

## TREATMENT OF DISEASED MICE WITH CARCINOMA BY ACTIVATING THE IMMUNE CELLS BY SOFT LASER WITHOUT DRUGS

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### Abstract

Regional immune response with mammary gland carcinoma has been studied statistically and histologically. However, the prognostic value remains conflicting. This work was conducted on 30 laboratory mice including healthy mice, and diseased mice with carcinoma not treated with laser (control group) and diseased mice with carcinoma treated with laser. The number of immune cells of the lymph nodes was estimated by using a mesh lens which inserted into the eyepiece of the microscope.

The results showed significant increase in the number of immune cells of the lymph node of the diseased mice with carcinoma treated with laser in comparison with that of healthy mice and diseased mice with carcinoma not treated with laser respectively.

The histological examination of the prepared sections of the lymph nodes of the diseased mice with carcinoma treated with laser showed various changes in the structure of the immune cells such as multiplication of the nucleus and cytoplasm cleavage due to laser stimulation.

The gross observations proved that there was a decrease in tumor size of the diseased mice with carcinoma after treatment with laser in comparison with not treated with laser.

The aim of the study was to evaluate the effect of low level laser therapy (LLLT) on increasing the response of immune system by stimulating the lymph node action in order to inhibit cancer cell activity which leads to decrease the tumor size in diseased mice.

**Keyword:** laser treatment, immune response, carcinoma.

### Introduction

Lymph nodes are encapsulated spherical or kidney-shaped organs. The nodes are found in the axilla and the groin, along the great vessels of the neck, and in large numbers in the thorax and abdomen, especially in mesenteries. Each node contains an outer cortex, an inner cortex, and a medulla. Within the cortical lymphoid tissue are spherical structures called lymphoid nodules. These nodules are rich in B lymphocytes that react with antigens, increase in size, and proliferate by mitosis, resulting in large basophilic cells with prominent nucleoli called immunocytes (1). A large majority of lymphocytes in lymph nodes are static. When the lymph nodes elicit the immune response, then the nodes enlarge in size (reactive hyperplasia) (2).

Low level laser therapy (LLLT) has been used successfully in biomedicine and some of the results are thought to be related to cell proliferation. The effect of LLLT on cell proliferation is debatable because studies have found both an increase and a decrease in proliferation of cell cultures. Cell culture is an excellent method to assess both effects and

dose of treatment (3). In both soft tissue and connective tissue injuries, LLLT can increase the final tensile strength of the healed tissue. By increasing the amount of collagen/production synthesis and by increasing the intra and inter-molecular hydrogen bonding in the collagen molecules, laser therapy contributes to improve tensile strength (5). LLLT has proved to be effective in treating and repairing biologically damaged tissue and to reduce pain. and also proven to be an efficient method for the prevention of oral mucositis (6). Several applications of lasers in clinical procedures for dental hard tissues are either currently in practice or being developed since newer wavelengths as well as different methods and delivery systems are being applied in the field of dentistry. In endodontic therapy lasers have been used as treatment coadjuvant with reference to both, low intensity laser therapy (LILT) and high intensity laser treatment (HILT) to increase the success rate of the clinical procedures. Low intensity laser therapy has the ability to produce analgesic, anti-inflammatory and biomodulation effects on the irradiated soft

tissue thereby improving the wound healing process and giving the patient a better condition of the postoperative experience (7).

The aim of the study was to evaluate the effect of low level laser therapy (LLLT) on increasing the response of immune system by stimulating the lymph node action in order to inhibit cancer cell activity which leads to decrease the tumor size in diseased mice.

### Materials and Methods

Thirty mice were used in this study: All inoculated with mammary gland carcinoma, and divided into five groups each of five mice.

The nodes appeared after 11 days of successful inoculation and the animals were ready for the experiment. The animals under study were anesthized and irradiated with laser that directed towards the cervical lymph node of the animal with spot diameter of 0.5cm and a distance from the laser source to the animal lymph node was 1cm and as follows:

Group A: irradiated with laser, for 15 minutes continuously, twice daily with time interval of one hour, and for five days.

The same procedure was applied for the other groups (B, C, and D) with different duration time (15, 25, and 35) days respectively and the fifth group (E) was kept as control group (not irradiated with laser). At the end of each duration time of treatment, the lymph nodes were taken for gross observation.

Sections of lymph nodes were made by using a routine procedure, and prepared for histological examination by light microscope. Photographs were made at different magnification. The number of immune cells of the lymph nodes was estimated by using mesh lens which inserted into the eyepiece of the microscope (8).

The laser type was Ga-Al-P (Gallium-Aluminum- phosphors) of wave length 810 nm in continuous mode, and of 3mw power. The tumor size of each node of the animals under study was measured by vernier, daily, starting from nodes appearance until the last day of the experiment (35 days).

The control group: inoculated with carcinoma and not irradiated with laser as mentioned above. Data was translated into code using as especially design of need code sheet and inserted into a computer system using spss version 13 software and time

dependent the most obvious and strong positive linear time dependent parameter as shown in Table (1, 2, and 3).

### Results and Discussion

The immune system comprises structures and cells that are distributed throughout the body: its principal function is to protect the body from invasion and damage by microorganism and foreign substances (4).

The results showed significant increase in the number of immune cells found in the lymph nodes of the diseased mice with carcinoma not treated with laser (control group) which was 12 as shown in tab. (2), whereas the number of immune cells of the lymph nodes of the healthy mice in the baseline was 7 as shown in tab. (1). This increase was due to immune response against cancer disease. The number of the immune cells of the lymph nodes of the diseased mice with carcinoma treated with laser in the control group was increased gradually to 16 due to laser stimulation as shown in tab. (3). And so on, there was always a gradual increase in the number of the immune cells of the lymph nodes of the diseased mice with carcinoma treated with laser from the beginning of the experiment until the last day of the experiment 35 which reached to 42 as shown in tab. (3) in comparison with the results of Tables (1 and 2), respectively. On the other hand, the histological examination of the prepared sections of the lymph nodes of the diseased mice with carcinoma treated with laser showed various changes in the structure of the immune cells such as multiplication of the nucleus and cytoplasm cleavage as shown in Figs. (2 and 3). Also, as shown in Fig.(1), the gross observations proved that there was a decrease in tumor size of the diseased mice with carcinoma after treatment with laser (right arrow ) in comparison with not treated with laser (left arrow).

It could be concluded from the results obtained as mentioned above that laser played an important role in increasing the immune response against cancer disease including an increase in the number of the immune cells of the lymph nodes and caused various changes in the structure of these cells such as multiplication of the nucleus and cytoplasm cleavage.

**Table (1)**  
**Immune Cells in healthy mice lymph nodes.**

	Duration time				
	Baseline	A 5 days	B 15 days	C 25 days	D 35 days
<b>Rang</b>	5-9	10-14	12-14	12-14	12-14
<b>Median</b>	7	12	14	14	14

$P < 0.001$

$N = 5.$

**Table (2)**  
**Immune Cells in mice infected with carcinoma.**

	Duration time				
	A 5 days	B 15 days	C 25 days	D 35 days	E Control group
<b>Rang</b>	14-20	20-25	25-28	26-30	10-14
<b>Median</b>	16	22	27	28	12

$P < 0.001$

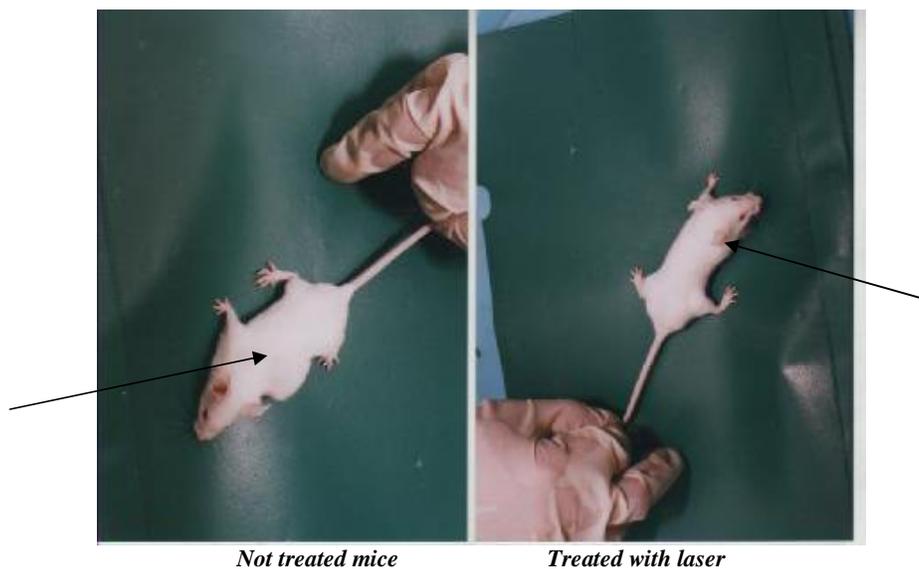
$N = 5.$

**Table (3)**  
**Immune Cells of mice infected with carcinoma and treated with laser.**

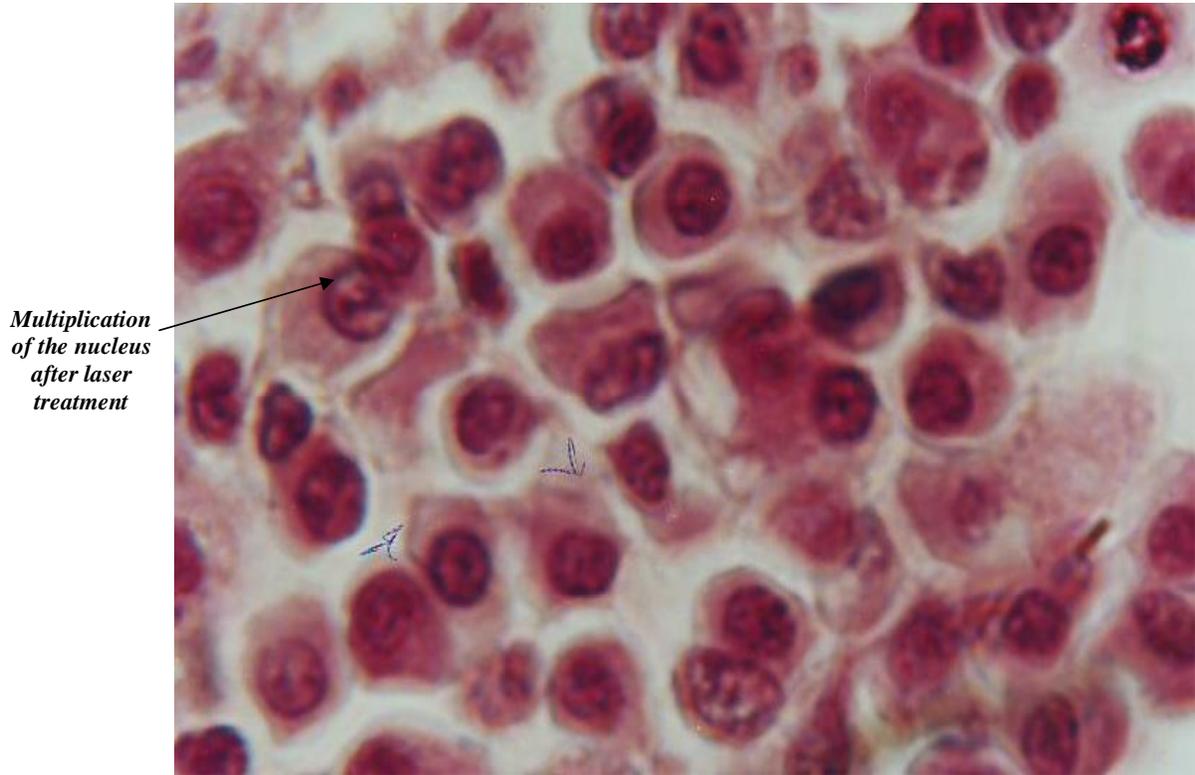
	Duration time				
	A 5 days	B 15 days	C 25 days	D 35 days	E Control group
<b>Rang</b>	20-24	28-32	30-36	40-45	14-22
<b>Median</b>	22	30	32	42	16

$P < 0.001$

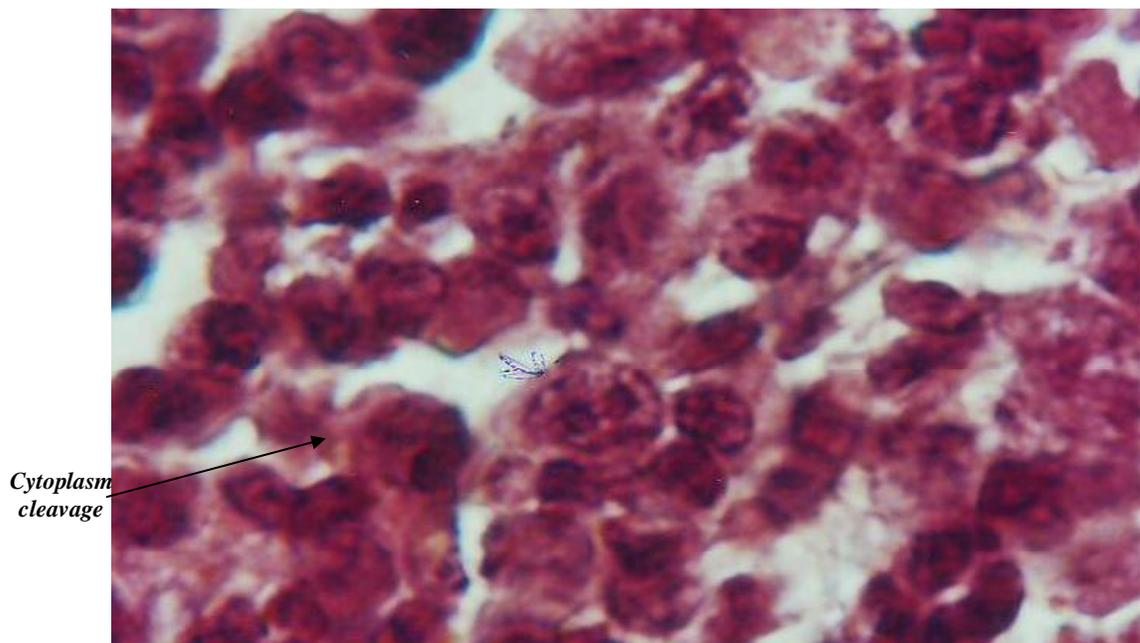
$N = 5.$



**Fig.(1):**Show gross observations of the diseased mice before and after treatment with laser.



*Fig.(2): Show the activation in the cell after laser treatment.*



*Fig.(3) : Show cytoplasm cleavage after laser treatment.*

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## الخلاصة

لقد تمت دراسة الاستجابة المناعية المحفزة باستخدام العلاج بالليزر واطى القدرة لفئران مختبرية محقونة بسرطان الغدة اللببية نسيجيا واحصائيا. اجري العمل على ثلاثين فارة مختبرية تضمنت مجموعة من الفئران السليمة ومجموعة من الفئران المصابة بالسرطان غير المعالجة بالليزر (مجموعة السيطرة) ومجموعة من الفئران المصابة بالسرطان و المعالجة بالليزر. تم حساب عدد الخلايا المناعية للعقد اللمفية باستخدام عدسة شبكية تم وضعها في العدسة العينية للمجهر.

اظهرت النتائج زيادة ملحوظة في عدد الخلايا المناعية للعقد اللمفية للفئران المصابة بالسرطان المعالجة بالليزر مقارنة بعدد الخلايا المناعية للعقد اللمفية للفئران السليمة والفئران المصابة بالسرطان غير المعالجة بالليزر (مجموعة السيطرة).

اظهر الفحص النسيجي لمقاطع العقد اللمفية للفئران المصابة بالسرطان والمعالجة بالليزر حدوث تغيرات مختلفة في تركيب الخلايا المناعية مثل انقسام النواة وحدث انشطار في السايوتوبلازم كنتيجة لتحفيز بالليزر.

اثبتت المشاهدات العيانية بان هنالك صغرى حجم الورم للفئران المصابة بالسرطان بعد معالجتها بالليزر مقارنة بتلك غير المعالجة بالليزر.

كان الهدف من هذه الدراسة هو معرفة تاثير العلاج بالليزر واطى القدرة على زيادة استجابة الجهاز المناعي بواسطة تحفيز فاعلية العقدة اللمفية لغرض تثبيط نشاط الخلايا السرطانية التي ادت الى صغر حجم الورم في الفئران المصابة.

من هذه الدراسة امكن الاستنتاج بان الليزر اداة فعالة في تحفيز الخلايا المناعية الموجودة في العقدة اللمفية والتي قامت بمهاجمة الخلايا السرطانية موقعا وبالنتيجة ادى الى صغر حجم الورم في الحيوانات المريضة.