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The effect of He-Ne laser and gold nanoparticles on expression of TGF beta in mice mammary adenocarcinoma

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ABSTRACT

Cancer cases are increasing all over the world due to many reasons; Transforming Growth Factor Beta (TGFβ) is a cytokine that plays a significant role in immunoregulation, cancer, and angiogenesis. The aim of the study was to examine the effects of He-Ne laser and Gold Nanoparticles (AuNPs) on TGF beta expression in mice with mammary adenocarcinoma. Fourteen albino female mice were selected, their ages were 3 to 4 weeks while their weights were ranged from 15 to 20 gm, and they were recruited from the Iraqi center for cancer and medical genetic research. The colloidal gold nanoparticles were prepared in a certain concentration and specifications, while Low Level Laser (LLL) was adjusted in a certain wavelength and power density. The tissue was taken from the breast of albino mice that are affected by adenocarcinoma, one group of the albino mice (7 mice) were irradiated by laser, while the other group (7 mice) was injected with the colloidal solution of nanogold particles. A low concentration of nanogold did not show any effect, thus only high concentration was used in the current study. LLL Therapy (LLLT) has more effect on TGFβ than nanogold by about 10%, however, the difference was not significant. The study came up with certain findings among them are; LLLT and AuNPs are used in cancer treatment, only high concentration of nanogold has effect on TGF-Beta. LLLT has more effect on TGF-Beta and is more active than nanogold.

Keywords: Albino mice; LLLT; AuNPs; TGF-Beta

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INTRODUCTION

Cancer cases are increasing all over the world mainly in the developing countries due to the pollution, style of life, stress and other factors. However the types of cancer are also increasing worldwide among them are; breast cancer, colon cancer, brain cancer, and skin cancer, all the statistics for male and female cancers are listed in the international agency for research on cancer [1]. Cancer is usually diagnosed as an abnormal growth in the cell that eventually turn to tumor that cannot be controlled and can damage the normal cell lines and tissues, also it can spread to all the body and this might happen when there is a genetic susceptibility [2].

Transforming Growth Factor Beta (TGFβ) is a protein that is produced by white blood cells, it is a cytokine that plays a significant role in immunoregulation, cancer, angiogenesis, and wound healing, it is exerted by the cells of the immune system to regulate the immune system and as an anti-inflammatory substance [3,4]. TGFβ has other functions such as controlling apoptosis, cell differentiation, cell proliferation, and growth thus, it is considered multifunctional and it is important for survival [5-7].

He-Ne laser is a gas laser that has high energy, which consists of neon and helium. Low intensity helium-neon laser might be used in many clinical applications. The laser light that is produced can be used in cancer treatment via destroying the tumor cells and limiting the spread of tumor cells in the body as well as reducing swelling, pain and improving wound healing [8,9]. Gold nanoparticles are also effective in cancer treatment by acting as drug carriers to target cancer cells, and to verify severity of the damage in the cells [10,11].

This study was carried out to investigate the effect of gold nanoparticles as well as the effect of using He-Ne laser radiation and photo thermal radiation on the level of TGF-Beta in mice with mammary adenocarcinoma.

MATERIALS AND METHODS

Experimental groups

Fourteen albino female mice were selected for the current study, they were aged 3 to 4 weeks and weighted 15 to 20 gm, they were provided from the Iraqi center for cancer and medical genetic research. Seven of them were treated with low laser level and 7 were treated with gold nanoparticles.

Gold nanoparticles

The colloidal Gold Nanoparticles (AuNPs) were prepared by Sigma

Aldrich company with the following description:

- Concentration~6.0E+12 partials/ml.
- Optical density=1 diameter=10 nm.
- Absorption=510-525 nm and storage 2-8°C.
- Shape: Spherical
- Dark red color.

Irradiation procedure

The following parameters were used in irradiation procedure *in vivo* study *via* Low Level Laser (LLL).

- The wavelength is equal to 532 nm.
- Output power is equal to 112 mW, Laser's power was determined using power meter.
- Laser beam diameter at aperture is equal to 4 mm.
- Exposure time for 8 minutes through 24 hrs.
- Power density (0.083W/cm^2) was calculated as follows:

Power density=Power (p)/Area (A) W/cm^2

Preparation of tissue block

This required correct selection of the tissue, fixation and slicing; formalin was used for tissue fixing. The tissue samples were submerged in paraffin wax. Sections were cut by a microtome and the range of cutting the specimens was 3 µm to 5 µm. Then the slices were placed on slides, dried *via* alcohol, washed by elevating the concentration (e.g. 50%, 75%, 90%, 95%, and 100%) and cleared by xylene prior to their examination under the microscope.

Anti-TGF beta antibody and expose mouse and rabbit specific HRP/DAB detection IHC kit from ABCAM Company, United Kingdom were used in this study. Following deparaffinizeation and rehydration by xylene, sequentially graded alcohol and distil water were used, incubation with 3 percent hydrogen peroxide for ten minutes was then done in order to block the activity of endogenous peroxidase. Slides were washed in phosphate buffered saline. Following their treatment with protein, they were blocked by incubating at 37°C for five minutes then rinsing with PBS was done. Primary antibody was used to cover the slices which were put for 1 hour in humidity chamber at 37°C (primary antibody was produced at dilution 1/100 for TGFβ).

Slides were washed carefully in PBS. Addition of the secondary antibody for ten minutes at room temperature was done, then addition of streptavidine-HRP antibodies for ten minutes at 37°C was performed. After rinsing, staining of the samples with DAB for 15-45 minutes at room temperature was done. Slides were counterstained with hematoxylin for 30 seconds and rinsed well in running tap water, then dried and mounted with permanent-mounting medium (DPX), after that, the slides were seen under light microscope at 10 magnification at the beginning, then they were seen at 40 magnification [12,13].

Statistical analysis

SPSS version 26 was used for the statistical analysis. Fisher exact test was performed to examine the effects of laser and nano particles on the expression of TGF-beta, P value less than 0.05 was considered significant.

RESULTS

This research focused on the comparison between using low level laser radiation and AuNPs in treating cancer and their effect on TGF Beta. Table 1 shows the association between the effect of low level laser and gold nanoparticles on TGF expression using the fisher exact test. The results showed that LLL Therapy (LLLT) has more effect on TGF Beta than nanogold (10%). The p-value for the fisher exact test was more than 0.05 (non-significant).

Figure 1 shows mice breast affected with adenocarcinoma and Figure 2 illustrates invasion of blood vessels by malignant cells.

Tab. 1. Association between the effect of low level laser and gold nanoparticles on TGF expression

	TGF positive	TGF negative	Total
low level laser	6	1	7
Gold nanoparticles	5	2	7
Total	11	3	14

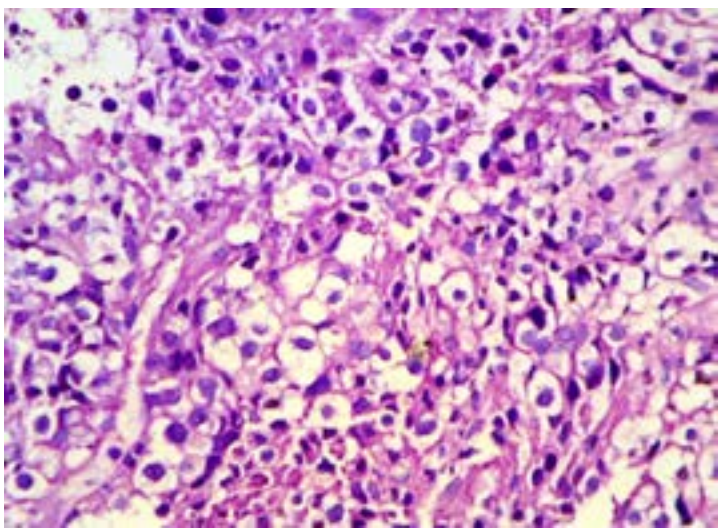

Fig. 1. Mice mammary adenocarcinoma (Presence of necrotic cells and malignant cells).

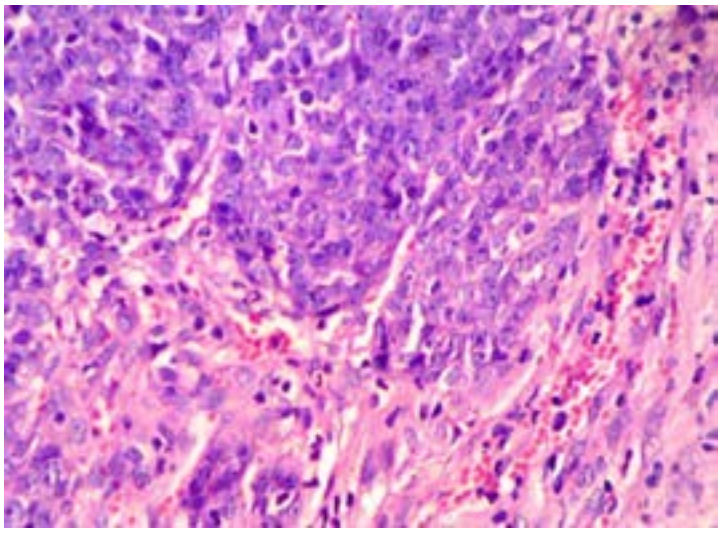

Fig. 2. Invasion of blood vessels by malignant cells (mice mammary adenocarcinoma).

After treating the tumor with low level laser radiation and with gold nanoparticles, the number of necrotic and malignant cells has declined as demonstrated in Figures 3 and 4 respectively.

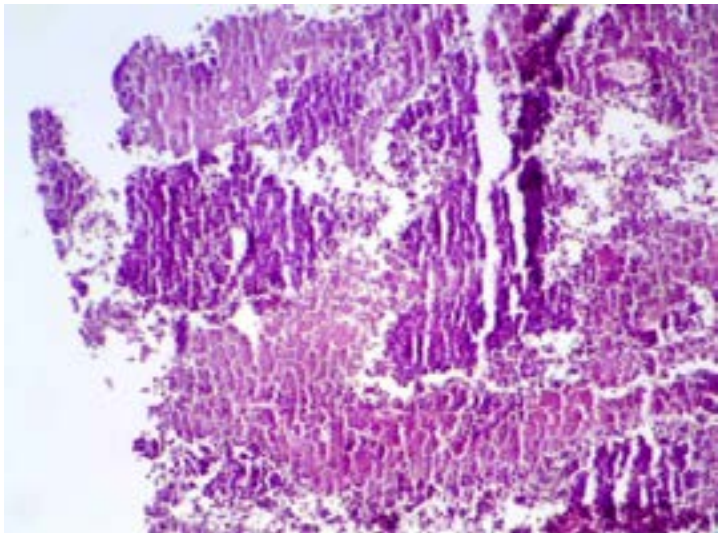

Fig. 3. Mice mammary adenocarcinoma. The tissue was treated by low level laser radiation.

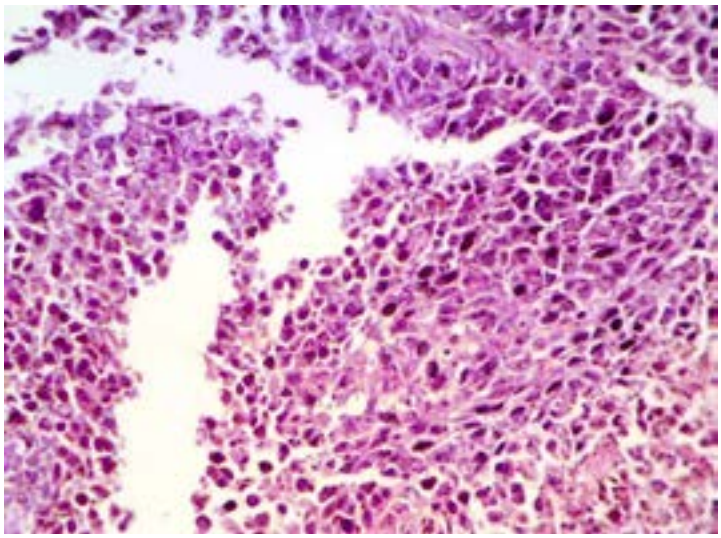

Fig. 4. Mice mammary adenocarcinoma. The tissue was treated by gold nanoparticles.

DISCUSSION

This study investigated the effect of using low level laser radiation and AuNPs in the treatment of cancer and their effect on TGF Beta expression in mice with mammary adenocarcinoma. In a study done on rats, it has been suggested that LLLT can modulate reactive oxygen species and the process of lipid peroxidation; thus it can help in tissue healing by reducing the time needed for tissue repair [14].

A study done in 2015, showed that chronic pain can be managed using certain techniques to enhance patients' quality of life and using LLLT can improve the patients' health [15].

Using LLLT as a therapy to a lot of pains, and minimizing the risk and the side effects of using laser radiation in treatment was taken into consideration in a study carried out by researchers in 2019 [16]. Another study found that LLLT can be used in treatment of Multiple Sclerosis (MS) [17]. Evidence showed that LLLT has been used to treat breast cancer [18].

Gold Nanoparticles (AuNPs) is applied biochemically in therapy as well as diagnosis [19]. Another study showed that nanogold is applied widely in the field of medical treatment, particularly in cancer treatment [20]. In a murine breast cancer model, it has been found that nanoparticle treatment might prevent lung metastasis and enhance survival [21]. Furthermore, the current study agreed with another study that considers nanoparticles promising in breast cancer treatment [22].

The current study is novel in comparing AuNPs and LLLT, it was found that LLLT shows effect on TGF-Beta around 10% more than AuNPs, in addition using LLLT is cheaper than using the nanogold and preparing the nanogold needs more time. The study has limitations including the small sample size. Further research, including randomized control trials, is mandatory in this field to reach more solid findings.

CONCLUSION

LLLT, and AuNPs are used in cancer treatment. Using nanogold is more costly than LLLT. The cancer treatment was investigated via the expression of TGF-beta, and LLLT was shown to be more active than nanogold.

AUTHORS CONTRIBUTION

Farah A. J. Al-Zahawi was responsible for the concept of the study, the interpretation of the data and the writing of the manuscript. Zainab M. Alawad has involved in the interpretation of the data, and the writing of the manuscript. Hanan L. AL-Omary has contributed to the statistical analysis and the writing of the manuscript. The final version of the manuscript was approved by all authors.

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