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# Study the Effect of Low Level Laser (LLL) on the Histological Changes of Lymph Node Tissue in Mice Inoculated with Mammary Gland Carcinoma

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## Abstract :

This study was conducted on normal mice. The mice were divided into three groups of five mice each (A,B and C ) and two control groups of three mice each. The second control group and the three other groups (A,B and C) of mice were inoculated with mammary gland carcinoma. After (11) days of successful tumour growth, the tumours appeared in different parts of the animal bodies in accordance with the gross observations. All the animal groups (A,B and C) under study were irradiated with laser in different duration times (1-3) weeks consecutively. According to the histological sections of the lymph nodes of the animals of the groups (A,B and C) the results showed various histological changes in the lymphocytes such as increased size and multiplication of the nuclei, cytoplasm cleavage in group (B) animals in comparison with the histological changes which were more abundant in the lymphocytes of the lymph nodes of group (C) animals that irradiated with laser of duration time of three weeks, whereas the histological changes that occurred in the lymphocytes of the lymph nodes of both group (A) animals which irradiated with laser of duration time of one week and the second control group of animals which inoculated with mammary gland carcinoma (non-irradiated with laser) were multiplication of the nuclei in some lymphocytes of the lymph nodes. The aim of this study was to evaluate the effect of low level laser (LLL) on the histological changes by stimulating the lymph node action in order to inhabit cancer cells activity.

***Key words: Low Level Laser, Lymph Node, Histological Changes after Laser Treated.***

## Introduction:

Low level laser therapy (LLL) is also known as "soft laser therapy" and bio-stimulation. The use of LLLT in health care has been documented in the literature for more than three decades. Numerous research studies have demonstrated that LLLT is effective for some specific applications in dentistry (1). A problem in dissecting this literature is the variation in methodology and dosimetry between different studies. Not only have a range of different wavelengths been examined, but exposure times and the frequency of treatments also vary. The inclusion of sham-irradiated controls in clinical studies is an important element, since placebo effects can be important, particularly in terms of the level of pain experienced and reported following treatment (1). Low level laser therapy has a range of dental, medical, physiotherapy, and veterinary applications. The latter group is of some interest, since when used in animals the possibility of any placebo effects of treatment (for example, on the perceptions of pain or discomfort) can be eliminated completely. Low Level Laser therapy benefits have been reported in both small and large animals (2,3). Early epithelialization, increased fibroblastic reactions, leucocytes infiltration, and neovascularization are seen in wounds irradiated using LLLT. Because of the overall impact of these influences, the time required for complete wound closure is reduced. Moreover, the mean breaking strength, as measured by the ability of the wound to resist rupture against force, is increased (4). Following LLLT, neural tissues show reduced synthesis of inflammatory mediators, as well as more rapid maturation and regeneration, particularly axonal growth. Low Level Laser therapy has also been proven to reduce pain in patients suffering from post-herpetic neuralgia, from cervical dentinal hypersensitivity (5), or from periodontal pain during orthodontic tooth movement (6). Low Level Laser therapy has proven to be very effective when applied to "trigger points" i.e., myofascial zones of particular sensibility and of high-

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est projection of focal pain points, due to ischaemic conditions. Results obtained after clinical treatment of patients with pain of varying origin (headaches and facial pain, skeletomuscular ailments, myogenic neck pain, shoulder and arm pain, epicondylitis humery, tenosynovitis, low back and radicular pain, Achilles tendinitis) using LLLT have been particularly promising. In fact, in one study, the author commented that the results "were better than we had ever expected" (7). In vivo studies of the analgesic effect of LLLT on nerves supplying the oral cavity have demonstrated that LLLT decreases the firing frequency of nociceptors, with a threshold effect seen in terms of the irradiance required to exert maximal suppression (8,9). In vivo, LLLT selectively inhibits a range of nociceptive signals arising from peripheral nerves, including neuronal discharges elicited by pinch, cold, heat stimulation, and chemical irritation (10, 11). In contrast, neuronal discharges induced by brush stimulation are not affected by LLLT. There is some evidence that laser irradiation may selectively target fibers conducting at slow velocities, particularly afferent axons from nociceptors (12, 13). It is understood that the behavior of cancerous cells significantly differs from that of normal ones.It is also well accepted that the observation of LLLT beneficial effects requires some type of deficiency because normal cells do not show any changes in their function after irradiation (14).The low level helium-neon (He-Ne) laser therapy during the radiotherapy treatment was found to be effective in preventing and treating the mucositis in head and neck cancer patients (15).

## Material and Methods:

Twenty one normal mice were used in this study (aged two months and body weight 150 gm). They were divided into three groups of five mice each (A, B and C) and two control groups of three mice each. This work was carried out in Iraqi centre for cancer and medical genetics research.

All the animal groups (A, B and C) were inoculated with mammary gland carcinoma (considered as a cell line), given as a gift for this work from the Iraqi centre that above mentioned. The same was done for the animals of the second control group (used as marker for comparison). After 11 days of successful transplantation (tumour growth), the tumours appeared and the animals were ready for the study in accordance with the gross observations.

All the animal groups (A,B and C) were anesthetized and irradiated with laser that was directed towards the cervical lymph nodes of the mice under study with a spot diameter of 1 cm and a distance from the laser source of 1 cm as in the following:-

Group A: irradiated with laser for 20 minutes continuously, twice daily with a time interval of one hour each and for one week. Same procedure was applied for the other groups (B and C) with different duration time( two weeks and three weeks) consecutively. The second control group of animals inoculated with mammary gland carcinoma (non-irradiated with laser and used as marker for comparison) as above mentioned. The first control group of normal mice (not inoculated with mammary gland carcinoma). At the end of each duration time of irradiation, two animals from each group under study were selected randomly and prepared for histological study. Sections of lymph nodes were made by using a routine procedure (16), and examined by light microscope. Photographs were made at particular magnification (40x).

The laser type used in this study was laser device of (Ga-P) (Gallium- Phosphorus) of wave length of 850 nm which made in the University of Technology (Iraq) worked in pulse mode of one second duration time.

## Results:

This study was conducted on normal mice. The mice were divided into three groups of five mice each (A, B and C) and two control groups of three mice each. According to the histological sections of the lymph nodes of the animals of the groups (A, B and C) the results showed various histological changes in the lymphocytes such as increased size and multiplication of the nuclei, cytoplasm cleavage. In the second control group of animals which inoculated with mammary gland carcinoma (non-irradiated with laser)and in group (A) animals which irradiated with laser of duration time of one week, the results showed multiplication of the nuclei in some lymphocytes of the lymph nodes as shown in figs (2 and 3) consecutively, whereas the histological changes that occurred in the lymphocytes of the lymph nodes of group (B) animals which irradiated with laser of duration time of two weeks were abundant such as increased size and multiplication of the nuclei, cytoplasm cleavage as shown in fig (4) and the histological changes that occurred in the lymphocytes of the lymph nodes of group (C) animals which irradiated with laser of duration time of three weeks were more abundant in comparison with that of group (B) animals such as increased size and multiplication of the nuclei, cytoplasm cleavage as shown in fig(5).

## 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 (17).This histological study has emphasized that there was a correlation between the duration time of irradiation of laser and the various histological changes that occurred in the structure of the lymphocytes of the lymph nodes of group (B) animals such as increased size and multiplication of the nuclei, cytoplasm cleavage as shown in fig (4) in comparison with the histological changes which were more abundant in the lymphocytes of the lymph nodes of group (C) animals as shown in fig (5), whereas the histological changes that occurred in the lymphocytes of the lymph nodes of group (A) animals were multiplication of the nuclei in some lymphocytes as shown in fig(3).

It could be said that with LLL, the immune ability increased and made lymphocytes activated and proliferate more quickly due to duration time of irradiation of laser that increased consecutively from 1-3 weeks as in the

groups (A,B and C) animals. In the second control group of animals which inoculated with mammary gland carcinoma (non-irradiated with laser), showed multiplication of the nuclei in some lymphocytes of the lymph nodes as shown in fig (2).This was due to immune response against cancer disease .The laser action can be described by increasing metabolic activity.

Furthermore, when the laser light is applied at an appropriate dose, laser can stimulate cell functions that are vital for the progress and resolution of the healing process via tissue biostimulation, such as increased mitochondrial ATP production, lymphocyte and mast cell activation , and proliferation of fibroblasts and other cells , besides promoting analgesia and anti-inflammatory effects (18,19).AS previously stated , the action of laser on tissues depends on the duration of emission of the different energy densities, and on the application area. Therefore, if these parameters are not duly verified and /or calibrated, treatment may be ineffective, compromising therapeutic success (20). That confirmed this study of histological changes of the lymph node tissue.

Last, but not least, it could be concluded from the results obtained as above mentioned that laser caused histological changes in the lymphocytes of the lymph nodes under study as an immune response against cancer disease such as increased size and multiplication of the nuclei, cytoplasm cleavage. Also these results may encourage the researchers to do further studies to use low level laser (LLL) as a therapeutic agent for cancer experimently.

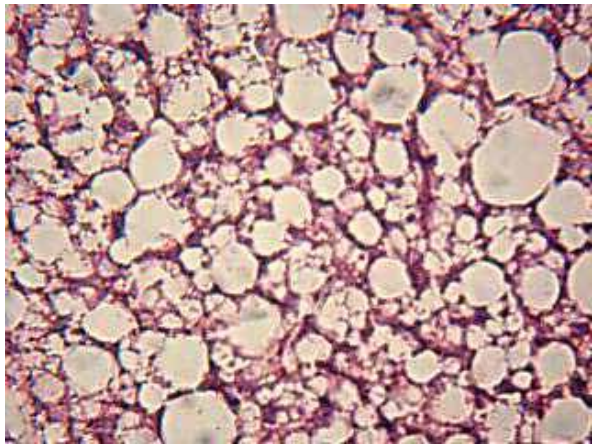

*Fig.1 40x Transverse section of lymph node tissue lymphocytes (in mice) First Control Group(normal)*

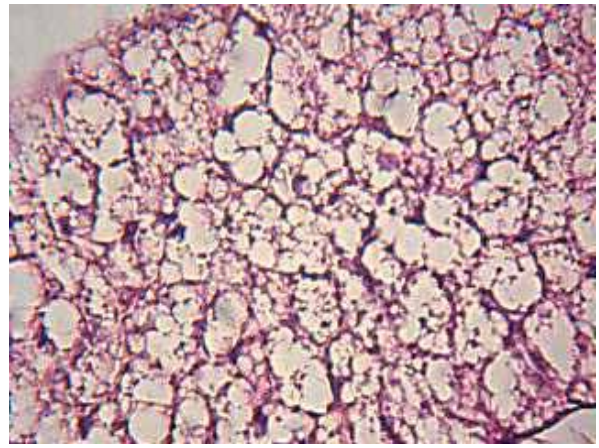

*Fig.2 40x Transverse section of lymph node tissue (lymphocytes) in mice showing multiplication of the nuclei in some lymphocytes Second Control Group (inoculated with mammary gland carcinoma)*

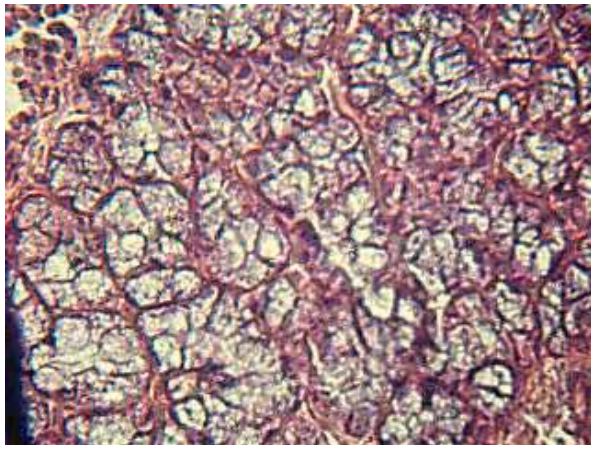

*Fig.3 40x Transverse section of lymph node tissue (lymphocytes) in mice showing multiplication of the nuclei in some lymphocytes Group (A) animals*

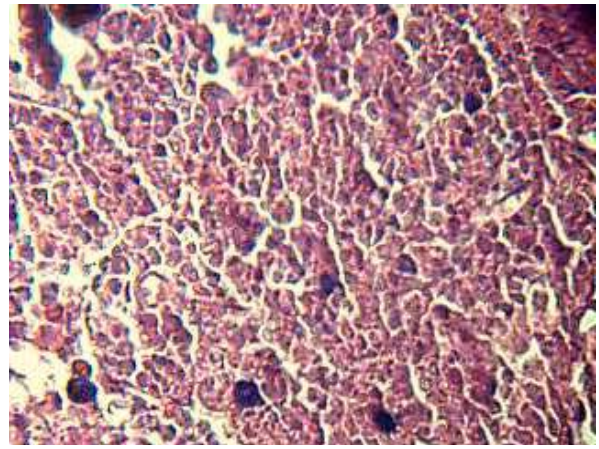

*Fig.4 40x Transverse section of lymph node tissue (lymphocytes) in mice showing abundant histological changes in the lymphocytes, such as increased size and multiplication of the nuclei, cytoplasm cleavage. Group (B) animals*

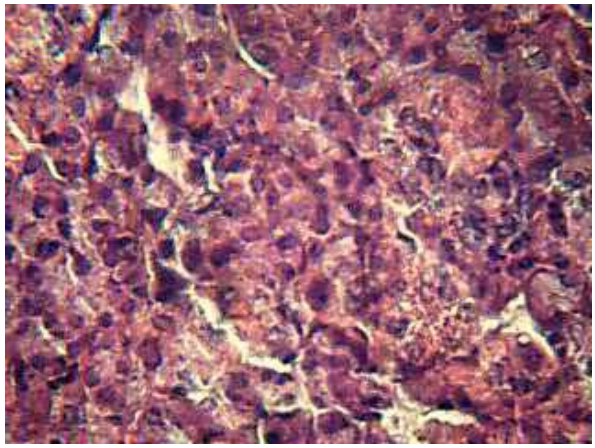

*Fig.5 40x Transverse section of lymph node tissue (lymphocytes) in mice showing more abundant histological changes in the lymphocytes, such as increased size and multiplication of the nuclei, cytoplasm cleavage. Group(C) animals*

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# دراسة تأثير ليزر واطئ القدرة (LLL) على التغيرات النسجيه لنسيج العقدة اللمفية في الفئران المحقونة بسرطان الغدة اللبنية

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## الخلاصة:

اجريت هذه الدراسه على فئران سليمة قسمت الفئران الى ثلاثه مجاميع (A,B and C) تكونت كل مجموعه من خمسه فئران ومجموعتي حيوانات السيطره حيث تكونت كل منهما من ثلاثة فئران. تم حقن مجموعه حيوانات السيطره الثانيه (استخدمت كمؤشر للمقارنه ) وحيوانات المجاميع الثلاثة (A,B and C)بسرطان الغده اللبنيه. بعد مرور (11) يوما على نمو الورم , ظهرت الاورام في اجزاء مختلفه من اجسام الحيوانات استنادا الى المشاهدات العيانيه. كل حيوانات المجاميع الثلاثة موضوعة الدراسه (A,B and C) شععت بالليزر في مدد مختلفه تراوحت بين (3-1) اسابيع بصوره متعاقبه. استنادا الى فحص المقاطع النسجيه للعقد اللمفيه لحيوانات المجاميع الثلاثة (A,B and C ) اظهرت النتائج تغيرات نسجية متباينه في خلايا اللمفوسايت كزيادة حجم الانويه وانقسامها, انشطار السايتوبلازم في حيوانات المجموعه (B) مقارنه مع تلك التغيرات النسجية التي كانت اكثر وضوحا في خلايا اللمفوسايت للعقد اللمفيه في حيوانات المجموعه C)) والتي شععت بالليزر لمده ثلاثة اسابيع, في حين كانت التغيرات النسجيه التي ظهرت في خلايا اللمفوسايت للعقد اللمفيه في كل من حيوانات المجموعه (A) والتي شععت بالليزر لمدة اسبوع واحد وحيوانات السيطره الثانيه والتي حقنت بسرطان الغده اللبنيه ( الغير مشععه بالليزر) مقتصره في بعض الخلايا على انقسام الانويه. كان الهدف من هذه الدراسه هو معرفة تاثير ليزر واطئ القدرة على التغيرات النسجيه من خلال تحفيز نشاط العقده اللمفيه لغرض تثبيط نشاط الخلايا السرطانيه .