

## LOW LEVEL LASER THERAPY AND OMEGA-3 IN THE MANAGEMENT OF RECURRENT APHTHOUS STOMATITIS

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### **ABSTRACT**

**Introduction:** In view of the importance and prevalence of Recurrent Aphthous Stomatitis (RAS) there is an urgent need for searching about an effective therapy that is less expensive, easy to use and readily available with no side effects. Low Level Laser Therapy (LLLT) and Omega-3 were considered as possible treatment options of RAS.

**Aim of the work:** The aim of the current study was to compare the usefulness of LLLT with or without omega-3 versus omega-3 alone in treatment of RAS and evaluate the immune-modulatory effect of systemically administered omega-3.

**Materials and method:** 45 patients were suffering from RAS were randomly assigned into either GI: (LLLT), GII: (omega-3) and GIII: (omega-3 plus LLLT). The comparison was assessed clinically via calculating: The reduction in ulcer surface area by using Visual Basic Program, the effectiveness indices (EI) of pain improvement at day 1 and day five, average duration of ulcer episodes, and the number of new ulcer outbreaks at base line and then monthly for the following 6 months. Immunologically via: Measuring the amount of IL2, determination of CD4+CD25<sup>high</sup> T regulatory cells in blood samples at base line (BL), 3 and 6 months.

**Results:** GI showed significant reduction in ulcer surface area and significant decrease in pain sensation, without significant change in all other parameters. GII showed no significant change in the degree of pain sensation in spite of reduction in ulcer surface area in addition to significant change in all other parameters. GIII showed significant improvement in all clinical parameters which is compatible with the changes occurred in the immunologic parameters. At BL no significant difference was observed in between the three groups. For the follow up measurements: The reduction in ulcer surface area and (EI) of pain improvement showed significant difference comparing GII with GI and GIII in favor of the last mentioned two groups with no difference in between both of them, regarding the monthly clinical variables and immunologic parameters showed significant difference comparing GI with GII and GIII in favor of the GII and GIII with no difference in between both of them.

**Conclusions:** Using LLLT with omega - 3 fatty acid in management of RAS gave the most favorable clinical and immunologic results. The used Visual Basic Program was effective in calculating the difference of ulcers surface areas and the digital data enabled fair statistical analysis.

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## INTRODUCTION

RAS is an inflammatory condition that affects the oral mucous membrane, characterized by a painful ulcer with an erythematous halo. The disease causes many problems for the patient owing to pain, leading to difficulties in eating, drinking, and maintaining oral hygiene.<sup>(1)</sup> It is the most prevalent oral mucosal disease which occur in otherwise healthy individuals.<sup>(2)</sup>

The etiopathogenesis of RAS is unclear, investigations have failed to determine the exact etiology and pathophysiology of the disease but they all agreed on that it is a multifactorial disorder with precipitating factors including: Immune dysregulation, such as T cell-mediated cytotoxicity as demonstrated by infiltrated mononuclear cells in RAS tissue specimens, presence of one or more autoantibodies also may be noted,<sup>(3)</sup> the involvement of interstitial collagenases(matrix metalloproteinase MMP 1 and 8) in tissue destructive events in RAU.<sup>(4)</sup> Other suggested the predisposing factors for the development of RAS include psychological factors, hormonal changes, trauma and allergy,<sup>(5)</sup> hematologic deficiency, familial tendency, medications and imbalance of oral microbiome.<sup>(6)</sup>

Management of aphthous stomatitis is either topical or systemic. Topical therapy includes local anesthetics as lidocaine gel, protective bioadhesives as cyanoacrylate, antiseptic or anti-inflammatory therapies as chlorhexidine gluconate, amlexanox, local antibiotics as tetracycline, and topical steroids as triamcinolone acetonide . Systemic therapy as colchicines, dapsone, cyclosporine is mostly used in severe cases of aphthous stomatitis.<sup>(7)</sup>

Laser therapy has been suggested as a treatment option for RAU, owing to the advantages of being noninvasiveness and easy management with anti-inflammatory, analgesic, and biostimulatory effects, making it useful clinical technique for wound-healing and tissue regeneration.<sup>(8)</sup> in addition to its ability to eliminate the potential adverse effects caused by drugs.<sup>(9)</sup> Low-level laser therapy has been used

in the treatment of inflammatory and painful conditions such as herpes labialis, RAS, oral mucositis, hypersensitive teeth, alveolitis, temporomandibular joint pain, and trigeminal neuralgia.<sup>(10)</sup>

Omega-3 fatty acids, also called  $\omega$ -3 fatty acids or n-3 fatty acids, are polyunsaturated fatty acids (PUFAs) that are essential to normal growth in young children and animals.<sup>(11)</sup> Mammals are unable to synthesize omega-3 fatty acids, but can obtain them through diet.<sup>(12)</sup>

Several human, animal and cell culture studies had suggested that omega-3 fatty acids had anti-inflammatory, and immunomodulatory effect as demonstrated by their inhibitory effect on the formation of pre-inflammatory cytokines<sup>(13, 14)</sup> the ability to decrease the inflammatory biomarkers in different types of inflammation.<sup>(14-16)</sup>

RAS is a common oral mucosal disorder adversely affects the quality of life produces pain and ulceration cause suffering of the affected individuals; add to that the suggested effectiveness of omega-3 polyunsaturated fatty acids and LLLT as treatment options gave us the bright light to carry out this study.

## MATERIALS AND METHODS

The purpose of the present study was explained to the patients and informed consents were obtained according to the guidelines on human research adopted by the Research Ethics Committee Faculty of Dentistry, Tanta University.

### **Patients were selected according to following criteria:**

1. Male or female, Ages ranged from 15 to 40 years old, nonsmoker.
2. No current or history of systemic medical problem, including any systemic disease that predisposing to RAS (e.g.Behçet disease)
3. Non-Pregnant and non-lactating.

- 4. No history of any earlier experiences of laser therapy.
- 5. Suffering from pain due to RAS ulceration on the oral mucosa with the following characteristics:
  - Duration of 2 days or less, the diameter  $\geq 4$ mm,
  - Not have been subjected to any treatment modalities for at least four weeks before the beginning of the study.

**The selected participants were randomly assigned into three groups each group included 15 patients:**

- **Group I : Low-level laser therapy**
  - Gallium-Aluminum Arsenide diode laser (GaAlAs)\* was applied with wave-length 806 nm for 80s (6.3 j/cm<sup>2</sup>), 4 times once daily from day 1 to day 4.
- **Group II: Omega-3 capsules**
  - Omega-3 systemic supply, soft gelatin capsules of 1000 mg each\*\*
  - Each participant received 1 capsule 3 times daily for 6 months.

- **Group III : Omega-3 capsules plus Low-level laser therapy.**

**Methods of assessment:**

**(A) Clinical evaluation: I:short term clinical assessment**

- 1- Objective evaluation: Ulcer surface area
  - The ulcers were photographed using a digital camera with high resolution.
  - The photographs were taken at:
    - Day 1: Base line
    - Day 5: After complete laser application.
  - A standardized object of known dimensions was placed near each ulcer, to act as a reference for dimensional changes in each photograph.
  - The ulcer surface area was measured using a Visual Basic Program in pixels.<sup>(17)</sup>

**Digital assessment of ulcer surface area in pixels<sup>(17)</sup>**

A Visual Basic Program was developed specially for the present research to calculate the surface

TABLE (1) The study design for patients.

Type of assessment	Time of assessment							
	BL	D5	1 <sup>st</sup> M	2 <sup>nd</sup> M	3 <sup>rd</sup> M	4 <sup>th</sup> M	5 <sup>th</sup> M	6 <sup>th</sup> M
<b>Clinical</b>								
Ulcer surface area	+	+						
Degree of pain sensation	+	+						
Average duration of ulcer episodes	+		+	+	+	+	+	+
Number of new ulcer outbreaks	+		+	+	+	+	+	+
<b>Immuonologic</b>								
ELISA serum IL2	+				+			+
CD4+CD25 <sup>high</sup> T regulatory cell proportion	+				+			+

\* Gallium-aluminum- arsenic diode semiconductor laser therapy with a glass rod T8 (Elexxion AG, Radolfzell, Germany), wavelength, 806 nm; power, 60 mW; duration, 80 seconds

\*\* Omega- 3 plus south Egypt Drug Industries Company (SEDICO) 6 October City Egypt

area. The software contained two image boxes, four labels and five buttons. The image boxes were used to obtain the ulcer images before and after treatment, while the 2 labels were used to indicate the data for the pretreatment images and 2 labels to indicate the data for the post treatment images.

The "Load" button was used to upload the images to the pictures boxes, the "Select" button was used to border the ulcer or the green marker, the

"Confirm" button was used to assure the bordering and selections, the "Count" button was assigned to count the number of pixels forming the bordered structures (ulcer or marker) and to display the results via the labels below the image boxes. The "Exit" button was assigned not only to terminate the program but to clear it from the RAM as well, so as not to alter the processor speed.

The surface area of the marker in the first image



Fig. (1) The Visual Basic Program layout.

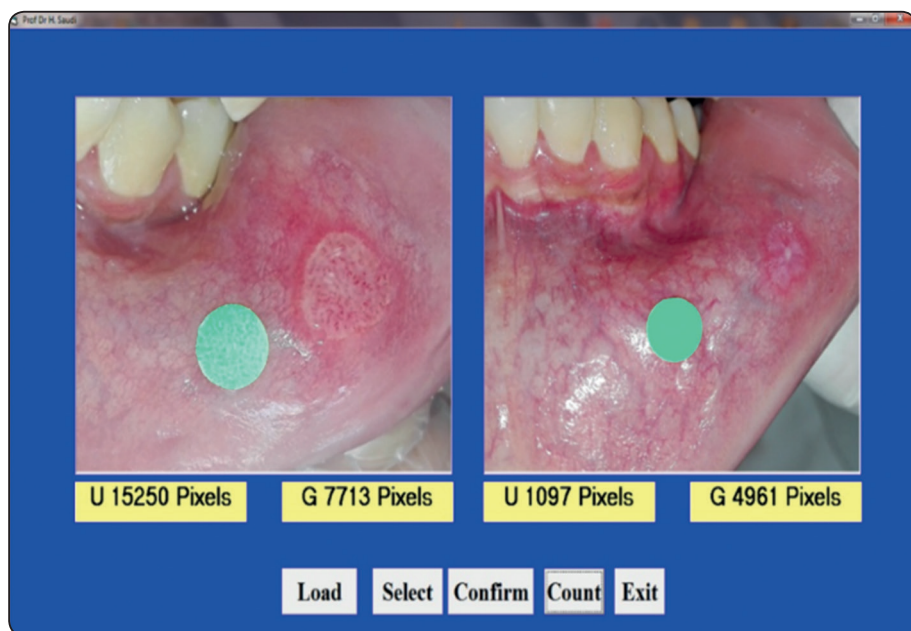


Fig. (2) The software layout for a case in group I (Laser group) displaying the pretreatment and post treatment ulcer images.

Labels indicate the number of pixels, as "U" for ulcer and "G" for green marker.

(pretreatment image) was taken as a reference for its surface area in the second image (post treatment image) either by magnification or surface reduction and the surface area of the ulcer in the pretreatment image was compared to that in the post treatment image so the percentage reduction or increase in the ulcer surface areas were controlled through the reference markers.

2-Subjective evaluation: Degree of pain sensation

- The degree of pain sensation was evaluated on visual analog scale (VAS).
- The degree of pain sensation was evaluated at:
  - Day 1: V1 VAS score at Base line
  - Day 5: V5 VAS score after complete laser application

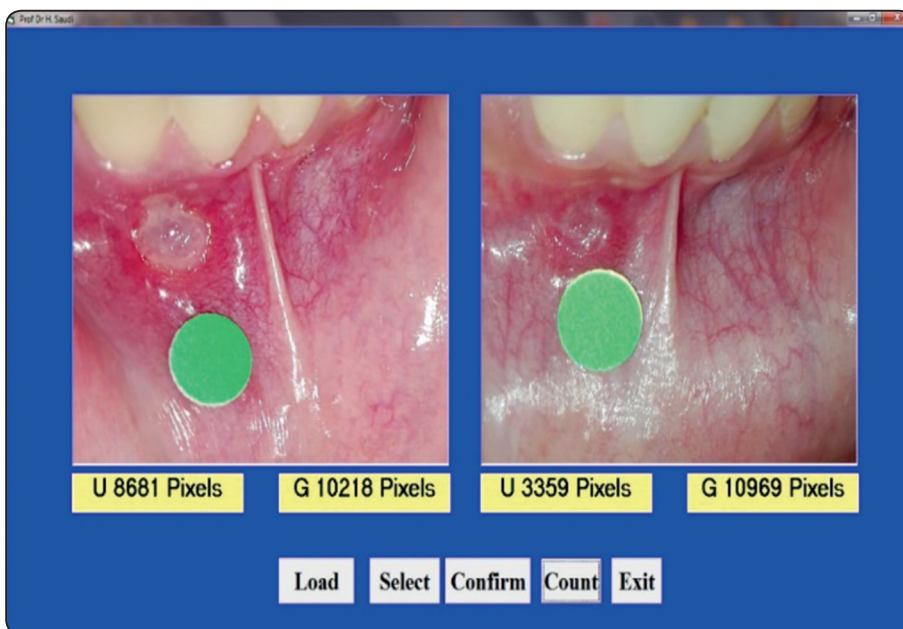


Fig. (3) The software layout for a case in group II (Omega group) displaying the pretreatment and post treatment ulcer images.

*Labels indicate the number of pixels, as “U” for ulcer and “G” for green marker.*

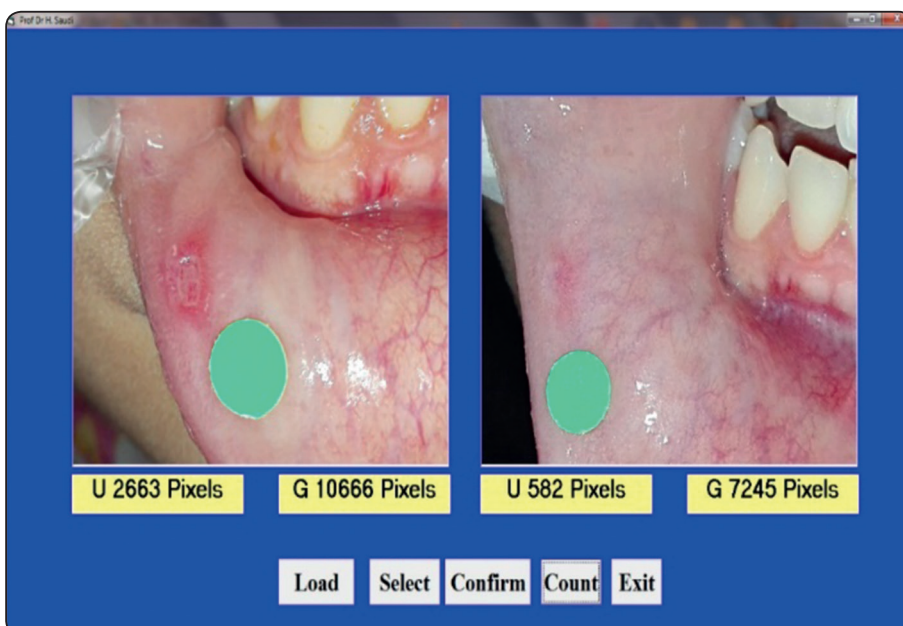


Fig. (4) The software layout for a case in group III (Omega plus group) displaying the pretreatment and post treatment ulcer images.

*Labels indicate the number of pixels, as “U” for ulcer and “G” for green marker.*

*28.3 pixels in each pixel;  
2.83 pixels in each 10 mm;  
nearly every 3 pixels for 1mm*



- The effectiveness index (EI) of pain improvement was calculated for each patient with the following formula.<sup>(18)</sup>

$$EI = [(V1 - V5) \div V1] \times 100\%.$$

- The effectiveness index was evaluated on a 4-rank scale.<sup>(19)</sup>

- (1) Heal: EI  $\geq$  95%;
- (2) Marked improvement: EI < 95%, but  $\geq$  70%;
- (3) Moderate improvement: EI < 70%, but  $\geq$  30%;
- (4) No improvement: EI < 30%.

## II: Long term clinical assessment

### 1-The average duration of ulcer episodes:

- At base line: Each participant was asked to estimate the average duration of ulcer episodes per month during the past year.
- The average duration of ulcer episodes was estimated by each participant monthly for the following 6 months

### 2-The number of new ulcer outbreaks:

- At base line: Each participant was asked to estimate monthly number of new ulcer outbreaks during the past year.
- The number of new ulcer outbreaks were estimated by each participant monthly for the following 6 months

**(B) Immunological assessment:** At Day 1: base line, then After 3 months and 6 months.

#### 1. Enzyme Linked Immunosorbent Assay (ELISA):

Peripheral blood samples were obtained from patients by venipuncture

Interleukin 2 was measured by using Human interleukin 2 ELISA (Enzyme-Linked) kit. It is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human interleukin 2 in human serum.

#### 2. Cell Flow Cytometric Analysis :

Venous blood samples were withdrawn with a disposable sterile plastic syringe from each subject and were used to analyze the expression CD4+CD25+ high T regulatory cells using flowcytometry.

#### Statistical analysis

The collected data were organized, tabulated and statistically analyzed using computer software Statistical Package for Social Science (SPSS).

## RESULTS

### (A) Clinical results:

#### (I) short term clinical parameters

- 1- **Objective evaluation:** Reduction of ulcers surface areas
- 2- **Subjective evaluation:** The effectiveness index of pain improvement

By comparing mean  $\pm$  SD reduction in the ulcer surface area and the effectiveness index of pain improvement (EI) between the study groups results showed statistically significant differences between GI and GII in favor of GI, also between GII and GIII in favor of GIII. No Statistically significant difference was found between Gr I and G III, see table 2.

#### (II) Long term clinical parameters

##### 1- The average duration of ulcer episodes.

##### 2- The number of new ulcer outbreaks.

#### The intra group comparison (see table 3)

- **GI:** showed that there was no statistically significant difference at any of assessment points as compared with baseline values.
- **GII and GIII:** The omega-3 regimen resulted in sustainable beneficial effects on those two clinical parameters with progressive

continuous shortening in ulcers duration and progressive continuous reduction in number of new ulcers and statistical significant difference at 3, 4, 5, and 6 months, at the 6-month follow-up, as compared with baseline values.

**The inter group comparison: (See table 4)**

- Showed that no significant differences were found when comparing the mean ± SD values at base line, 1<sup>st</sup> and 2<sup>nd</sup> month’s period of evaluation between the all groups.
- For the 3-, 4-, 5-, and 6-month follow-up appointments, the results showed significant difference between (GI), & (GII) in favor of (GII), and also the same significant difference was found between (GI), & (GIII) in favor of (GIII), with no significant difference regarding the comparison between (GII), & (GIII), which gave strong support to the suggested progressive sustainable beneficial effects of omega-3 therapy.

**(B) Immunological assessment:**

1. **IL-2 Concentration in serum.**
2. **CD4+CD25<sup>+</sup>high T regulatory cells.**

**Intra group comparison: (See table 5)**

- **GI:** showed no statistical significant difference in the mean ± SD values at base line 3 months and 6 months point of assessment.
- **GII and GIII:** sustained increase in cell number and decrease in IL2 concentration with statistical significant from base line to 3 months and 6 months.

**Inter group comparison: (See table 6)**

- Baseline measurements showed no statistical significant differences between all groups as evident by close similarity of their mean values (P > 0.05).
- At 3 months and 6 months points of evaluation results showed significant differences between GI and GII also between GI and GIII in favor of GII and GIII with no statistical significant differences between G II and G III.

TABLE (2) Shows inter group comparison of the mean± SD reduction in ulcer surface area and the EI of pain improvement for the study groups.

Parameter	The groups (Mean ± SD)		F test	P value
Reduction in ulcer surface area	G I (4352± 1222)	G II (2935% ± 965)	5.72	0.021*
	G I (4352 ± 1222)	G III (4418 ± 1105)		0.990
	G II (2935 ± 965)	G III (4418 ± 1105)		0.015*
EI of pain improvement	G I (87.01% ± 2.9)	G II (3.67 %± 3.99)	286.5	0.001*
	G I (87.01% ± 2.9)	G III (87.19% ± 3.43)		0.892
	G II (3.67% ± 3.9)	G III (87.19% ± 3.43)		0.001*
Statistically significant at the 5 % level of significance SD = standard deviation				

\* Statistically significant at the 5% level of significance. SD: Standard Deviation

TABLE (3) Intra group comparison of the mean $\pm$  SD values of average duration of ulcer episode and No. of new ulcers outbreak among the study groups at baseline and at 1-, 2-, 3-, 4-, 5-, and 6-month follow-up periods

The group		BL	1 <sup>st</sup> M	2 <sup>nd</sup> M	3 <sup>rd</sup> M	4 <sup>th</sup> M	5 <sup>th</sup> M	6 <sup>th</sup> M
Duration of ulcers Laser	Range	6 – 11	6 – 11	6 – 10	6 – 11	6 – 10	5 – 8	6 – 9
	Mean	7.07	7.07	7.60	7.53	7.40	6.73	7.00
	S. D	1.33	1.33	1.59	1.36	1.18	0.88	1.00
	P value			1.0	0.615	0.313	0.471	0.471
Duration of ulcers Omega	Range	5 – 9	5 – 9	5 – 10	3 – 9	3 – 7	0 – 5	0 – 5
	Mean	6.93	6.67	7.07	5.60	4.33	2.80	1.20
	S. D	1.16	0.98	1.71	1.72	1.05	1.61	1.66
	P value			0.615	0.801	0.001*	0.001*	0.001*
Duration of ulcers Laser & Omega	Range	6 – 7	6 – 7	5 – 9	4 – 6	3 – 5	0 – 5	0 – 4
	Mean	6.33	6.33	6.73	4.80	3.87	2.27	1.67
	S. D	0.49	0.49	1.53	0.86	0.52	1.79	1.72
	P value			1.0	0.362	0.001*	0.001*	0.001*
No. of new ulcers outbreak Laser	Range	4 – 8	3 – 8	3 – 7	3 – 7	4 – 7	3 – 7	4 – 8
	Mean	4.60	4.53	4.00	4.27	4.33	4.27	4.53
	S. D	1.06	1.13	1.07	0.96	0.82	0.88	1.06
	P value			0.856	0.104	0.364	0.468	0.364
No. of new ulcers outbreak Omega	Range	4 – 8	4 – 8	3 – 7	2 – 4	1 – 3	0 – 2	0 – 1
	Mean	4.87	4.73	4.40	2.53	1.67	0.87	0.40
	S. D	1.06	1.10	1.06	0.64	0.62	0.52	0.51
	P value			0.659	0.124	0.001*	0.001*	0.001*
No. of new ulcers outbreak Laser & Omega	Range	3 – 7	3 – 7	3 – 6	1 – 4	1 – 2	0 – 1	0 – 1
	Mean	4.67	4.40	4.33	2.47	1.73	0.80	0.40
	S. D	0.90	0.91	0.90	0.74	0.46	0.41	0.51
	P value			0.314	0.208	0.001*	0.001*	0.001*



TABLE (4) Shows inter group comparison of the mean± SD values of the duration of ulcers and No. of new ulcers outbreak among the study groups at baseline and at 1-, 2-, 3-, 4-, 5-, and 6-month follow-up periods

Time of assessment Average duration of ulcer episode	The groups (Mean ± SD)		F test	P value	Time of assessment No of new ulcers	The groups (Mean ± SD)		F test	P value
Base line	G I (7.07±1.33)	G II (6.93±1.16)	2.037	0.732	Base line	G I (4.60±1.06)	G II (4.87±1.06)	0.584	0.473
	G I (7.07±1.33)	G III (6.33±0.49)		0.065		G I (4.60±1.06)	G III (4.67±0.90)		0.857
	G II (6.93±1.16)	G III (6.33±0.49)		0.129		G II (4.87±1.06)	G III (4.67±0.90)		0.590
1 <sup>st</sup> M	G I (7.07±1.33)	G II (6.67±0.98)	2.042	0.277	1 <sup>st</sup> M	G I (4.53±1.13)	G II (4.73±1.10)	0.383	0.605
	G I (7.07±1.33)	G III (6.33±0.49)		0.053		G I (4.53±1.13)	G III (4.40±0.91)		0.730
	G II (6.67±0.98)	G III (6.33±0.49)		0.364		G II (4.73±1.10)	G III (4.40±0.91)		0.389
2 <sup>nd</sup> M	G I (7.60±1.59)	G II (7.07±1.71)	1.101	0.371	2 <sup>nd</sup> M	G I (4.00±1.07)	G II (4.40±1.06)	0.674	0.285
	G I (7.60±1.59)	G III (6.73±1.53)		0.149		G I (74.00±1.07)	G III (4.33±0.90)		0.372
	G II (7.07±1.71)	G III (6.73±1.53)		0.575		G II (4.40±1.06)	G III (4.33±0.90)		0.858
3 <sup>rd</sup> M	G I (7.53±1.36)	G II (5.60±1.72)	16.005	0.001*	3 <sup>rd</sup> M	G I (4.27±0.96)	G II (2.53±0.64)	24.854	0.001*
	G I (7.53±1.36)	G III (4.80±0.86)		0.001*		G I (4.27±0.96)	G III (2.47±0.74)		0.001*
	G II (5.60±1.72)	G III (4.80±0.86)		0.115		G II (2.53±0.64)	G III (2.47±0.74)		0.819
4 <sup>th</sup> M	G I (7.40±1.18)	G II (4.33±1.05)	60.031	0.001*	4 <sup>th</sup> M	G I (4.33±0.82)	G II (1.67±0.62)	82.780	0.001*
	G I (7.40±1.18)	G III (3.87±0.52)		0.001*		G I (4.33±0.82)	G III (1.73±0.46)		0.001*
	G II (4.33±1.05)	G III (3.87±0.52)		0.190		G II (1.67±0.62)	G III (1.73±0.46)		0.779
5 <sup>th</sup> M	G I (6.73±0.88)	G II (2.80±1.61)	40.634	0.001*	5 <sup>th</sup> M	G I (4.27±0.88)	G II (0.87±0.52)	145.086	0.001*
	G I (6.73±0.88)	G III (2.27±1.79)		0.001*		G I (4.27±0.88)	G III (0.80±0.41)		0.001*
	G II (2.80±1.61)	G III (2.27±1.79)		0.330		G II (0.87±0.52)	G III (0.80±0.41)		0.776
6 <sup>th</sup> M	G I (7.00±1.00)	G II (1.20±1.66)	69.791	0.001*	6 <sup>th</sup> M	G I (4.53±1.06)	G II (0.40±0.51)	156.442	0.001*
	G I (7.00±1.00)	G III (1.67±1.72)		0.001*		G I (4.53±1.06)	G III (0.40±0.51)		0.001*
	G II (1.20±1.66)	G III (1.67±1.72)		0.397		G II (0.40±0.51)	G III (0.40±0.51)		1.0
<p><i>Statistically significant at the 5 % level of significance SD = standard deviation</i></p>									

TABLE (5) Intra group comparison of the Mean  $\pm$  SD values of the IL-2 Concentration in serum and number of CD4+CD25+ high T regulatory cell among the study groups at the 3, 6 months

The groups	Time of assessment		F test	P value
IL2 concentration Laser Group I	base line (91.52 $\pm$ 1.71)	3 months (91.32 $\pm$ 1.42)	2.285	0.798Ns
	base line (91.52 $\pm$ 1.71)	6 months (90.00 $\pm$ 2.00)		0.060Ns
	3 months (91.32 $\pm$ 1.42)	6 months (90.00 $\pm$ 2.00)		0.099Ns
IL2 concentration Omega Group II	base line (90.90 $\pm$ 1.67)	3 months (60.25 $\pm$ 2.55)	1463.841	0.001*
	base line (90.90 $\pm$ 1.67)	6 months (43.70 $\pm$ 1.57)		0.001*
	3 months (60.25 $\pm$ 2.55)	6 months (43.70 $\pm$ 1.57)		0.001*
IL2 concentration Laser and Omega Group III	base line (91.88 $\pm$ 2.21)	3 months (60.90 $\pm$ 1.66)	1471.645	0.001*
	base line (91.88 $\pm$ 2.21)	6 months (43.1 $\pm$ 2.18)		0.001*
	3 months (60.90 $\pm$ 1.66)	6 months (43.1 $\pm$ 2.18)		0.001*
CD Cells NO Laser Group I	base line (18.13 $\pm$ 1.55)	3 months (18.07 $\pm$ 1.62)	0.009	0.910Ns
	base line (18.13 $\pm$ 1.55)	6 months (18.07 $\pm$ 1.62)		0.910Ns
	3 months (18.07 $\pm$ 1.62)	6 months (18.07 $\pm$ 1.62)		1.0Ns
CD Cells Omega Group II	base line (18.27 $\pm$ 1.53)	3 months (20.73 $\pm$ 1.62)	27.001	0.012*
	base line (18.27 $\pm$ 1.53)	6 months (22.73 $\pm$ 1.83)		0.001*
	3 months (20.73 $\pm$ 1.62)	6 months (22.73 $\pm$ 1.83)		0.002*
CD Cells Laser and Omega Group III	base line (18.33 $\pm$ 1.63)	3 months (20.80 $\pm$ 1.70)	27.2016	0.008*
	base line (18.33 $\pm$ 1.63)	6 months (22.93 $\pm$ 1.79)		0.001*
	3 months (20.80 $\pm$ 1.70)	6 months (22.93 $\pm$ 1.79)		0.004*
Statistically significant at the 5 % level of significance; SD = standard deviation				

TABLE (6) Inter group comparison of the Mean ± SD values of the IL-2 Concentration in serum and number of CD4+CD25+<sup>high</sup> T regulatory cell among the study groups at the 3, 6 months

The parameter	The groups (Mean ± SD)		F test	P value
IL-2 Concentration in serum 3 months	G I (91.32 ± 1.42)	G II (60.25 ± 2.55)	836.781	0.001*
	G I (91.32 ± 1.42)	G III (60.90 ± 1.66)		0.001*
	G II (60.25 ± 2.55)	G III (60.90 ± 1.66)		0.460
IL-2 Concentration in serum 6 months	G I (90.00 ± 2.00)	G II (43.70 ± 1.57)	1935.294	0.001*
	G I (90.00 ± 2.00)	G III (43.1 ± 2.18)		0.001*
	G II (43.70 ± 1.57)	G III (43.1 ± 2.18)		0.494
CD4+CD25+ high T regulatory cell proportion 3 months	G I (18.07 ± 1.62)	G II (20.73 ± 1.62)	13.404	0.001*
	G I (18.07 ± 1.62)	G III (20.80 ± 1.70)		0.001*
	G II (20.73 ± 1.62)	G III (20.80 ± 1.70)		0.912
CD4+CD25+ high T regulatory cell proportion 6 months	G I (18.07 ± 1.62)	G II (22.73 ± 1.83)	37.094	0.001*
	G I (18.07 ± 1.62)	G III (22.93 ± 1.79)		0.001*
	G II (22.73 ± 1.83)	G III (22.93 ± 1.79)		0.756
<i>Statistically significant at the 5 % level of significance; SD = standard deviation</i>				

**DISCUSSION**

RAS is a widespread and extremely bothersome disorder, as even in the case of small ulcers which may not exceed 1-2 mm in diameter, the patient may feel severe pain added to that it's recurrent pattern and impairment of oral functions accompanying it.<sup>(6)</sup>

Many factors supported the choice of LLLT as a treatment option for RAS such as the simplicity and safety by which this treatment modality is characterized.<sup>(1)</sup> Laser therapy is said to be effective in tissue repair and pain control,<sup>(20)</sup> and had been suggested to reduce or eliminate inflammation,<sup>(21)</sup> also significantly reduced discomfort when eating, drinking, and brushing teeth.<sup>(1)</sup> It is considered non-invasive therapy, required no anesthetic agent and had no observable adverse effects on the mucosa.<sup>(22)</sup>

*Serhan*<sup>(23)</sup> suggested that omega-3 polyunsaturated fatty acids had anti-inflammatory and immune-regulatory actions that can be attributed to the production of the proresolving lipid mediators,

resolvins and protectins, during their metabolism, these lipid mediators can enhance resolution of inflammation and help healing process.<sup>(23)</sup>

➤ The results of the current study showed that of LLLT result in rapid healing with marked reduction of the ulcer surface areas. Such results agreed with *De Souza, et al.*,<sup>(24)</sup> who proved the positive effect of LLLT on healing in RAS. They used triamcinolone acetonide and GaAlP diode laser. Both treatments were applied until the disappearance of the lesions. The ulcer surface areas were determined every day, by using a millimeter ruler. They concluded that LLLT can induce healing of RAS with rapid reduction of ulcer surface area compared to the corticoid group.

Our results agreed with a randomized, controlled clinical trial that was conducted by *Saudi et al.*,<sup>(17)</sup> to compare CO2 laser with cyanoacrylate tissue adhesive as local treatment modalities for RAS. A surface area measuring software had been used

to trace the healing pattern of the ulcers from the digitalized photographs which were loaded into the computer memory. Their results stated that CO<sub>2</sub> laser therapy produced healing ulcers with minimal edema.<sup>(17)</sup>

Our results agreed with *Aggarwal et al.*,<sup>(25)</sup> they performed a research aim to clinically evaluate the LLLT as treatment option for RAS. The test group used diode laser 810 nm and the other one in to control group received no treatment. The sizes of the present ulcers were measured using periodontal probe before and after the treatment. The results showed that the LLLT group had a statistically significant reduction in ulcer surface area as compared to the control group.

Our results also agreed with *Elshenawy H.M et al.*,<sup>(26)</sup> their results indicated that application of diode laser (970nm) can be considered as an effective adjunctive therapy help in accelerate healing and reducing ulcers surface area. There was a statistically significant decrease in mean ulcers surface area after treatment.

Agreed with our results *Prasad and Pai* <sup>(22)</sup> their study included two groups, study group treated with CO<sub>2</sub> laser and the other group used as control received placebo (inactive laser probe). They concluded that LLLT using CO<sub>2</sub> laser in recurrent aphthous recurrent aphthous stomatitis (RAS) accelerate healing and decrease healing time.

The results of the present study was on a line with *Zand et al.*,<sup>(27)</sup> Their study included 10 patients each patient have suffering from two separate ulcers, one of them used as study where LLLT was applied and the other one used as placebo. They found that the shorter healing time present for the ulcers treated by laser compared to those where no active laser applied

➤ Our results also showed that LLLT has an analgesic effect in patients with recurrent aphthous stomatitis (RAS), it reduced the pain sensation with

significant change in the EI of pain improvement. Such results agreed with *De Souza et al.*,<sup>(24)</sup> they compare the topical application of corticosteroid and the use of LLLT. They concluded that the use of LLLT resulted in analgesic effects with regard to RAS. Also *Aggarwal et al.*,<sup>(25)</sup> as they observed Immediate complete pain relief following the LLLT application, they concluded that LLLT was effective in pain relieving during the management of RAS.

*Takashi et al.*,<sup>(28)</sup> suggested that The analgesic effect of laser therapy can be attributed to the inhibitory effect of nerve conduction due to a reversible conformational change in the voltage-gated Na-K channels, caused by LLLT, an effect resemble local anesthesia.

Agreed with our results *Albrektson, et al.*,<sup>(1)</sup> Their study was conducted on 40 patients were suffering from RAS divided in two groups the study group, had received LLLT (Diod laser 809 nm, 80 seconds per treatment) daily for three consecutive days, and the control group, did not receive laser treatment. They found that significant pain relieving, and significant reduction in the difficulty of drinking, speaking, eating, and teeth brushing in the study group compared to the control group.

*Prasad and Pai* <sup>(22)</sup> Pain levels in RAS patients were evaluated immediately, and after 24 hours a single session of carbon dioxide (CO<sub>2</sub>) laser application. Mean pain scores in the laser group were significantly reduced immediately after treatment compared with pretreatment. While the placebo group showed little difference in pain scores between pretreatment and immediately after treatment.

In our study daily omega-3 treatment produced a significant reduction in number of ulcers, duration of ulcers. The helpful positive clinical effects of the omega-3 supplements can be attributed to their suggested partial inhibitory effect of some aspects of the inflammatory process such as: Leucocyte chemotaxis, adhesion molecule expression and

leucocyte endothelial adhesive interactions, production prostaglandins and leukotrienes from arachidonic acid, production of inflammatory cytokines and T cell reactivity.<sup>(29)</sup>

Such results agreed with *ElKhouli and El-Gendy EA.*,<sup>(30)</sup>. The results showed significant reduction in number of ulcers, healing time, and level of pain, by 3 months that persisted for 6 months. The results of the current study are in accordance with *Duffy et al.*,<sup>(31)</sup> they suggested that taking omega-3 would significantly influence the inflammatory symptoms of systemic lupus erythematosus.

Agreed with the results of the current study *Nosratzahi and Akar.*,<sup>(5)</sup>. They used omega 3 capsules to treat patients were suffering from RAS. They recorded the pain sensation, size, duration and recurrence rate of ulcers, weekly in the first month and then monthly in the next 5 months. In the omega-3 group significant change was observed in the aforementioned variables compared with the placebo group.

In the study under discussion omega 3 result in anti-inflammatory and immune-omodulatory effects evident by the significant decrease in IL2 concentration and increase in CD4+CD25+<sup>high</sup> T regulatory cells proportion, these actions had been translated into clinical effects.

The anti-inflammatory effects of omega-3 fatty acids have been proven by several animal studies through their role in increasing EPA-derived eicosanoids with less pro-inflammatory effects, and increasing the generation of EPA- and DHA-derived anti-inflammatory resolvins, decreasing arachidonic- acid-derived eicosanoids with pro-inflammatory properties, in addition to their ability to decrease the levels of the inflammatory cytokines.<sup>(32, 33)</sup>

Our results are supported by *Lewkowicza. et al.*,<sup>(34)</sup> they conducted an immunological study on people who suffer from RAU in a repeated manner,

They found that there is some immune imbalance which can affect the maintenance of peripheral tolerance represented in the predominance of IL-2 and other proinflammatory cytokine such as IFN, TNF.

agreed with our results *Buno et al.*,<sup>(35)</sup> they had conducted an immunologic research on RAU patients, with Final conclusion that there is an imbalance in cytokine distribution in oral mucosa in RAU has shown in elevated levels IL-2, and other cytokines in lesional and non-lesional mucosa of RAU patients than in healthy individual. They explained that this predominance of pro-inflammatory cytokine resulted from increased production both by the cells from peripheral blood and by oral mucosa, and result in maturation and activation of many types of immune cells.

*Szamel M.*,<sup>(36)</sup> had conducted a study to assess the role of polyunsaturated fatty acids in regulation of T cells function and proliferation results stated that incorporation of polyunsaturated fatty acids in cellular plasma membrane is necessary for activation of early signal for T cell activation and proliferation.<sup>(37)</sup>

Our results is supported by *stentz FB et al.*,<sup>(38)</sup> they conducted an in vitro study to evaluate the effect of various types of fatty acids with different concentrations their overall, data suggested that fatty acids have the ability to modulate T cells functions and induce their proliferation.<sup>(37)</sup>

*Byleveld et al.*,<sup>(39)</sup> have demonstrated that mice fed fish oil have increased T cell proliferation the effect of a fish oil diet on lymphocyte proliferation was investigated. Mice were fed fish oil beef tallow for 14 days before intranasal challenge with influenza virus. Bronchial lymph node cell proliferation to virus was significantly higher. In mice fed fish oil, spleen cell proliferation to virus was also significantly higher.

## CONCLUSIONS

- LLL application induce tissue repair and stimulate quick healing of ulcers compare to using omega alone
- A potential therapeutic role can be achieved using omega-3 capsules in treatment of RAS with beneficial clinical effects increasing over time.
- Using LLLT with omega 3 fatty acid is the best choice for management of RAS as it gives the most favorable clinical and laboratory results.
- The used Visual Basic Program was effective in calculating the difference of ulcers surface areas and the digital data enabled fair statistical analysis.

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