International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 1, p. 65-71, 2018

RESEARCH PAPER OPEN ACCESS

Histological evaluation of the thymocyte composition in the thymus gland of mice by using soft laser

Ahmed Anwar Albir*

Department of Basic Sciences, College of Dentistry, University of Baghdad, Baghdad, Iraq

Key words: Medical Laser, Thymocytes of the thymus gland, Histological alterations, Mice.

http://dx.doi.org/10.12692/ijb/13.1.65-71 Article published on July 15, 2018

Abstract

Background: Thymus is composed of two lobes and it is located in the mediastinum, each one is split up into many lobules and each of them is separated from another by trabeculae that consist of connective tissue. The author used thymus gland as a target for soft laser in order to achieve better results due to hypothetical effects on its composition mainly thymocytes.The presented research was aimed to find the possible effect of soft laser on the cellular composition of the normal thymus gland of mice. Thirty three of normal male from Swiss albino mice at 9 weeks of age and body weight 41-49 gm were the targets for this experimental study. These mice were separated into three groups (11mice/group). The first group was kept as a control and was not subjected to radiation of the laser, while the rest of the groups were exposed to laser irradiation. The outcomes of this study show how laser caused marked alterations in the cellular contents of the thymus glands of the second group of mice mainly the thymocytes which included increased size of the thymocytes and multiplication of the cellular nuclei. The mentioned alterations were more plentiful in the thymocytes composition of the thymus glands of the last set of mice irradiated with laser. Soft laser proved the hypothesis and caused noticeable atterations in the thymocytes structure and that in turn could support the immune system as well.

* **Corresponding Author:** Ahmed Anwar Albir ✉ ahemdalbir@yahoo.com

Introduction

The thymus is located in the mediastinum (Junqueira and Carneiro, 2003; Mescher, 2013). It is composed of two lobes, each one is divided into many lobules and each of them is separated from another by trabeculae that consist of connective tissue. Every lobule consists of two main components, an external cortex and an internal medulla (Elgbratt, 2007).

The cortex and the medulla meet at region called corticomedullary junction (CMJ) which is considered as the point of entrance and the way out for developing thymocytes (Prockop and Petrie, 2000). T cell precursors come into existence in the bone marrow and circulating from the bone marrow to violate the thymus by the corticomedullary junction, and through the intrathymic trip, T cells are termed thymocytes (Naquet *et al.,* 1999; Wu *et., al* 1991; Godfrey *et al.,* 1993; Godfrey, *et al.,* 1994). In thymus and throughout the development, growing thymocytes shift from cortex to medulla (van Ewijk, 1991; Gill *et al*., 2003; Petrie, 2003). It shouldn't be ignored that the thymus is able to generate T cells thymus through the life span (Aspinall *et al.,* 2002; Taub and Longo, 2005). In addition to the greater composition of thymocytes, the thymus microenvironment has different kinds of thymic epithelial cells (TEC), stroma cells, macrophages, fibroblasts and dendritic cells. All these cells contribute in thymocyte maturation process (Elgbratt, 2007).

There are thymic (Hassall) corpuscles in the thymic medulla which have variable size and contain large sums of thymic epithelial cells (TECs). Those cells secrete many cytokines that are important for the control of the activity of dendritic cells as well as the differentiation of systematic T cells (Mescher, 2013). Laser phototherapy has a main role in the medical fields including: promoting wound healing, tissue repair and prohibition tissue from death, relief of inflammation in chronic diseases and injuries with its associated pain and edema, relief of neurogenic pain and some neurological problems (Hamblin and Waynant, 2006).

The purpose of this experimental study was to address the question of whether the soft laser could affect the cellular structure of the thymus gland particularly thymocyte. In reality, the presented work is a hypothesis needs to be proved.

Materials and methods

33 normal male of Swiss albino mice at 9 weeks of age and body weight 41-49gm were selected to be the goals for this empirical research, and this number of mice that could be sufficient was chosen in order to ensure that our presented work will achieve better results. This mentioned number of mice was divided into three targeted groups (n=11 each).

The first targeted group of mice was kept as unirradiated control in order to compare the results of other groups of mice irradiated with laser with that of the unirradiated control group of mice. Both second and third targeted groups of mice that constitute irradiated groups with laser were anaesthetized and exposed to a low power gallium aluminum arsenide laser (Ga Al As) of wavelength (lambda = 830nm). The beam of mentioned laser was directed above the heart (location of the thymus gland) of the targeted mice and the object was one centimeter distant from the laser source. Once daily irradiation with laser was performed for 30 and 35 minutes to the targeted mice which included both second and third irradiated groups respectively.

The entire period of experimentation (laser irradiation) lasted 12 consecutive days. Both time of receiving laser irradiation and entire period of experimentation (laser irradiation) were arranged for each irradiated group of mice as in the Table 1. After the end of the entire period of experimentation (laser irradiation), all the mice including both normal control group and the other irradiated groups were killed and their thymus glands were rapidly obtained. Sections of thymus glands were prepared by using a routine procedure aiming at studying the histological evaluation of the thymocyte composition by light microscopy. Photographs were made at original magnification.

Statistical factors

Mean

The average pixel value taken to be equal to the average brightness or intensity and computed using equation (1), (Gomes and Velho, 1997):

$$\mu = \frac{1}{MN} \sum_{x=1}^{M} \sum_{y=1}^{N} I(x, y) \quad (1)$$

Where M, N are the dimensions of figure and I(x,y) is the pixel intensity value (0-255 for a 256 bit figure).

Variance

Coefficient of variance is defined as the ratio of standard deviation to the mean and calculated from following equation (Gomes and Velho, 1997):

$$C_v = \frac{\sigma}{\mu} \times 100\ \% \quad (2)$$

Sometime C_v is assumed as a measure to difference in digital figure.

Standard deviation (Std)

The standard deviation is the most commonly used index of variability and is a measure related to the average distance of the scores from their mean value. This is also an indicator of contrast in the figure.

It is computed using the following result (Gomes and Velho, 1997):

$$\sigma = \sqrt{\frac{1}{MN} \sum_{x=1}^{M} \sum_{y=1}^{N} (I(x, y) - \mu)^2} \quad (3)$$

The standard deviation is important in identifying the details content in the figure.

Results

The findings of our experimental work confirmed the existence of marked effects of soft laser on the thymocytes structure.

Table 1. Time of receiving laser irradiation and entire period of experimentation (laser irradiation) for each irradiated group of mice.

Group	Number of mice	Time of receiving laser irradiation	Entire period of experimentation (laser irradiation)
First (unirradiated control)	11	________	________
Second (irradiated with laser)	11	30 minutes once daily	12 days
Third (irradiated with laser)	11	35 minutes once daily	12 days

Figure 1 represented the histology of the thymus gland lobule of unirradiated group (control group of mice), showed normal composition of the thymocytes. Figure 2 showed alterations in the composition of the thymocytes of the thymus gland of the second group of mice irradiated with laser included increased size of the thymocytes and multiplication of the cellular nuclei. Figure 3 showed more obvious alterations in the composition of the thymocytes in the thymus gland of the third group of mice irradiated with laser included increased size of the thymocytes and multiplication of the cellular nuclei.

Table 2. The histological evaluation of the effects of soft laser on the thymocyte composition.

Number of mice per group = 11			
Name of the group	Time of receiving laser irradiation	Entire period of experimentation (laser irradiation)	Histological evaluation of the composition of the thymocyte
First (unirradiated control)	________	________	Normal composition of the thymocytes (Figure 1)
Second (irradiated with laser)	30 minutes once daily	12 days	Increased size of the thymocytes, and multiplication of the cellular nuclei were clearly marked (Figure 2)
Third (irradiated with laser)	35 minutes once daily	12 days	Increased size of the thymocytes, and multiplication of the cellular nuclei were more clearly marked (Image 3)compared with the second group of mice irradiated with laser (Figure 3)

Table1 showed time of receiving laser irradiation and entire period of experimentation (laser irradiation) for each irradiated group of mice, whereas Table 2 showed the histological evaluation of how thymocytescomposition affected by irradiating with soft laser, and table 3 showed the comparison between mean, standard deviation and variance values of the three figures.

Table 3. Mean, Std and Variance values of the three figures of their R, G, and B bands.

Figure No.	Bands	Mean	Stdev	Variance
Figure 1	R	165.150983	46.764264	71.8267061873
	G	108.742503	58.336759	43.8720096846
	B	157.461023	37.617848	43.8720096846
Figure 2	R	171.112174	54.092200	50.5496214409
	G	118.491401	74.016735	49.6635284603
	B	158.950830	47.340910	28.4631612353
Figure 3	R	163.338496	36.398892	22.4798948187
	G	93.329292	53.928686	28.9758167776
	B	141.079776	35.272071	16.4025370093

Discussion

The data that was gathered as mentioned in Tables 1, 2, and 3 and also the Figures 1, 2, and 3 all of them showed clearly noticeable effects of laser irradiation on the thymus gland, in the second group of mice which irradiated with laser for 30 minutes once daily during the entire period of experimentation (12 consecutive days), the laser produced an effective alterations the structure of the thymocytes of the thymus glands particularly increased size of the thymocytes and multiplication of the cellular nuclei as shown clearly in Figure 2 comparing with the unirradiated group (control group) as depicted in Figure 1.

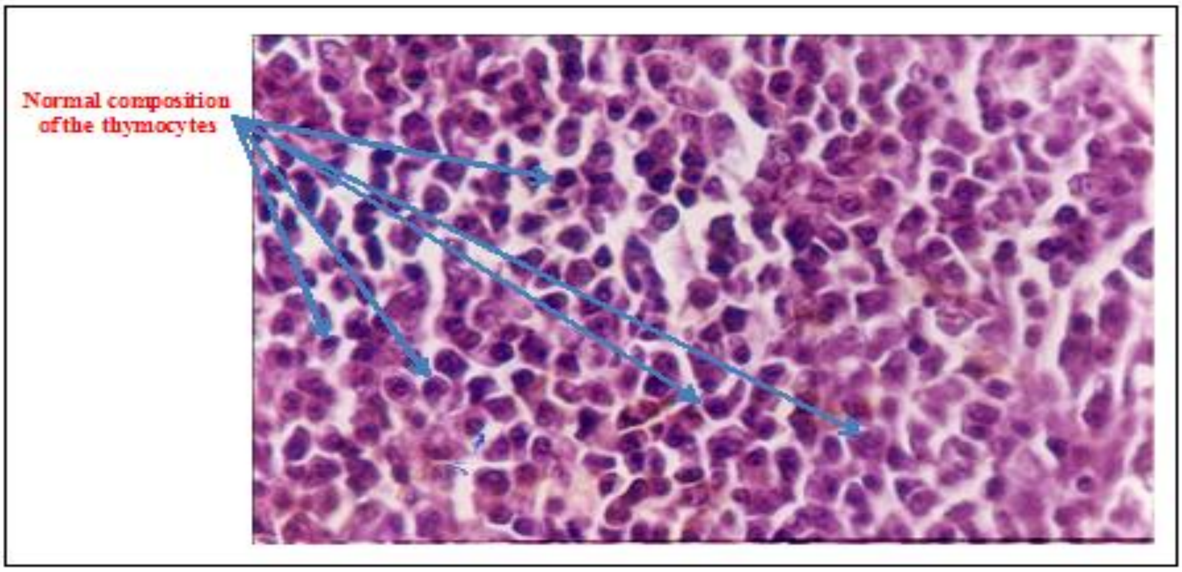

Fig.1. Histology of the lobule of the thymus gland of the first irradiated control group of mice showing normal composition of the thymocytes. H & E, 40^Magnification.

In the third group of mice which irradiated with laser for 35 minutes once daily for 12 days as well, the laser produced more effective alterations in the composition of the thymocytes of the thymus glands particularly increased size of the thymocytes and multiplication of the cellular nuclei as shown clearly in Figure 3 comparing with the second group of mice as shown clearly in Figure 2. It could be said that these alterations that occurred in the composition of the thymocytes were due to the following:

The selective laser of the presented work was suitable for stimulation of the cellular composition of the thymus gland particularly stimulation of the thymocytes components.

The gradual increasing in times of our doses of laser irradiation was appropriated because these mentioned times gave the laser the opportunity to alter the thymocytes components.

The entire period of experimentation (laser irradiation) of the presented work which lasted 12 consecutive days was sufficient to achieve noticeable results.

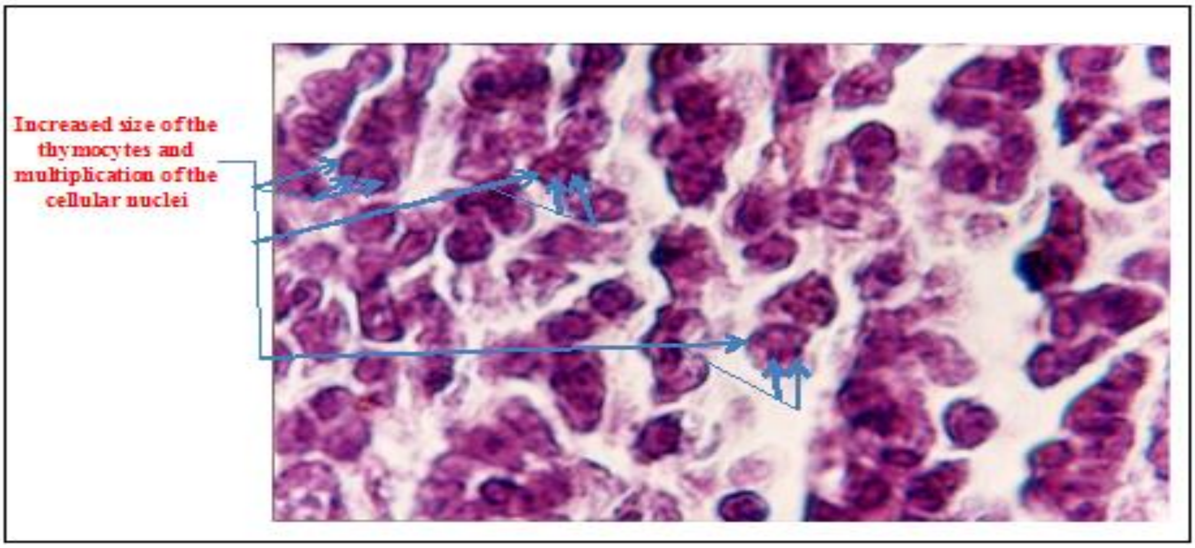

Fig. 2. Histology of the lobule of the thymus gland of the second group of mice irradiated with laser showing abundant alterations in the composition of the thymocytes included increased size of the thymocytes and multiplication of the cellular nuclei. H & E, 40^Magnification.

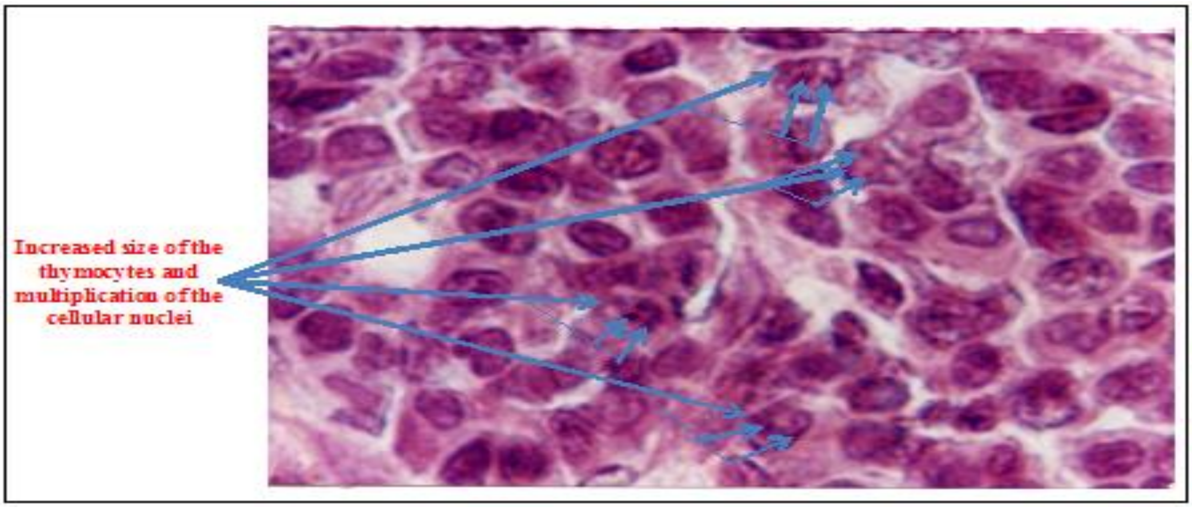

Fig. 3. Histology of the lobule of the thymes gland of the third group of mice irradiated with laser showing more abundant alterations in the composition of the thymocytes included increased size of the thymocytes and multiplication of the cellular nuclei. H & E, 40^Magnification.

As it is clearly noticed from Table 3 and the statistical features (mean, variance, std) of R, G and B bands of 3 figures before and after different times of receiving laser irradiation, it showed no much different values.

So the figures preserve their main characteristics except the alterations resulted from stimulating state of laser irradiation (Gomes and Velho, 1997).

The current experimental study performed a hypothesis that established probable effects of laser irradiation when utilized to a normal tissue like the thymus gland structure of mice. So, our feedback proved this hypothesis and the laser caused notable changes in the cellular structure of the thymus gland particularly the thymocyte structure. In another words, soft laser can stimulate cellular functions by applying it at an appropriate dose (Catão, 2004; Gomez-Villamandos *et al.,* 1995), however, the principles of laser action in cells and tissues are still not well known (Rocha Júnior *et al.,* 2007).

Finally, many questions remain unanswered about the mechanism of laser action and its effects on diverse sorts of cells and tissues. These inquiries should be answered through time and careful investigation.

Conclusion

Soft laser was a successful biostimulative tool in proving our hypothesis and caused alterations in the composition of the thymocyte which included increased size of the cell and multiplication of the cellular nucleus. Eventually, soft laser will enhance the immune reactions of the thymus gland and will quicken the treatment of dangerous diseases especially cancerous diseases.

Conflict of interest

No conflict of interest

References

Junqueira LC, Carneiro J. 2003. Basic histology. Text & Atlas.10thedn.Chapter 14. Lange NY, 265-290.

Mescher AL. 2013.Junqueira's Basic Histology. Text & Atlas 13thedn. Chapter 14. Lange NY, 262-288.

Elgbratt K. 2007. Characteristics and Consequences of Thymic Involution in Inflammatory Bowel Disease: Experimental studies in Gαi2-deficient and DSS-induced Colitis as well as in IBD patients. Thesis. Department of Microbiology and Immunology, Göteborg University.12, 13.

Prockop S, Petrie HT. 2000.Cell migration and the anatomic control of thymocyte precursor differentiation, Semin Immunol. **12**,435-444.

Naquet P, Naspetti M, Boyd R. 1999.Development, organization and function of the thymic medulla in normal, immunodeficient or autoimmune mice. Semin Immunol. **11**, 47-55.

Wu L, Scollay R, Egerton M, Pearse M, Spangrude GJ, Shortman K. 1991. CD4 expressed on earliest T-lineage precursor cells in the adult murine thymus. Nature, **349(6304),** 71-74.

Godfrey DI, Kennedy J, Suda T, Zlotnik A. 1993.A. developmental pathway involving four phenotypically and functionally distinct subsets of CD3-CD4-CD8-triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. J. Immunol. **150(10),** 4244-52.

Godfrey DI, Kennedy J, Mombaerts P, Tonegawa S, Zlotnik A. 1994.Onset of TCR-beta gene rearrangement and role of TCR-beta expression during CD3-CD4-CD8- thymocyte differentiation. J. Immunol. **152(10),** 4783-92.

vanEwijk W. 1991. T-cell differentiation is influenced by thymic micro environments. Annu. Rev, **9,** 591-615.

Gill J, Malin M, Sutherland J, Gray D, Hollander G, Boyd R. 2003.Thymic generation and regeneration. Immunol. Rev. **195**, 28-50.

Petrie HT. 2003. Cell migration and the control of postnatal T cell lymphopoiesis in the thymus. Nat. Rev. Immunol. **3,** 859-866.

Aspinall R, Andrew D, Pido-Lopez J. 2002. Age-associated changes in thymopoiesis. Springer Semin. Immunopathol. **24(1),** 87.

Taub DD, Longo DL. 2005. Insights into thymic aging and regeneration. Immunol.Rev. **205**, 72.

Hamblin MR, Waynant RW."Mechanisms for Low-Light Therapy."Proc. of SPIE 6140614001-614010.

Gomes J, Velho L. 1997. Image Processing for Computer Graphics, Silvio Levy, New York, Inc.

Catão, MHCV. 2004. Osbeneficios do laser de baixa intensidade na clinica odontológica na estomatologia. Rev. Bras. Patol.Oral.**3**, 214-8.

Gomez-Villamandos RJ, Santisteban Valenzuela JM, Ruiz Calatrava I, Gomez-Villamandos JC, Avila Jurado I. 1995. He-Ne laser therapy by fibroendoscopy in the mucosa of the equine upper airway. Lasers Surg. Med. **16,** 184-8.

Rocha JúniorAM, Vieira BJ, de Andrade LCF, Aarestrup FM. 2007. Effects of low-level laser therapy on the progress of wound healing in humans: the contribution of in vitro and in vivo experimental studies. J. Vasc. Bras.**6**, 258-266.