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EFFECTS OF X-RADIATION ON THE SALIVARY COMPOSITIONS

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ABSTRACT

Background: Human saliva performs an important role in preserving healthy teeth and oral tissues. Many factors have effects on saliva compositions and may interfere with function of saliva. X-radiation consider one of the most important factors which have important effects on the saliva. Understanding the consequences of radiation still valuable to many interested investigators. **Aim of study:** Monitor irradiation influence in radio-treated individuals who had head and neck malignancies on the most organic and inorganic constitutes of human saliva. **Material and Methods:** The study undertaken on individuals (80 male) aged between 30-45 years who referred to Radiation and Nuclear Medicine Hospital in Baghdad for the period between Dec. 2013 to Oct. 2016, Forty of them are patients with malignancies in head and neck after 12 weeks radiotherapy. The other forty were healthy individuals. A whole dosage was between 40 - 44 Gray (5 fractions weekly with specific dosage of 2.0 daily). Assessment of most salivary organic and inorganic components were carried out for all individuals after radiotherapy. The levels of salivary total protein, albumin, immunoglobulin A, M and G, amylase, calcium,, phosphate, magnesium in all subjects were measured utilizing specific tests. **Results:** The study showed a significant difference in the most salivary organic constitutes between radio-treated patients group and healthy group, so the study showed a significant increase of the levels of salivary total protein and albumin in patients with radiotherapy in compared to healthy individuals. A significant reduction in the levels of salivary IgA and IgM in patients with radiotherapy with a non-significant increase in the levels of salivary IgG in compared to healthy individuals. The study also showed a significant decrease in the levels of salivary amylase in patients with radiotherapy in compared to healthy individuals. Also, there was no significant difference of most inorganic constitutes in saliva between radio-treated patients group and healthy group, so the levels of salivary Ca, P and Mg in radio-treated individuals in opposite to healthy individuals. **Conclusion:** Long term exposure of head and neck region to x-radiation have adverse influence on most component of saliva.

KEYWORDS: Saliva, Radiotherapy, Salivary component, radiation.

1. INTRODUCTION

Human saliva composed of approximately ninety-nine percent water and less than one percent of dry components, like protein and salts.[1] It is secreted by the salivary glands, in addition to their function in the lubrication of the mouth,the salivary glands offer other functions like antiviral, antibacterial, antifungal properties and also adjust pH, moreover individual saliva considered as a diagnostic fluid, because its easily to compile non-invasively.[2,3] The daily mean of saliva production in a good health person varies from 1 to 1.5.[4] The three pairs of major salivary glands (parotid, submandibular, and sublingual) are responsible for whole saliva production in addition to hundreds of small salivary glands diffused in labial, buccal, lingual, palatal regions.[5] The majority of unstimulated saliva amount produced by submandibular glands and majority of stimulated saliva amount produced by parotid glands.[6,7]

1.1. Composition of Saliva

The secretory human saliva may be serous, mucous, or mixed. Serous secretions secreted at most by the parotid glands and they are abundant with ions and enzymes. Mucous saliva are abundant with glycoproteins and produced mainly by small glands. The submandibular and sublingual glands considered as a mixed glands and the salivary secretions is influenced by the secreted rates of serous and mucous cells.[8,9,10]

The individual saliva consists of a big quantity of various protein composed in the acinar cells.[11] Part of these proteins have physical properties as lubrication of bolus and oral tissue through mastication, helping swallowing food and speech. The other proteins have biochemical properties and act as biological systems for the oral mucosa protection and dentitions against pathological changes.[12] The saliva contain also specific components have immunity abilities as immunoglobulin A which acts against the foreign bodies inside the body and prevent its

activities.[13] Other salivary immunologic components, such as immunoglobulin G and immunoglobulin M, but they are in small amount and could be from gingival fluid.[4,14] Salivary amylase is also consider the most numerous components in saliva, it is about 10-20 % of the total proteins which contribute in digestive function[15,16] Saliva is in charge of the primary digestive function of starch, as a result of activity salivary amylase.[17]

1.2. Radiotherapy

Radiotherapy as a procedure of treating malignancies originated shortly after the x-radiation discovery. Radiotherapy was initially introduced in the treatment of patients with cancer in the beginning of 1896.[18] The consequences of X-radiation on salivary gland are of special importance for radiotherapy of head and neck tumors.[19]

Irradiation considered as an important treatment method for head and neck malignancies 26. Wang et al stated that Radiotherapy of head and neck malignancies includes most major and minor salivary glands according for the location of tumor.[20]

1.3. Effects Of X-Radiation On Living Tissues And Salivary Glands

The effect of radiation on living tissues was achieved either by direct ionizations of living tissues which considered as direct effect. In turn, the absorption of photon by water within the living tissues and cause ionizing a part of its water molecules. The producing ions created free radicals that subsequently that produce disturbing on biological molecules. Because of this disturbing including water molecules imposed to modify the biologic molecules, this sequence of happenings is termed indirect.[21]

Salivary glands are more radio-sensitive, and it different from the other radiosensitive tissues, because of their tissues proliferate slowly and are composed of highly differentiated cells.[22] Salivary glands consider the most part subjected to radiotherapy during treatment of head and neck malignancies[23] The parenchymal part of salivary glands is more sensitive to radiation. Parotid glands are more radiosensitive than submandibular and sublingual glands, that is mean, the serous cells are more sensitive to radiation than mucous cells, so that the remaining saliva will be more viscous and the reduced level of saliva in radio-treated patients which comprise the major salivary glands is variable from normal in this way.[21]

2. MATERIAL AND METHODS

A study was undertaken on individuals (80 males aged between 30-45 years who were referred to Radiation and Nuclear Medicine Hospital in Baghdad, for the period between Dec. 2013 to Oct. 2016). Forty individuals included in this study were patients selected with malignancies in head and neck region on radiotherapy for twelve successive weeks. while the forty other selected as health individuals. A whole dosage was about 40 - 44 Gray, (5 fractions weekly with specific dosage of 2.0 daily).

All individuals included in the study are informed of the likelihood that the results may be used for research objectives.

2.1. Criteria of Individual Selection

All individual must have relatively healthy periodontal status, with no history of previous radiotherapy, non-smokers and non-drinkers, no impairment of salivary gland, have no any current of systemic disease and must not be on drugs that may have influence on salivary glands.

2.2. Collection of Saliva

This was done on unstimulated saliva and collected at morning to reduce daily variation.[24,25]

The individuals required to sputter the saliva into a disposable plastic measured jar for period of ten minutes.

About ten ml of unstimulated saliva collected from each subject. The gathered saliva putted for centrifuging (25.00 r.p.m for 5 minutes at temperature 4°C), then the required laboratory tests were done or the remaining saliva frozen at -70°C for future analysis.

The total protein concentration determined by using Biuret test. The copper atoms of Biuret solution will react with peptide bonds and changing color, so the deep violet color marks the presence of proteins. The Albumin amount determined by a photometric color test, so Albumin with bromocrosol green will form a green complex in an acidic pH.[26,27]

The IgA, IgG and IgM amounts were evaluated by Turbidimetric method[28,29] (using kit bio system). These parameters evaluated by auto-analyzer.

To measure the salivary amylase, the collected salivary samples were diluted with normal saline (saliva to saline ratio was 1:10) and by utilizing manual spectrophotometry the amylase values are obtained in IU/L.

For determination of the salivary calcium, phosphate and magnesium levels, each salivary sample was centrifuged at room temperature-1800 rpm for about 6 minute. The procedure was done by taking a 3 ml of superficial layer. This weighted by electronic balance and separated into two parts, one part used for P measurement, and this done by spectrophotometer and the other part used for Ca and Mg measurement, this done by utilizing air-acetylene, atomic absorption spectrophotometer. The resultant collected in graduate tubes and the volume measured to the closest 0.05 ml.

3. RESULTS

Levels of salivary organic constitutes (salivary total protein, Albumin and Immunoglobulins) and levels of salivary inorganic constitutes (calcium, magnesium and potassium) were statically analyzed and the findings expressed as the mean ± standard deviation.

Levels of salivary total protein and albumin showed a significant increase in patients group with radiotherapy in opposite to healthy group. Table 1,2.

Table 1: Salivary total protein levels of healthy and patient subjects.

	N	Level (g/L) Mean ± SD	t- test	P-value
Healthy	40	2.98 ±1.13	t = 2.4818	**0.015**
Patients	40	3.65 ± 1.28		

Table 2: Levels of salivary albumin of healthy and patient subjects.

	N	Level (g/L) Mean ± SD	t- test	P-value
Healthy	40	0.25 ± 0.94	t = 2.47	**0.01**
Patients	40	0.81 ± 1.08		

Levels of salivary IgA and IgM in patients group with radiotherapy showed a significant reduction with no-significant increase of salivary IgG levels in opposite to healthy group. Table 3.

Table 3: Levels of salivary IgA, IgG, IgM of healthy and patient subjects.

	N	Level (mg/dl) Mean ± SD	t- test	P-value
		IgA		
Healthy	40	25.12 ± 2.88	t = 8.85	**0.0001**
Patients	40	19.26 ± 3.04		
		IgG		
Healthy	40	0.8 ± 1.32	t = 1.50	**0.13**
Patients	40	1.2 ± 1.04		
		IgM		
Healthy	40	0.68 ± 0.92	t = 3.1069	**0.0026**
Patients	40	0.1 ± 0.74		

In relative to the levels of salivary amylase, a significant decrease in the levels of salivary amylase in patients group with radiotherapy in opposite to healthy group. Table 4.

Table 4: The levels of salivary amylase of patient and healthy subjects.

	N	Level (IU/L) Mean ± SD	t- test	P-value
Healthy	40	2724 ± 544.8	t = 14.9473	**0.0001**
Patients	40	1242.4 ± 310.5		

In addition, the study showed non-significant differences in the levels of salivary Ca, Mg and P in patients group with radiotherapy in opposite to health group. Table 5.

Table 5: Levels of salivary Ca, Mg and P in patients and healthy group.

	N	Level (mg/dl) Mean ± SD	t- test	P-value
		Calcium		
Healthy	40	5.3 ± 1.26	t = 0.640	**0.523**
Patients	40	5.1 ± 1.52		
		Magnesium		
Healthy	40	0.25 ± 0.08	t = 1.3950	**0.1670**
Patients	40	0.22 ± 0.11		
		Phosphate		
Healthy	40	18.32± 8.66	t = 0.8211	**0.4141**
Patients	40	16.54 ± 10.63		

4. DISCUSSION

The effects of x-radiation on salivary glands are of special importance in radiotherapy of patients with head and neck malignancies due to the undesirable effects of radiotherapy, as the modified salivary components, the reduced salivary flow rate leading to irreversible problems such as burning mouth, oral dryness, impeded oral functioning, night oral disturbance,, disturbed social activities and vulnerability to oral infections and dental caries.

Clinical radio-treated patients not only exhibit significantly reduced levels of saliva, but also exhibit specific alterations such as electrolyte levels of saliva, changed viscosity, pH and immunoglobulin.

The study monitored influence of radiation on most salivary components in patients with radiotherapy.[30]

It had found that there is significant elevation in the levels of salivary total protein and albumin levels significantly in patients group with radiotherapy in compared to healthy group. Similar study showed a non-significant elevation in the levels of total protein and albumin[31] while other studies this elevation was significant.[32,33,34]

Many study discussed this increase of level of total protein in patients with oral malignancies which interpreted that due to the minimal amount of saliva secreted in opposite to synthetic protein in saliva.[35,36,37]

Other studies discussed the elevation of albumin level in oral cavity. They supposed that the elevation of salivary albumin was due to impairment of oral epithelium which lead to infiltration of albumin from blood to saliva through gingival curricular fluid.[38]

There was a significant decrease of salivary concentration of IgA and IgM in patients group with radiotherapy and non-significant increase in the levels of

salivary IgG in compared to healthy group in present study.

Similar study showed that the levels of salivary IgA and IgG levels have a trend to decrease while salivary IgM levels was significant decreased.[39]

Hershkovich and Nagler stated that the salivary IgA governed by interaction between T & B lymphocytes.[40] Ortholan et al stated that the high dose of radiation has an effect on T & B cells and in turn affect IgA level.[41]

Salivary IgA play an important role in the protection of mucosa against microbial infections. Secretory IgA is produced by plasma cells and through the epithelial cells transport into lumens of salivary gland.[40] Donia et al stated that radiation might cause damage to cells of epithelium and impair movement o immunoglobulin A into saliva.[41]

Salivary IgM are originally formed in serum and transport to saliva through gingival crevicular fluid. Nagler suggested that a weak oral mucosa and neuropathy after radiotherapy was due to impair movement of serum immunoglobulin M to saliva.[42]

Others stated there was no or little elevation of salivary IgG pre-and post-radiotherapy due to mucosal and sulcular movement of serum IgG into saliva.[43]

This study also showed a significant reduction in the levels of salivary amylase after radiotherapy in compared to healthy group. The result was similar to many studies.[31,33,39,44,45]

Salivary amylase is an effective enzyme to determine the work of serous cells. Since the parotid gland acini are mostly serous type, so the parotid glands are more radiosensitive in compared to other major salivary glands.[46] This reduction of amylase was due to probability of salivary gland impairment and minimal dietary through radiotherapy,[43] other study stated that salivary gland dysfunction was the only cause in the significant decrease of salivary amylase.[39]

Another similar study showed a significant reduction in the levels of salivary amylase following three weeks after radiotherapy in patient with head and neck malignancies.[45]

On other side, the present study showed non-significant difference in levels of salivary Ca, P and Mg in radio-treated patients in opposite to healthy subjects. These results agreed with many studies.[33,47,48,49]

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