Effect of Laser light on lymphocyte Apoptosis

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Abstract

Background: Apoptosis is a physiological type of cell death characterized by certain morphological nuclear and biochemical changes. This process deletes the unwanted cells by a clean mechanism that does not evoke any inflammating changes.

Objective: To show the effect of laser light on the lymphocyte apoptosis.

Subject & Methods: The study was conducted on lymphocyte apoptosis. The taken number of apoptotic lymphocyte was estimated before and after exposure.

Results: Results showed that there was a significant increase in the percentage of lymphocyte apoptosis after exposure to 630nm laser light, which was further increased by increasing the time of exposure.

Conclusion: Lymphocyte apoptosis can be induced by low dose of laser and increasing the exposure time can increase this.

Keywords: He-Ne Laser light, lymphocyte apoptosis

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Introduction

Apoptosis is a genetically controlled process that's why it's called programmed cell death; it plays an important role in tissue homeostasis; differentiation and development like in the separation of fingers during limb development [1].

Apoptosis can be triggered by different stimuli which can be intracellular or extracellular; some known extracellular signals include receptor ligand such as TNF-α, Fas-ligand as well as signaling proteins that cross the membrane such as Granzyme- B and non-protein activators as calcium and radiation [2]. The intracellular apoptotic signals as reactive oxygen species or laser therapy trigger apoptosis at the mitochondrial membrane, this cause opening of mitochondrial membrane transition pore, which lead to release of cytochrome C- and execution of apoptotic process. [3], both, intracellular and extracellular, passes in two phases: an initial commitment phase (when the cell responds to signal that commit the cell to undergo self destruction) and an executional phase (when cell death can not be stopped) [4], which results in typical morphological changes of apoptotic cell death; as plasma membrane blebbing, shrinkage of the cell, nuclear chromatin condensation, lastly fragmentation into apoptotic bodies that will be phagocytosed by neighboring cells like macrophages [5].

Surface changes are probably due to the cleavage of cytoskeletal protein, such as fodrin, gelsolin, pectin, actin and cytokeratin [6].

Materials& Methods

Fresh blood samples were obtained from the anti cubital vein of twenty six healthy subjects with sterile EDTA tube, the sample diluted 1:1 with isotonic phosphate buffered saline (PH 7.4). Two milliliters of this mixture were carefully layered on the top of

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four milliliters of ficoll, in a ten milliliters siliconized glass centrifuge tube, lymphocyte separation done according to method of Boyum and Scan., 1968 [7]. The above compound was centrifuged in cold centrifuge at (3100 rpm), for 30minutes at 24°C. After centrifugation, lymphocyte forms a white buffy coat at the interface of the blood plasma which was aspirated by Pasteur pipette and transferred into a ten ml siliconized tube, washed three times by PBS for 20min until a pellet was formed, the lymphocyte pellet re-suspended in 0.5 ml of PBS.

The lymphocyte then was exposed to laser beam (633nm) for 15 minutes and for 30 minutes and both conditions were studied as follows:

A. Cell count and viability:

The number of lymphocytes were counted using Heamocytometer counting chamber, the results were expressed as cell/mm³ [8]. Trypan blue exclusion test was done to assess cell viability. A known volume of lymphocyte suspension (100µl) was mixed with an equal volume of trypan blue dye (the concentration of trypan blue 0.2%) and examined immediately under light microscope.

One ml of the rest volume of the lymphocyte suspension drawn to a Westergren tube fixed by holding it directly in front of the laser beam so that the beam passes directly through the opening end of the tube and thus the whole suspension was exposed to the light for (15minutes and 30minutes.), then after each of the different exposure time, cell count, viability were estimated.

Morphology of lymphocyte was studied after staining of lymphocytes by acridine orange according to procedure of Vacca (1985) [9]. Slides were examined by fluorescent microscope.

Mitochondria membrane permeability changes were studied using mitolight (mitochondrial permeabilization detection kit (chemicon).

In healthy cells, the dye accumulates and aggregates in mitochondria giving off a bright red fluorescence
(λem = 585-590 nm), while in apoptotic cell the mitochondrial membrane brakes down and so the dye remains in the cytosol in its monomeric form giving off a green fluorescence (λem = 527-530 nm).

B. Cellular morphology:

The morphological characteristics of lymphocyte apoptosis were assessed according to the method of Willingham (1999) [10]. The morphological changes related to apoptotic process as stated by Collins [11], these changes include: Membrane blebbing, cell shrinkage, chromatin condensation and fragmentation of nuclear material, DNA fragmentation, and lastly formation of apoptotic bodies. Cellular morphology was assessed after staining the cells by acridine orange (DNA binding specific dye) [8], and examined freshly by fluorescent microscope.

Results

T-test was used for the analysis of the results. The results of the lymphocyte apoptosis was assessed before and after irradiation with 630nm laser light, included, cellular changes related to the staining cell by trypan blue and examined by light microscope, (figure 1). The percentage of lymphocyte has apoptosis increased significantly ($P < 0.0005$) after irradiation as shown in (figure 2).

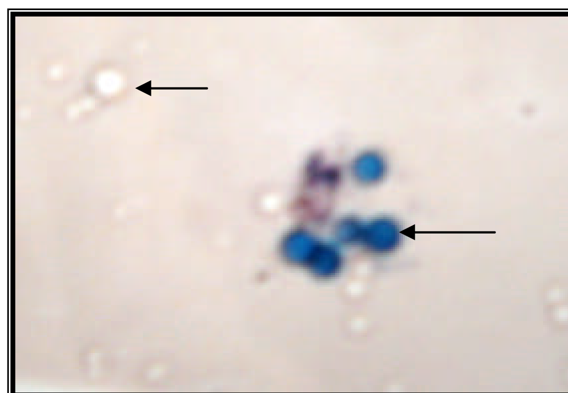


Figure 1: Trypan blue keeping out test white cell normal, dark cell (apoptotic) stained blue. Light microscope (10X)

The morphological changes included: membrane bleb formation, nuclear changes like condensation of nuclear material, kidney-shaped nucleus, fragmentation of nuclei, the percentage of these cells increased significantly (P< 0.005) after irradiation, (figure 3). After increasing the exposure time of lymphocyte to laser (30 minutes), the percentage of lymphocyte apoptosis significantly increased (P< 0.0005), (figure 4).

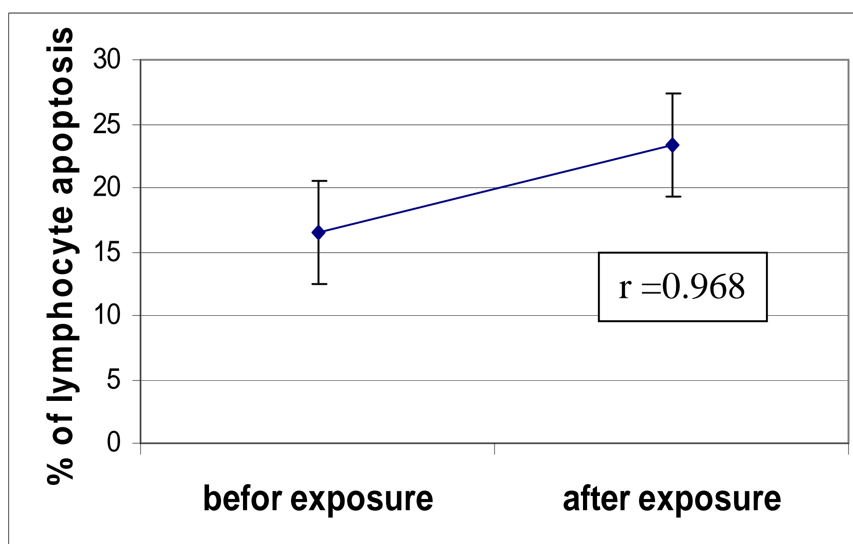


Figure 2: Comparison of the percentage of lymphocyte apoptosis before and after the exposure to laser by Trypan blue exclusion test using light microscope

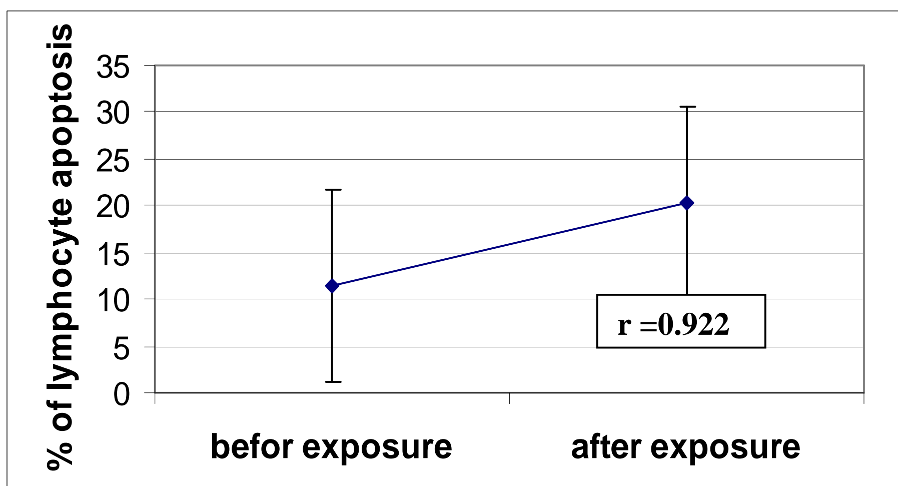


Figure 3: Comparison of the percentage of the lymphocyte apoptosis stained by acridine orange before and after the exposure to laser by using fluorescent microscope.

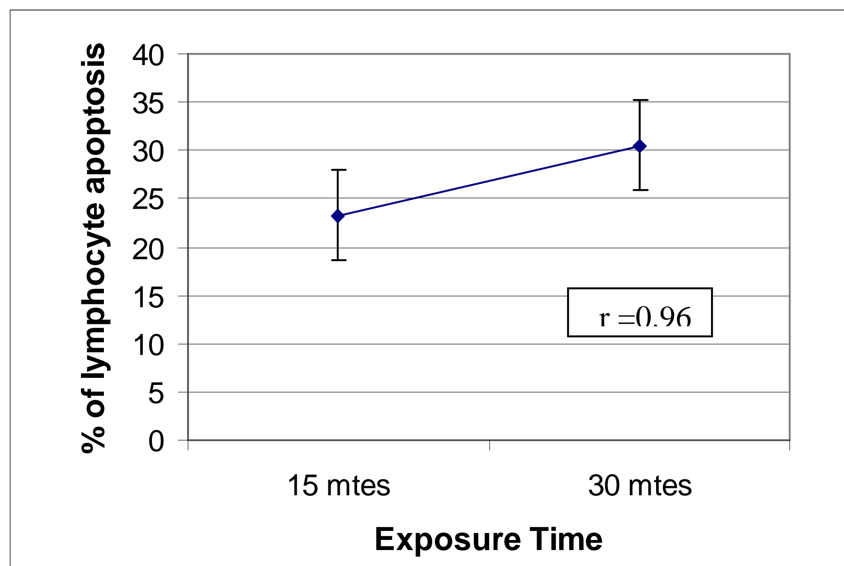
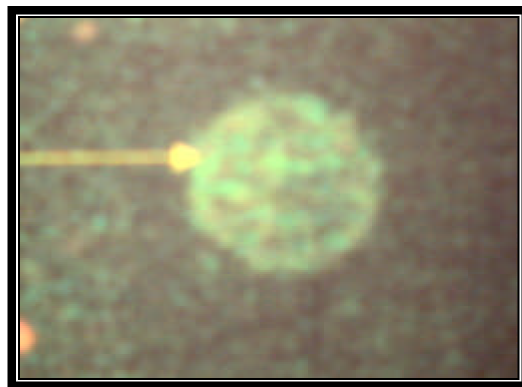


Figure 4: Effect of increasing the exposure time on lymphocyte apoptosis by morphological changes.

A morphological change was observed for lymphocyte having apoptosis, which stained by achridine orange and examined by fluorescent microscope (figure 5).

(Figure 5) shows lymphocyte stained by DNA specific dye (Acridin Orange): (5.a) the normal cell shows intact rounded cell with yellow fluorescence. While in figure (5.b) shows apoptotic cell with membrane bleb formation then separation of cell into two fragments as shown in figure (5.c).

The morphological changes related to mitochondria are shown in (figure 6). In normal lymphocyte the dye accumulate in mitochondria giving off a bright red fluorescence (6.a) while in apoptotic cell the mitochondrial membrane disturbed and the dye will gives off a green fluorescence (6.b).



(a)

(b)

(c)

Figure 5: Lymphocyte stained by acridin orange (DNA binding specific dye) shown by fluorescent microscope 40X.

a. Normal lymphocyte cell.

b. Membrane bleb formation.

c. Fragmentation of nucleas into two parts.

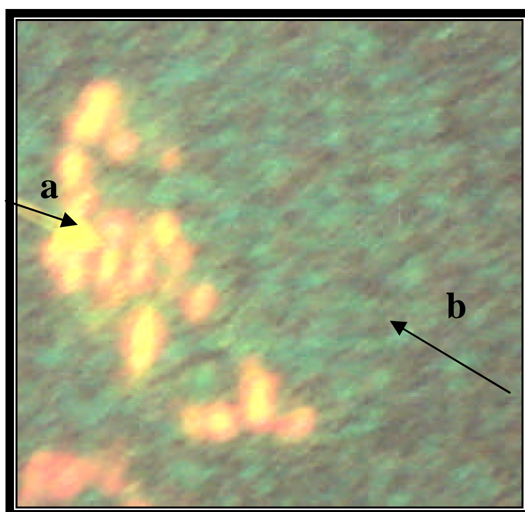


Figure 6: Mitochondria (mitolight detection kit)
a. Normal cell mitochondria (red).
b. Arrow apoptotic cell show green fluorescence of mitochondria shown by fluorescent microscope (40X).

Discussion

Lymphocyte apoptosis can be induced by many factors some acts on cell membrane and can trigger lymphocyte apoptosis by external pathway like fas ligand and granzyme-B, or withdrawal of growth factors like IL-2. All these can stimulate lymphocyte apoptosis by external pathway that lead to activation of caspases 8, 9, 7 and other down stream caspases [(12)].

Other factors can stimulate lymphocyte apoptosis after penetrating cell membrane and act directly on internal apoptosis pathway or mitochondrial pathway, this trigger like laser.

Results in figures 2, 3 and 4 shows that there is an increase in lymphocyte apoptosis caused by the exposure to laser light, which increased further by increasing time of exposure. This could be interpreted by: the mechanism of photon energy conversion in laser medicine is heating. A photobiological reaction involves the absorption of a specific wavelength of light by the functioning photoreceptor (photoaccceptor) molecule (molecules capable of absorbing the wavelength used for irradiation resulting in a photobiological response), after absorbing the light of the wavelength used for irradiation this molecule assumes an electronically excited state [(13)]. So the supplied heating due to irradiation with laser light 633 nm will penetrate lymphocyte membrane and cause disruption of mitochondrial membrane potential caused changes in mitochondrial optical properties [(14)] which may lead to release of cytochrom-C that stimulate down stream caspases 3 and 6 and lead to lymphocyte apoptosis [(15)]. This was proved by mitolight detection kit in this study, which showed changes in color upon changes in mitochondrial membrane potential which was induced by laser therapy

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