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Activation of the Immune Response of the Spleen by Using Low Level Laser (LLL) for Treatment of Mice Inoculated with Mammary Gland Carcinoma

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Abstract:

Regional immune response with mammary gland carcinoma was studied statistically. However the prognostic value remains conflicting.

This study was conducted on thirty normal mice which were inoculated with mammary gland carcinoma. The tumour size of the animals under study were measured before and after laser irradiation by using a vernier and compared with that of non irradiated animals with laser (control group).

The aim of this study was to evaluate the effect of low level laser (LLL) on increasing the response of immune system by stimulating the spleen action to inhibit the cancer cell activity and then decrease the tumour size of inoculated mice.

The results of the gross observation showed enlargement in the irradiated spleen in comparison with that of non irradiated ones. It could be said that the enlargement which occurred in the spleen proved that there was an increase in immune response by using laser stimulation.

From this study, it may concluded that low level laser(LLL) was an efficient tool in stimulating the defense cells that found in the spleen which attack the cancer cells locally which resulted in decreasing the tumour size of the irradiated inoculated mice.

Key words: *Low Level Laser (LLL), Mammary Gland Carcinoma, Spleen*

Introduction:

The spleen is the largest accumulation of lymphoid tissues in the body. Because of its abundance of phagocytotic cells and the close contact between these cells and circulating blood, the spleen is an important defense against microorganisms that penetrate the circulation. It is also the site of destruction of aged erythrocytes. As is true of all other lymphoid organs, the spleen is a production site for activated lymphocytes, which pass into the blood. The spleen reacts promptly to antigens carried in the blood and is an important immunologic blood filter and antibody- forming organ (1).In humans, it is located in the left upper quadrant of the abdomen. It removes old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is subsequently shuttled to the liver for removal (2). It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation. The spleen is brownish (2, 3). Recently, it has been found to contain in its reserve half of the body's monocytes within the red pulp. In mice, the spleen stores half the body's monocytes so that upon injury they can migrate to the injured tissue and transform into dendritic cells and macrophages and so assist wound healing (4). These monocytes, upon moving to injured tissue (such as the heart), turn into dendrites cells and macrophages while promoting tissue healing (4,5). It is one of the centers of activity of the reticuloendothelial system and can be considered analogous to a large lymph node, as its absence leads to a predisposition toward certain infections(6).

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Low level laser therapy (LLLT) has proved to be effective in treating and repairing biologically damaged tissue and to reduced pain. LLLT has also proven to be an efficient method for the prevention of oral mucositis (7).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 coadjutant 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(8).

Material and Methods:

Thirty normal mice were used in this study (aged two months and body weight 40-50 gm). They were divided into five groups of three mice each (A, B, C, D and E) and five control groups of three mice each also (A1, B1, C1, D1 andE1). This work was carried out in the Iraqi center for cancer and medical genetics research.

All the animal groups (A, B, C, D and E) were inoculated with mammary gland carcinoma (considered as a cell line), given as a gift for this work from the Iraqi center that above mentioned. The same was done for the control groups of animals (used as markers for comparison) as shown in table (1). After 11 days of successful transplantation (tumour growth), the tumours appeared and the animals were ready for the experiment in a accordance with the gross observations.

All the animal groups(A,B,C,D and E) were anesthetized and irradiated with laser that was directed towards the spleen 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 15 minutes continuously, three times daily with a time interval of one hour each and for three days. Same procedure was applied for the other groups (B,C,D and E) .with different duration time (six days, nine days, twelve days and fifteen days) consecutively as shown in table (2). The control groups of animals inoculated with mammary gland carcinoma(non-irradiated with laser and used as markers for comparison) as shown in table(1) as above mentioned. The tumour size was measured by using vernier starting from the tumour appearance until the last day of the experiment (15 days). The laser type used in this study was laser device of (Ga-Al-P) (gallium- aluminum-phosphorus) of wave length of 810 nm which made in the university of technology (Iraq) worked in pules mode of one second duration time. 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.

Results:

This study was conducted on normal mice. The results of the five control groups of three mice each (A1,B1,C1,D1 and E1) inoculated with mammary gland carcinoma showed gradual increase in the tumour size due to the increase of duration time of tumour growth starting from the tumour appearance until the last day of the experiment (15 days) as shown in table 3(A) whereas the results of the other five groups of three mice each (A,B,C,D and E) irradiated with laser showed decrease in tumour size due to the increase of duration time of irradiation of laser (3,6,9,12 and15 days) consecutively as shown in table 3(B).

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 (9). Several studies have emphasized the important prognostic role of certain immunomorphologic reaction of spleen with various types of carcinoma. However, assessing the degree of the immune response is not objective and has not reached a reproducible result (10, 11) in the present study when the laser treatment was used as a stimulatory procedure. The laser light induced a particular effect on the cell proliferation and cell membrane, so the importance of electromagnetic radiation in the form of laser was to stimulate the macromolecules and initiate the confirmatory changes in proteins that cause transfer of energy to electrons within the cells (12).

The laser action can be described by increasing metabolic activity; the result is that significant effect on damaged carcinoma cells by body defense (immunity) caused a decrease in tumour size as shown in table(3). The clinical reduction in tumour size could be due to laser immunomodulation .This controversial concept of laser on tumour tissue can be clarified by the idea that laser may reduce tumour growth (10,13) as shown in table(3). The mean of the tumour size was measured for the first control group of mice inoculated with mammary gland carcinoma of duration time of 3 days which was 2.3 mm, whereas there was a decrease in the mean of the tumour size of the first treated group of mice with laser of duration time of 3 days to 1.6mm .There was also a decrease in the mean of the tumour size of the second treated group of mice with laser of duration time of 6 days to 1.2 mm in comparison with the second control group of mice inoculated with mammary gland carcinoma of duration time of 6 days which was 2.3 mm .There was an increase in the means of the tumour size in both third and fourth treated groups of mice with laser of duration time of 9 and 12 days which were 1.4 and 1.4mm consecutively .This inactivation of the defense system was related to many reasons according to the physical agents around the animals such as,

atmospheric pressure, hot, dusty weather and the given does of the laser in the same time not enough to reactivate the immune system at these conditions, but these means in the same time were still less than that of the means of the tumour size in both third and fourth control groups of mice inoculated with mammary gland carcinoma of duration time of 9 and 12 days which were 3.5 and 2.7 mm consecutively as shown in table (3) .There was a decrease in the mean of the tumour size of the fifth treated group of mice with laser of duration time of 15 days to 1.2 mm .This decrease was a result of an efficient dose of laser that was given to the inoculated mice in order to activate the immune system again.

Last, but not least, low level laser (LLL) can open a new era in the treatment of cancer and also gives evidence that stimulation of the spleen activity by irradiation with this laser can be used to accelerate the healing process by stimulating the defense cells (plasma cells and macrophages)) that found in the irradiated spleen and as a result stimulate the immune system which will attack the cancer cells and inhabit their activity.

Table (1): The control groups of mice inoculated with mammary gland carcinoma (untreated with laser)

Groups	A1	B1	C1	D1	E1
Duration time of tumour	Three days	Six days	Nine days	Twelve days	Fifteen days
Number of mice	3	3	3	3	3

Table (2): The groups of mice inoculated with mammary gland carcinoma (treated with laser)

Groups	A	B	C	D	E
Treatment	Carcinoma irradiated with laser for three days	Carcinoma irradiated with laser for six days	Carcinoma irradiated with laser for nine days	Carcinoma irradiated with laser for twelve days	Carcinoma irradiated with laser for fifteen days
Number of Mice	3	3	3	3	3

Table (3): Diameter of the tumour size (mm) before and after treatment with laser for different duration times.

Control groups	(A) .Tumour size /mm of the control groups of mice inoculated with mammary gland carcinoma				
	days 3	days 6	days 9	days 12	١٥ days
Range	3.3 –1.1	3.5 – 1.2	3.6 – 1.56	3.7 – 1.8	٢– ٣,٩
Mean	2.2	2.3	3.5	2.7	٢,٨
SD	1.1	1.2	1.1	1	1
R = 0.32	(P= 0.25(NS				
B= 0.03	(P= 0.38(NS				
Treated mice with laser	(B) Tumour size/mm of different duration time of irradiation of laser.				
Range	2.5 – 0.7	1.9 – 0.4	2.4 – 0.4	2.4 – 0.4	1.8– 0.4
Mean	1.6	1.2	1.4	1.4	1.2
SD	0.9	0.8	1	1	0.7
R = - 0.10	P= 0.73 (NS)				
B= - 0.01	P= 0.77 (NS)				

R-The ability of applied this relation on the popular.
B -The outcome with respect to income

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تنشيط الاستجابه المناعيه للطحال بأستخدام ليزر واطئ القدرة (LLL) لمعالجة الفئران المحقونه بسرطان الغده اللبنيه

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الخلاصه:

لقد تمت دراسة الاستجابه المناعيه المحفزه بأستخدام ليزر واطئ القدره أحصائيا. أجريت هذه الدراسه على ثلاثين فأره سليمه حقنت بسرطان الغده اللبنيه. تم قياس حجم الورم بأستخدام الفيرنيه للحيوانات موضوعة الدراسه قبل وبعد التشعيع بالليزر ومقارنة النتائج مع مجموعة الحيوانات غير المشعه بالليزر(مجموعة السيطره).

الهدف من هذه الدراسه هو تقييم تأثير ليزر واطئ القدره على زيادة أستجابة الجهاز المناعي وذلك بتحفيز فعالية الطحال لغرض تثبيط نشاط الخلايا السرطانيه الذي يؤدي بعد ذلك الى تقليل حجم الورم في الفئران المحقونه.

أوضحت نتائج المشاهده العيانيه حصول كبر في حجم الطحال المشعع نتيجة التحفيز بالليزر مقارنة بذلك الغير مشعع. يمكن القول بأن كبر حجم الطحال انف الذكر أثبت بأن هنالك زياده في الاستجابه المناعيه بأستخدام التحفيز بالليزر.

من هذه الدراسه, أمكن الاستنتاج بأن الليزر واطئ القدره ((LLL كان اداة فعاله في تحفيز الخلايه المناعيه الموجوده في الطحال والتي هاجمت بدورها الخلايه السرطانيه موقعيا وأدت الى صغر حجم الورم في الفئران المحقونه المشععه بالليزر.