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Therapeutic Effect of Curcumin Oral Gel on Salivary Epidermal Growth Factor and Lactate Dehydrogenase Levels in Relation to Oral Mucositis in Head and Neck Cancer Patients Undergoing Concurrent Chemoradiation

Rouaa S. Farhan [1], Fawaz D. Al-Aswad [2]

[1,2] College of Dentistry, University of Baghdad, Baghdad, Iraq.

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Correspondence: Rouaa S. Farhan
Email: dr.rouaa.alkhaledy@gmail.com

Website: https://djm.uodiyala.edu.iq/index.php/djm

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Abstract

Background: Curcumin is a traditional herbal medication, which has shown promise in a variety of pharmacologic applications. Epidermal growth factor an amino acid polypeptide found in saliva and other bodily fluids, which promotes cell proliferation and renewal. Lactate dehydrogenase is classified as altered protein markers present in saliva, and its levels showed significantly elevated in head and neck cancer. To study the effect of curcumin oral gel on levels of salivary epidermal growth factor and lactate dehydrogenase in head and neck cancer patients receiving concurrent chemoradiation induced oral mucositis.

Patients and Methods: Ninety head and neck cancer patients receiving concurrent chemoradiation, 45 patients in each group. Saliva levels of lactate dehydrogenase and epidermal growth factor measured by an enzyme linked immunosorbent assay. WHO scale used to assess oral mucositis.

Results: Highly significant increase in salivary epidermal growth factor and decrease in lactate dehydrogenase levels with less severe oral mucositis in group treated with curcumin. Significant differences were found between the two groups in the WHO scale at two weeks (P = 0.041) and six weeks (P=0.02).

Conclusion: The current study concludes that curcumin oral gel can increase epidermal growth factor and reduce lactate dehydrogenase levels in saliva, and it may be used as an alternate treatment for oral mucositis-induced by chemoradiation.

Keywords: Head and neck cancer, Curcumin, Oral mucositis.

Introduction

Curcumin goes under another name, The Zingiberaceae family includes turmeric. One to two percent curcuminoids and three to twelve percent volatile oil are the two main components of the root. A phenolic compound with possible health benefits, dimethylsulfoxide is also known as curcumin (1, 2). Numerous clinical investigations have shown the extensive variety of pharmacologic capabilities

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exhibited by Curcumin oral gel. These features include the ability to enhance wound healing, anti-inflammatory, antifungal, antibacterial, and anticarcinogenic actions (3). Curcumin improves epithelialization and wound healing by protecting and activating keratinocytes while acting as a reactive oxygen species scavenger and an antioxidant (4, 5). Curcumin may potentially increase the effectiveness of morphine by decreasing pain transmission channels and promoting the production of serotonin, dopamine, and noradrenaline at large dosages (6). Head and neck cancers (HNCs) encompass a wide range of malignancies that can develop in the oral cavity, nasal cavity, paranasal sinuses, pharynx, larynx, and salivary glands (7). Chemotherapy combined with radiotherapy is the first line of treatment when head and neck cancer has spread locally (8).

Cytotoxic concurrent chemoradiotherapy causes oral mucositis (OM), an inflammatory disorder of the mouth and throat that is a major problem in oncology (9). Confluent and deep ulcers are the most severe symptoms of oral mucositis. When a patient has pain due to mucositis, it may greatly compromise a patient's functional status and quality of life (10).

Epidermal growth factor (EGF) promotes cell renewal and proliferation (11). Evidence reveals a link between low-grade clinical oral mucositis and increased epidermal growth synthesis (12). Curcumin has a notable effect on numerous growth factors, including EGF (13).

Lactate dehydrogenase (LDH) is essential enzyme catalyzed the reversible conversion of pyruvate and lactate during glycolysis and gluconeogenesis (14). Under typical, healthy conditions, lactate dehydrogenase is located in the cytoplasm of cells. LDH is released into the extracellular environment when cells are exposed to cytotoxic substances (15). Salivary LDH levels may change in several oral diseases, including malignancy, periodontitis, and gingivitis. According to reports, it is theorized that severe mucositis cases would be associated with higher LDH levels due to the cytotoxic nature of chemoradiation (16).

To our knowledge, no previous studies have studied the effect of curcumin oral gel on salivary epidermal growth factor and salivary lactate dehydrogenase levels in HNC patients undergoing concurrent chemoradation-induced oral mucositis. Therefore, this study aimed to determine the effect of curcumin oral gel on levels of salivary epidermal growth factor and lactate dehydrogenase in head and neck cancer patients under concurrent chemoradiation-induced oral mucositis.

Patients and Methods

Study design: From March 2023 to June 2024, this study was carried out. There were 90 HNC patients that took part in this study. There were two groups created: the study group and the control group. For the trial, 45 patients were given oral gel containing curcumin, whereas 45 patients were given magic-solution as a control.

Inclusion criteria: Included patients between the ages of 30 and 70, diagnosed with head and neck cancer and scheduled for concurrent chemoradiotherapy. Patients were also required to wear a head and neck mask during radiation treatments, and their oral cavity mucosa had to be within the radiation range. Chemotherapy was cisplatin 40 mg/m2 administered weekly, and radiotherapy consisted of 33 fractions scheduled five days a week for six weeks with 50-70 Gray (Gy).

Exclusion criteria: included patients receiving palliative radiation or radiation treatment alone.

Assessment of oral mucositis clinical: On the 2nd week of chemoradiation and the last day of the chemoradiation treatments, patients were examined and scored on a scale from 0 to 4 developed by the World Health Organization. With a score of 0, no symptoms are present; with a score of 1, the oral mucosa is red and

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uncomfortable; and with a score of 2, the mouth is ulcerous and makes it hard to eat normally. When score reaches 3, the ulcer has already developed and the patient is limited to drink fluids; when it reaches 4, the patient is completely unable to consume any food or liquids (17).

Curcuma longa oral gel: The subjects in the curcumin group were given Curenext®, aTn product made by (Abbott Healthcare, India), which includes 10 milligrams of Curcuma longa root extract (rhizome) per gram of gel. From the beginning with the initial saliva sample collection until their chemoradiotherapy treatment was finished, patients were told to use a cotton swab or their fingers to apply the gel three times a day (18, 19). A standard mouthwash consisting of nystatin, dexamethasone, lidocaine, and tetracycline was administered to patients in the magic-solution group.

Saliva sample collection and storage: Each of the 90 patients had three complete saliva samples taken: once before chemoradiation, once after the second week of treatment, and again at the six-week chemoradiation. Patients spat into a plastic tube that was marked with their name, group, and visit date in order to collect their unstimulated saliva. The next step was to place it in an icebox and freeze it at -80°C until analysis.

Laboratory analysis: The levels of EGF and LDH in the saliva were determined using an enzyme-linked immunosorbent assay (ELISA). In accordance with the manufacturer's instructions, commercial quantitative sandwich (ELISA) kits from Cloud-Clone Corp (CCC, USA) were used. Salivary samples were taken to determine EGF and LDH levels using phosphate-buffered saline as a negative control and a manufacturer-supplied standard curve.

Statistical analysis

Data were handled in an Excel spreadsheet. Analysis was carried out using SPSS version 22, the Statistical Package for the Social Sciences. Tests used are the Wilcoxon Signed Ranks, Bonferroni, a paired t-test, an independent t-test, and chi-square ($\chi 2$) test. A P-value below 0.05 was defined significant.

Results

The results of this study showed that when comparing the two groups according to age and sex, no statistically significant differences were found.

Salivary epidermal growth factor (EGF): The comparison between the two studied groups with respect to the EGF marker, over different experimental periods, results show that mean values increase clearly over time, and at higher levels with respect to those treated with the curcumin group (Table 1 and Figure 1).

Table (1). Summary Statistics of EGF (pg/ml) marker along studied periods of the studied groups.

Periods	Groups	No.	Mean	Std. D.	Std. E.	95% C.I. for Mean		Min.	Max.
						L.b.	U.b.		
Initiation Period (P1)	Curcumin G1	45	122.6	10.64	1.6	119.4	125.8	100.8	152.4
	Magic Solution G2	45	118.6	9.86	1.5	115.6	121.5	93.6	136.9
After 2 weeks (P2)	Curcumin G1	45	250.0	56.3	8.4	233.1	266.9	129.1	404.5
	Magic Solution G2	45	200.1	42.1	6.3	187.5	212.8	122.7	280.9
After 6 weeks (P3)	Curcumin G1	45	407.5	61.7	9.2	389.0	426.1	304.2	545.8
	Magic Solution G2	45	257.6	50.2	7.5	242.6	272.7	170.4	349.8

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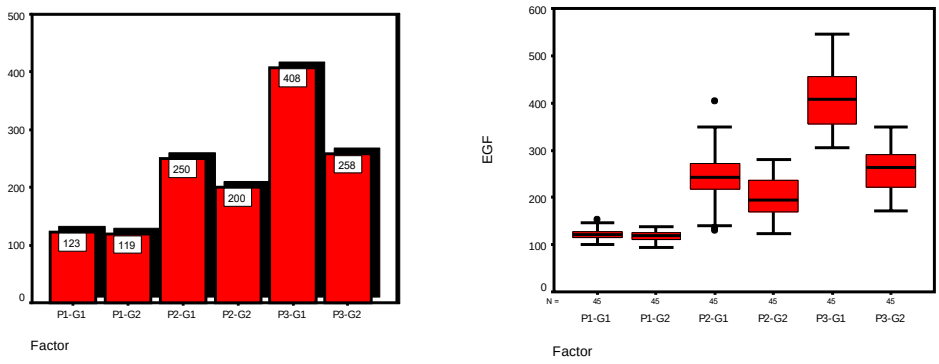

Figure 1. Stem-Leaf Plot with Bar Chart, and stem-leaf plot to explore the behavior of EGF marker readings' distribution along the study of sequential periods in each group.

Means of salivary EGF were highly significantly (P = 0.000) increased two and six weeks after chemoradiotherapy compared to that before chemoradiotherapy and six weeks after chemoradiotherapy compared to that at two weeks after chemoradiotherapy in both study groups. The increment in EGF two and six weeks after chemoradiotherapy was significantly greater in the group given curcumin than that treated with magic solution compared to that before chemoradiotherapy (Table 2).

Table 2. Significant levels for testing repeated measurements of EGF marker readings in each group independently over the sequential of studied periods.

Groups	Pairwise Comparisons		Mean Diff. (I-J)	Std. Error	Sig. Level	95% C. I. for Diff.	
	(I) EGF	(J) EGF				L.b.	U.b.
Curcumin	Initiation	After 2 w.	-127.5	10.25	0.000	-148.1	-106.8
		After 6 w.	-285.0	10.25	0.000	-307.6	-262.4
	After 2 w.	After 6 w.	-157.5	10.25	0.000	-187.2	-127.8
Magic Solution	Initiation	After 2 w.	-81.5	8.06	0.000	-97.1	-65.9
		After 6 w.	-139.1	8.06	0.000	-157.5	-120.6
	After 2 w.	After 6 w.	-57.5	8.06	0.000	-80.8	-34.2
(*) HS: Highly Significant at P<0.01; Testing are based on repeated measurers of several related groups, through using adjustment for multiple comparisons by "Bonferroni" test.							

Salivary lactate dehydrogenase (LDH): The comparison between the two studied groups with respect to the " LDH " marker, over the course of an experimental period. Results show that mean values are decreasing clearly over the time periods, and at a lower level with respect to those treated with curcumin (Table3 and Figure 2).

Table 3. Summary Statistics of LDH (ng/mL) marker along different periods of the studied groups.

Periods	Groups	No.	Mean	Std. D.	Std. E.	95% C.I. for Mean		Min.	Max.
						L.b.	U.b.		
Initiation period	Curcumin	45	5.290	0.86	0.13	5.03	5.55	3.65	6.64
	Magic Solution	45	5.286	1.07	0.16	4.96	5.61	3.03	6.97
After 2 weeks	Curcumin	45	4.04	0.42	0.06	3.92	4.17	3.18	4.74
	Magic Solution	45	5.16	0.81	0.12	4.92	5.40	3.83	7.06
After 6 weeks	Curcumin	45	3.06	0.45	0.07	2.93	3.20	2.28	3.74
	Magic Solution	45	3.49	0.78	0.12	3.26	3.72	2.01	4.64

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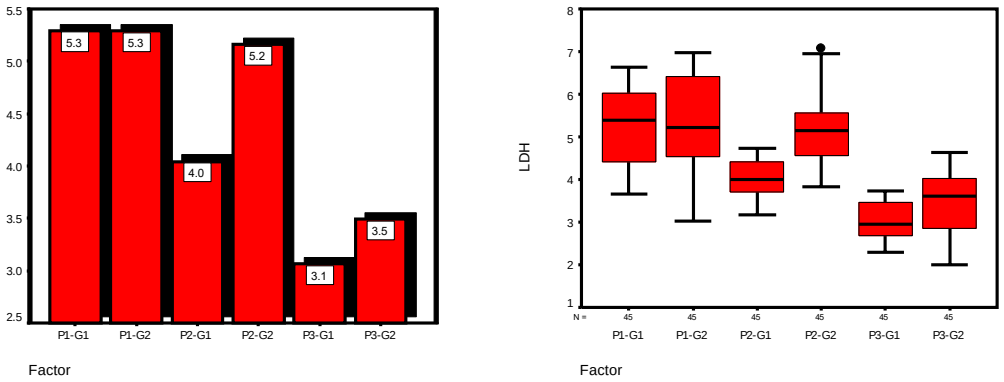

Figure 2. Bar Chart, and a stem-leaf plot for exploring the behavior of LDH marker readings' distribution along the study of sequential periods in each group.

The results in Table 4 showed that the mean salivary LDH was significantly decreased at two and six weeks after chemoradiotherapy compared to before chemoradiotherapy and at six weeks after chemoradiotherapy compared to two weeks after chemoradiotherapy in both study groups. The decrement in LDH two and six weeks after chemoradiotherapy in the patients treated with curcumin had a much greater decrease than those treated with the magic solution compared to that before chemoradiotherapy.

Table 4. Significant levels for testing the covariate of LDH (ng/mL) marker's readings in each group independently over the sequential periods.

Groups	Pairwise Comparisons		Mean Diff. (I-J)	Std. Error	Sig. Level	95% C. I. for Diff.	
	(I) LDH	(J) LDH				L.b.	U.b.
Curcumin	Initiation	After 2 w.	1.245	0.118	0.000	0.951	1.540
		After 6 w.	2.227	0.139	0.000	1.881	2.572
	After 2 w.	After 6 w.	0.981	0.062	0.000	0.826	1.136
Magic Solution	Initiation	After 2 w.	0.127	0.199	1.000	-0.368	0.621
		After 6 w.	1.797	0.192	0.000	1.318	2.275
	After 2 w.	After 6 w.	1.670	0.164	0.000	1.263	2.077
(*) HS: Highly Significant at P<0.01; NS: Non-Significant at P>0.05; Testing is based on repeated measures of several related groups, using adjustment for multiple comparisons by the "Bonferroni" test.							

Clinical evaluation of oral mucositis world health organization scale: The results in Table 5 and Figure 3 showed that during the 2-week and 6-week chemoradiation visits, the curcumin group had a lower mean WHO score than the magic-solution group with regard to oral mucositis.

Table 5. Summary Statistics of Grade of Mucositis WHO score along different periods of the studied groups.

Groups	Statistics	Periods		
		Initiation	After 2 weeks	After 6 weeks
Curcumin	Mean of Score	0.000	1.667	1.178
	Interquartile Range	0.000	1.000	1.000
	Minimum score	0.000	1.000	1.000
	Maximum score	0.000	3.000	2.000
Magic Solution	Mean of Score	0.000	1.689	1.378
	Interquartile Range	0.000	0.000	1.000
	Minimum score	0.000	1.000	1.000
	Maximum score	0.000	3.000	3.000

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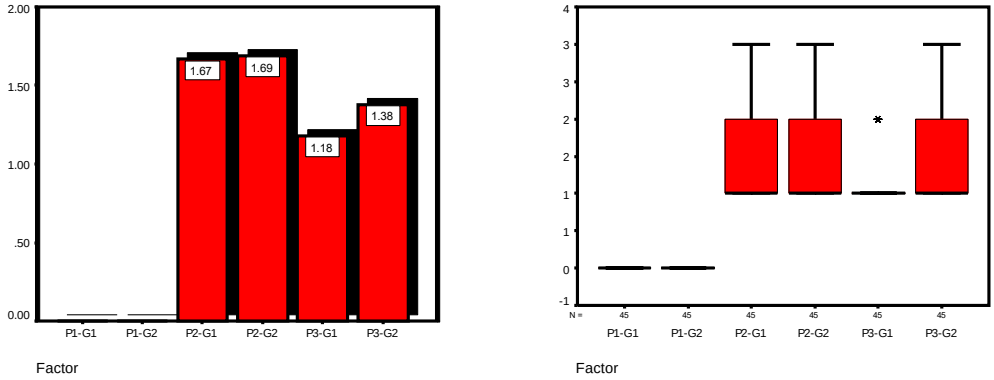

Figure 3. Bar Chart, and stem-leaf plot for exploring behavior of WHO Score reading's distribution along the studied of sequential periods in each group.

Results in Table 6 represent the WHO score's readings at a significance level of (P=0.000), there were highly significant differences with respect to all probable pairwise comparisons grade of mucositis GOM , either for curcumin or magic solution groups % independently.

Table 6. Significant levels for testing of the WHO score readings in each group independently over the sequential periods.

Groups	Pairwise Comparisons		Z-value	Sig. Level
	(I) GOM	(J) GOM		
Curcumin	Initiation	After 2 w.	-5.964	0.000
		After 6 w.	-6.283	0.000
	After 2 w.	After 6 w.	-4.491	0.000
Magic Solution	Initiation	After 2 w.	-5.970	0.000
		After 6 w.	-6.081	0.000
	After 2 w.	After 6 w.	-3.300	0.001
(*) HS: Highly Significant at P<0.01; Testing are based on the "Wilcoxon Signed Ranks" test.				

Grade of mucositis between study groups: The results in Table 7 showed the comparison in the grade of mucositis between the study group and the magic solution group after chemoradiotherapy. After two weeks, 66.7% of patients in the curcumin group were graded I compared to 42.3% in the magic solution group, with a p-value = 0.041 indicating statistical significance. After six weeks, 82.2% of patients in the curcumin group were graded I compared to 60% in the magic solution group, a statistically significant difference (P=0.02).

Table 7. Comparison between study groups by grade of mucositis.

Grade of mucositis (WHO)	Study group		X2 test	P - Value
	Curcumin (%) n= 45	Magic Solution (%) n= 45		
Two weeks after chemoradiotherapy				
1	30 (66.7)	19 (42.3)	6.351	0.041
2	11 (24.4)	15 (33.3)		
3	4 (8.9)	11 (24.4)		
Six weeks after chemoradiotherapy				
1	37 (82.2)	27 (60.0)	7.75	0.02
2	8 (17.8)	13 (28.9)		
3	0 (0)	5 (11.1)		
WHO: World Health Organization. $\chi 2$: chi-square test.				

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Discussion

Since it is simpler to apply, absorbs quickly, topical curcumin treatment, in the form of an oral gel, offers several benefits over systemic curcumin because it interacts with surrounding tissues, prolonging the contact period that increases its benefits, and because it has fewer evident bad effects. Patients with dysphagia or gastrointestinal issues may potentially benefit from oral gel formulations in reducing undesirable effects (17-20).

Concurrent chemoradiotherapy causes basal epithelial cell death, which may occur as a result of free radical production. These free radicals activate second messengers which carry messages from the cellular surface receptors to the inner cell environment, resulting in increased production of pro-inflammatory cytokines, tissue damage, and cell death (21).

In this study the majority of patients using curcumin group experienced only mild mucositis grade1 at the end of the chemoradiotherapy sessions; a few had grades 2 but none had severe mucositis (grades 3 and 4) whereas patients in magic solution group experienced grade 2 and grade 3 mucositis. The results agree with results of Alsalim et al., 2024, most patients treated with curcumin did not have any mucositis (grade 0) throughout their radiation treatments and small number of patients did experience mild mucositis (grades 1 and 2), but none of them had severe mucositis (grades 3 and 4) (22). A study done by Arun et al., 2020 revealed that the majority of patients in the curcumin group experienced only grade 1 mucositis after four weeks of treatment (23). Also, our findings are in

agreement with those of the Shah trial, which also indicated that, Grade 3 mucositis did not occur in the curcumin group, unlike the control group (1). Additionally, Patil's research showed that the two groups' WHO ratings were significantly different (1, 24). Oral mucositis caused by chemoradiation was less severe in HNC patients treated with curcuma long a gel compared to placebo gel (19), chlorhexidine gel (18).

Salivary EGF levels in HNC patients treated with chemoradiotherapy showed that lower EGF levels during treatment exacerbated OM severity, perhaps as a result of reduced cell proliferation and suppression of mucosal repair (25). In this study, when curcumin oral gel was applied a significant increase in salivary EGF after chemoradiation compared to before, as well as a reduction in the severity of OM, provide evidence that EGF may aid in speed up the healing process after chemoradiation damage to the mucosa and promote the oral mucosa's recovery (26). The enhanced salivary EGF and improved wound healing that occurred in the curcumin group may have been caused by curcumin's anti-inflammatory and combined antibacterial characteristics. Curcumin has many effects that promote healing and tissue remodeling, including promoting epithelization, restoring collagen architecture, and accelerating angiogenesis (27). These findings are in line with those of the research conducted by Alsalim et al. in 2024, which shown that use of curcumin oral gel significantly reduced the severity of OM and resulted in a significant increase in salivary EGF after radiation compared to before radiation (22).

Cancer cells often exhibit elevated levels of LDH activity, which facilitates promote uncontrolled cell proliferation and migration, especially those in hypoxic conditions (28). Patients with HNC had

significantly higher salivary LDH levels, according to previous studies (29, 30). Radiation exposure was linked to higher levels of salivary LDH, which in turn exacerbated the severity of oral mucositis, according to a study by Shivashankara et al. 2019 (31).

Also, in this study, when curcumin oral gel was applied, a significant decrease in salivary LDH after

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chemoradiation compared to before, and the severity of OM was reduced, suggesting that curcumin exhibits anti-oxidant properties and helps in the prevention of free radicals and toxic products resulting from oxidative stress, which contribute to cancer development. According to preclinical studies, curcumin inhibits reactive oxygen species (ROS) and free radicals, which protect DNA from damage by oxidative stress. This stress may be induced by ionizing radiation and other oxidative causes (32). The nuclear factor – kappaB NF κ-B plays an important role in the production of oxidative stress and nitric oxide synthase, which may cause cancer. Curcumin inhibits NF-kappaB production, which in turn suppresses the development of cancer growth (32). It has shown anti-cancer benefits under concurrent chemotherapy and radiotherapy .Studies have shown curcumin reduces reactive oxygen species (ROS) levels and decrease lactate dehydrogenase (LDH) release (33). According to Han et al., 2023, curcumin shows promise as a cancer treatment (34). Study by Chandrashekar et al., 2021 showed that patients with oral submucous fibrosis OSMF had significantly reduced LDH levels after treatment with curcumin oral gel (35). No previous studies have evaluated the therapeutic effect of curcumin oral gel on salivary LDH levels in HNC patients under concurrent chemoradiotherapy-induced oral mucositis.

Conclusions

Significant increase in the level of salivary EGF and significant reduction in salivary LDH level in HNC patients undergoing concurrent chemoradiotherapy after using topical curcumin oral gel compared to the magic solution, suggesting that it is effective and could be used as an alternative treatment in preventing and managing oral mucositis caused by concurrent chemoradiation. In addition, it's recommended for head and neck cancer patients to use curcumin oral gel as a preventive agent for chemoradiation-induced oral mucositis before concurrent chemoradiotherapy.

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Ethical clearance: This study was approved by a protocol number 934724 by the Research Ethics Committee of the University of Baghdad, College of Dentistry, Iraq.

Conflict of interest: None.

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التأثير العلاجي لهلام الكركمين الفموي على مستويات عامل نمو البشرة اللعابي ونازعة هيدروجين اللاكتات وعلاقته بالتهاب الغشاء المخاطي الفموي لدى مرضى سرطان الرأس والرقبة الذين يخضعون للعلاج الكيميائي الإشعاعي المتزامن

[١] رؤى شاكر فرحان، [٢] فواز داود الاسود

الملخص

خلفية الدراسة: الكركمين هو دواء عشبي تقليدي أظهر نتائج واعدة في مجموعة متنوعة من التطبيقات الدوائية. عامل نمو البشرة هو حمض أميني متعدد الببتيد موجود في اللعاب وغيره من سوائل الجسم، والذي يعزز تكاثر الخلايا وتجددها. نازعة هيدروجين اللاكتات يصنف على أنه علامات بروتينية متغيرة موجودة في اللعاب، وقد أظهرت مستوياته ارتفاعًا ملحوظًا في سرطان الرأس والرقبة.

اهداف الدراسة: دراسة تأثير هلام الكركمين الفموي على مستويات عامل نمو البشرة اللعابي و نازعة هيدروجين اللاكتات في مرضى سرطان الرأس والرقبة الذين يتلقون العلاج الكيميائي الإشعاعي المتزامن الناجم عنه التهاب الغشاء المخاطي الفموي.

المرضى والطرائق: تسعون مريضًا بسرطان الرأس والرقبة يتلقون علاجًا كيميائيًا إشعاعيًا متزامنًا، ٤٥ مريضًا في كل مجموعة. تم قياس مستويات اللعاب من نازعة هيدروجين اللاكتات وعامل نمو البشرة باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم .مقياس منظمة الصحة العالمية استخدم لتقييم التهاب الغشاء المخاطي الفموي.

النتائج: زيادة كبيرة في عامل نمو البشرة اللعابي وانخفاض في مستويات نازعة هيدروجين اللاكتات مع أقل حدة في التهاب الغشاء المخاطي الفموي في المجموعة المعالجة بالكركمين. تم العثور على اختلافات كبيرة بين المجموعتين في مقياس منظمة الصحة العالمية عند أسبوعين (**P = 0.041**) و ٦ أسابيع (**P = 0.02**).

الاستنتاجات: خلصت الدراسة الحالية إلى أن هلام الكركمين الفموي يمكن أن يزيد من عامل نمو البشرة ويقلل من مستويات نازعة هيدروجين اللاكتات في اللعاب ، ويمكن استخدامه كعلاج بديل لالتهاب الغشاء المخاطي الفموي الناجم عن العلاج الكيميائي والإشعاعي.

الكلمات المفتاحية: سرطان الرأس والرقبة، الكركمين، التهاب الغشاء المخاطي الفموي.

المؤلف المراسل: رؤى شاكر فرحان

الايميل: dr.rouaa.alkhaledy@gmail.com

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[٢،١] كلية طب الاسنان - جامعة بغداد - بغداد - العراق.