-6 in HNSCC patients (24±10.4) is significantly higher than its level in control group (20±7.7), with P-value (0.03) as shown in table 3.

There is statically significant elevation (P-value=0.04) of IL-6 level in first patients group after finishing 3DCRT (30.7±11.3) when compared to this cytokine level (25.2±9.7) before starting treatment. Similar elevation is recorded in this study in second group of patients, as the salivary IL-6 before IMRT (23.5±11.2) and increased till reach to (31.7±15.5) after the last day of RT with P-value (0.023).

In comparison between the levels of IL-6 in both treatment groups, there is no difference statically with P-value=0.79.

Table 3: presents the mean ± SD of salivary IL-6 with p-value in patient pre and post radiotherapy.

Marker	Groups	Number	Mean ± SD	Range	P-value
Salivary IL-6 (pg/ml)	Control	30	20±7.7	9.6-39	0.03 S
	Total patients pre-radiotherapy	60	24.4±10.4	5.5-48	
	Patients pre-3DCRT	30	25.2±9.7	9.5-45	0.04 S
	Patients post-3DCRT	30	30.7±11.3	15.4-53	
	Patients pre-IMRT	30	23.5±11.2	5.5-47	0.023 S
	Patients post-IMRT	30	31.7±15.5	11.7-61.8	
	Patients post-3DCRT	30	30.7±11.3	15.4-53	0.79 NS
	Patients post-IMRT	30	31.7±15.5	11.7-61.8	

S=Significant, HS=highly significant, NS=Non-significant.

DISCUSSION

The study shows a significantly elevation of salivary TNF-α in HNSCC than control, this agree with many studies such as Korostoff et al., (24), Juretic et al., (25) and Ameena and Rathy, (26) when they illustrated similar increase in patients with oral cancer and Małgorzata et al., (27) as they mentioned an increased level of salivary TNF-α in oropharyngeal squamous cell carcinoma patients.

This elevation of salivary TNF- α could be explained by the fact that this cytokine secreted by several cells including tumor cells, so became a part of cancer environment. (28, 29) Tumor necrosis factor - α is related to DNA damage mediated by oxidative stress and also works as cancer promoting agent and is linked to each step of carcinogenesis including transformation, proliferation, angiogenesis, also invasion and distant metastasis in different cancer types. (30, 31)

The present study find a significant increase in salivary IL-6 in patients group before RT than control, this match with Cheng et al., (32) and Vesty et al., (33) when they detect salivary IL-6 most frequently in patients with HNSCC than control and could be considered as potential salivary biomarker. Also other studies mentioned significant increase in salivary Il-6 on patient with oral cancer in comparison to control group. (24, 34, 35, 36, 37, 38)

The increase of IL-6 is probably from local production from different cells like inflammatory cells and especially tumor cells, it's inhibit body immune response against cancerous cells by inhibiting differentiation of dendritic cells and enhance tumor metastasis. (39 40)

The present study shows a significant elevation in both salivary TNF- α and IL-6 after RT, this match with Bossi et al., (41) and Russo et al., (42) as they reported in their studies an elevation in the level of different salivary cytokines including IL-6 and TNF-α in HNSCC patients after RT when compared to their level pre-treatment, but other study conducted by Citrin et al., (43) when they illustrated an increase in cytokines level but statically not significant.

This elevation can be explained by: radiotherapy cause direct DNA damage which result in cell injury or death and cause generation of free radicals, excessive DNA damage could lead to what's called cellular senescence which is a state of fixed cellular proliferative arrest, this damage will affect epithelial cells, fibroblast and endothelial cells. All these events will lead to up-regulation of inflammatory process and secretion of many pro-inflammatory cytokines such as IL-6 and TNF- α from immune cells like macrophage. (44, 45, 46, 47)

The study explanation of non-statically difference between 3DCRT and IMRT is that even less radiation dose is delivered to normal tissues in IMRT, at the same time this radiation dose to both malignant and normal tissues can up-regulate the above mentioned inflammatory response and lead to increase in salivary cytokines levels.

In conclusion the salivary IL-6 and TNF- α are high in patients with HNSCC than in healthy individuals, so they can be used as biomarkers for early detection of HNSCC.

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