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CYTOGENESES CHANGES ON LYMPHOCYTE BY USING LOW LEVEL LASER (LLL)

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ABSTRACT
In the last three decades, the laser had significance in medicine and industry, the soft laser is used in therapy of different disease, viral infection, bacterial infection that was painful in the oral cavity. The aim of this study is to determine the genetic effect of low level laser (LLL) on lymphocyte. As whole blood was taken from 30 healthy volunteers and irradiated with both pulse and a continuous mode of the same wavelength of laser of 650 nm output power of 25mw, at different duration time(15, 20, 25, 30min) in comparison with control (no irradiation). The results showed no differences between both groups in chromosomes number or and chromosome shape. In conclusion, soft laser with this wavelength was safe and it can be used in treatment of oral disease or wound healing.

KEY WORDS: lymphocyte, laser, chromosome changes, genetic changes.

INTRODUCTION

Laser recently becomes extremely important both in medicine and sciences. Soft laser have been used to achieve very precise therapeutic effects, such as bio stimulating cells and for anti-inflammatory effects [1, 2]. Cellbio stimulation provide by soft laser is reflected throughout the formation of the following mechanisms, reduction of cellular cariokynesis time which leads to faster wound healing; the increase of cellular ATP, stimulation of intra and extra fluid ions. So that the potential cell energy is increased, this supports the bipolarization and then helps in cellular exchange stimulation of specific cellular elements that depending on their absorption potentials, regarding some wavelength[3]. All of these mechanisms of stimulation and regulation produce effects which favor wound healing and swelling reduction that leads to total improvement of both arteriovenal and lymphatic nutrition and microcirculation[4]. The observation that chromosome damage can't be caused by exposure to soft laser beam among the first reliable evidence that soft laser or low level laser cant cause major alteration to the genetic material of eukaryotic cell[5]. Although our understanding of chromosome structure is incomplete evidence suggest that chromosome abnormalities are direct consequence and manifestation of damage at the DNA level[6]. In the classical cytogenetic techniques, chromosome are studied directly by observing and counting aberration in metaphases[7]. The aim of this study is to determine the effects of the soft laser on the DNA at the chromosome level as an essential part of genetic toxicology because chromosomal mutation is an important event in carcinogenesis[8,9,10].

MATERIALS & METHOD

Materials

1- A fresh blood was collected in plane tubes.
2- Laser device of 658nm, power density10mw.
3- Lymphocyte culture medium (15/Bovin serum in RPMI/640 with 0.3mg Pha(by Iraqi center for cancer research and medical genetic
4-5mg of cyt-B, FUMhoob

Method

1- One ml of fresh blood is withdrawn from 30 volunteers, divided into two tubes containing heparin as anticoagulant, each with 0.5 ml of blood, one was served as control (not irradiated) and the other exposed to laser beam in continues mode as the following groups:
A-group irradiated for 15mn
B-group irradiated for 20mn
C-group irradiated for 25mn
D-group irradiated for 30mn
2-Both groups C&D irradiated with the same laser but with pulse mode of 5 pulse/ sec.
3- Blood of the four groups after irradiation were incubated in lamplight lymphocyte culture medium (15/Bovine serum in RPMI/640 with 0.3mg PHA and put in incubator at 37ºc for 72hr.
4-After 28 hours of adding cyst-B cells are harvested by cytocentrifuge, after that cells are gently suspended in the tubes, cell suspension is then transferred to cytocenterifuge and exposed to dry air for 20min, then fixed for 10 min in absolute menthol.
5- The cells were stained by using DIFF Quick.
6-After staining, the slides are examined at 100X magnification by using fluorescence microscope.
6-The number of micronuclei were counted in both control and the irradiated samples.

RESULTS & DISCUSSION

This study showed that after irradiation of whole blood there is no changes in chromosomes number or and chromosome breadless or any infringements of chromosomes less than what is recognized that chromosome loss and malsegregation of chromosomes

(non-disjunction) are important event in cancer and ageing and that these are probably cause by defect in the spindle Centro meter or as consequence of under condensation of chromosome structure before metaphase[7].The same number and shape in both samples are appearing as show in (Fig.1). Furthermore, when it compared with the control they show the same number (Fig-2). From this counting it was noticed that the no. of chromosome in the control and each sample group is the same. In the method (MNI) of micronuclei with the above dose remains fixed, we can say with safe dose (Fig.1-Fig.2). Many theories have been postulated about the mechanism of action for low level laser especially about the exact mechanism of action and the physiological changes occurring at the cellular level. According to Russian researchers, DNA replication emits light at 658nm. Since this is very close to the Wavelength of the He-Ne laser light, it is postulated that laser may accelerate DNA replication via photo stimulation Laser irradiation at this frequency is said to be non-mutagenic since it is not in the range to alter the genetic program by affecting chromosomal ultra-structure. The latter is more likely to occur at Ultra violet light irradiation at 300-400nm.

The method of using soft laser in wound healing and pain relief has not got clarified complete but Escola[5] have reported that soft laser with the above specification with these duration time had no effect on genetic material also there is no changes in nuclei no. According to the results that obtained in this work, the soft laser with this wavelength was safe and it is recommended for treating oral disease or wound healing for herpes simplex labial therapy which is safety within the above dose, also we conclude that this type of laser cannot be carcinogenic and or one of causes of chromosome abnormalities.

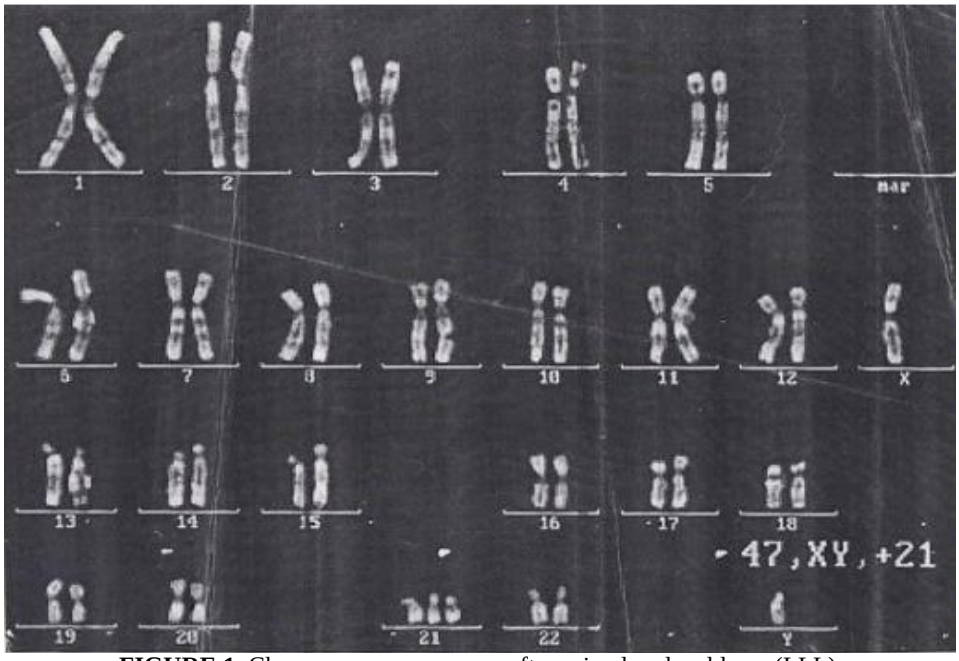

FIGURE 1: Chromosome appearance after using low level laser (LLL)

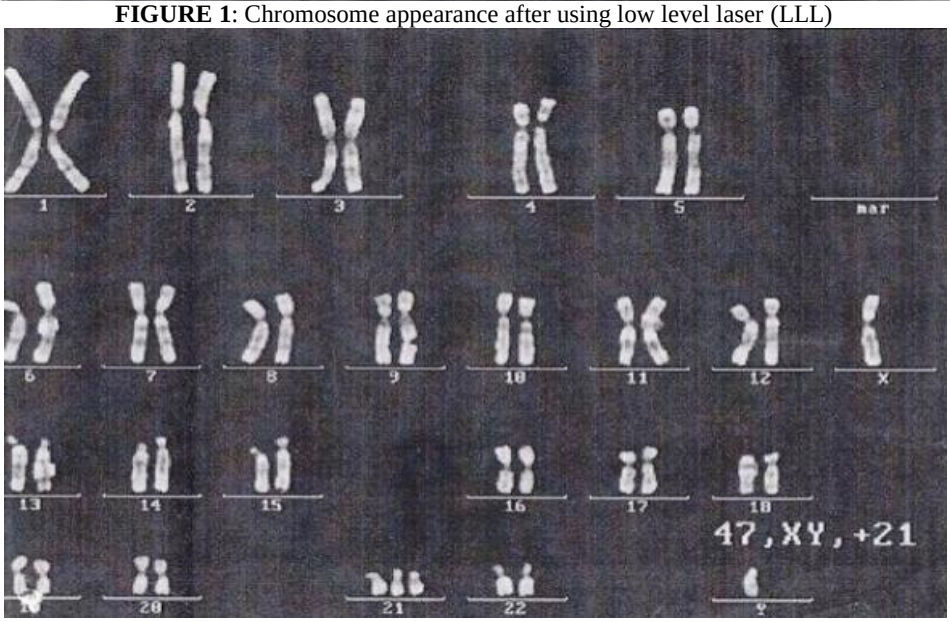

FIGURE 2: Chromosome appearance of control group (no laser treatment)

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