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Immuno-hematological and biochemical changes in diabetic retinopathy patient`s blood after treatment by Nd-YAG laser

Farah A.J Al-Zahawi **Ali S. Mahmood**

Institute of Laser for Postgraduate Studies, University of Baghdad, Baghdad, Iraq

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Abstract: Diabetic retinopathy is one of most important complications of diabetes mellitus that can be treated by Nd:YAG laser. Laser is used in ophthalmic practice for photocoagulation and photodisruption. The purpose of this study is to evaluate changes in immunological ,hematological and biochemical values after treatment of diabetic retinopathy by laser. Blood samples from 10 patients suffering from diabetic retinopathy were taken before and after laser treatment to coagulate retina to prevent leakage and hemorraghe to avoid deterioration of vision.In group one (4 patients = 40%), blood tests were done one day after treatment. In group two (6 patients =60%) tests were done 7 days after treatment with laser. The study showed no clear changes in the values of biochemical tests(except glucouse is already high) and hemtologyical tests (except PT, PTT, clotting time and bleeding time for those patients taking l00mg acetylsalicylic acid or warfarin) after irradiation with laser, probably because that main organs that affecting these tests or the elements that involve in these tests have not been irradiated directly. The main changes were in mmunoglobulin values which increased as noticed in both groups, because all light-induced biological effects depend on the parameters of irradiation, the results suggest that the laser irradiation may play two principal roles in immunological changes. First, is stimulation of cellular proliferation, and stimulation of cellular differentiation that is responsible for different types of immunoglobulin.The other suggestion is that laser is acting as a triggering factor which induces systematic effects through the circulation when laser interacts with living cells so it has systemic effects through circulating blood.

Introduction

The absorbed portion of a laser radiation can produce wavelength dependent and wavelength independent interaction mechanisms. A wavelength dependent interaction mechanism involves, photochemical signs such as photodynamic therapy, biostimulation and photochemical ablation due to volume stress. On the other hand the wavelength dependent mechanism also involves photothermal interaction leading to coagulation, vaporization, carbonization, melting and photothermal ablation due to thermal stress.When the laser pulses are extremely of short pulse duration a wavelength independent interaction mechanism occurs and different phenomena manifest themselves such as photodisruption and plasma induced ablation. Interaction of ultrashort pulsed laser at high intensities causes optical breakdown at the tissue surface. The electrons from molecules in target tissue that gain energy will be freed and produce a collection of free electrons, and ions, called plasma (Markolf, 2003). In Photodisruption **r**apid expansion of plasma creates acoustic and shock waves that are combined with latent tissue stress incise the target tissue, producing photodisruption (Hamilton, and ubig 1996). In general photodisruption may be regarded as a multi-

cause mechanical effect starting with optical breakdown (Fuller, 1987) The primary mechanisms are shock wave generation and cavitation, completed by jet formation if cavitations collapse in fluids and near a solid boundary. In this way the tissue is disrupted mechanically. Nd-YAG laser is most useful when very high energies are required as in photodisruption procedures. Such high energy emissions can only be sustained for picosecond and femtosecond (Beesley, 1978)

The most important application of disruptive interaction is posterior capsulotomy of the lens-frequently being seen as a complication after cataract surgery and also laser induced lithotripsy of urinary calculi. Laser used in clinical ophthalmologic practice can be subdivided into those for photocoagulation and for photodisruption. If the raised temperature reaches a certain critical level coagulation of the tissue will occur. To photocoagulate the retina, energy needs to be absorbed in sufficient quantity to cause significant local temperature rise. An average luminance from a continuous wave laser, set at 1mW, during photocoagulation is 10000 mW/cm^2. This is sufficient to raise the temperature to 30 degree of Celsius and to coagulate retinal pigment epithelium. (Baxter, 1999).

In the present work a comparison of values before and after treatment with laser for a diabetic retinopathy is presented.

It involves the evaluation of biochemical values, which can be obtained by many tests involving Ca^{+2}, total protein, albumin, globulin, glucose and lipids (Zilva, 1984). Also hematological evaluations that can be made by RBC count, ESR, Hb, PCV, WBC tests (Dacie, and Lewis, 1970) and Finally Immunological investigations (IgG, IgM, and IgA) carried out by immunoglobulin tests (Parslow, 2001).

Materials and methods

Blood samples were taken from 10 patients inflected with diabetic retinopathy before and after laser treatment (at medical city- teaching hospital, specialties hospital). Blood samples for immunological, hematological and biochemical values were taken from four patients (first group), one day after post-irradiation and blood samples from the other six patients (second group), were taken seven day after post irradiation. The average age was between 60-70 years. Each patient was treated with certain laser parameters as shown in Table (1)

Table (1): Laser parameters for each patient

Case No.	Pulse duration sec.	Spot size µm	No. of shoot	Duration of operation min.	power mW
1	0.2	100	143	15	200
2	0.2	80	121	10	220
3	0.2	300	1000	20	400
4	7 n sec.	80	45	1	842
5	0.2	100	56	20	350
6	0.2	200	683	25	700
7	0.2	200	720	20	300
8	0.2	100	140	10.	200
9	0.2	60	120	20	400
10	0.2	200	150	15	200

Radial immunodiffusion (RID) plates (for IgG, IgA and IgM) were used. Kits for calcium, total protein, albumin, globulin, triglycerides, glucose, and cholesterol were used. For hematological tests, disposable syringe, EDTA tube, trisodium citrate tube, plane tube, slides, cover slides, microscope, centrifuge, capillary tube, micropipette, tip water bath,

spectrophotometer, Hb meter. W.B.C chamber and Westergren mechain were used. A 5 W maximum power, CW mode, Nd: YAG medical laser system used in this study, with diode laser of 633nm as aiming beam.

Results

There was no clear changes in the values of biochemical test (except glucouse is already high) after irradiation with laser in both groups as shown in Tables 2 and 3. Also there was no clear changes in the values of hematological tests after irradiation with laser in both groups as shown in tables 4 and 5

Table (2): Biochemical values after one day post irradiation (1st group)

Case No.	Triglyceride mg/dl		Cholesterol mg/dl		Ca+ mg/dl		Albumin g/dl		Globulin g/dl		Total protein g/dl	
	before	after	before	after	before	after	before	after	before	after	before	after
1	196	195	260	263	9.1	9.4	4.9	5.2	3	3.1	7.5	7.5
2	90	90	170	170	10.5	10.5	4.2	4	3.4	3.7	6.9	6.7
5	100	98	401	396	8	8	5.1	4.9	5.1	4.8	8	8.3
10	110	107	250	240	7.6	7.8	3.9	3.5	4	4.2	73	7.3

Table (3): Biochemical values after 7 day post irradiation (2nd group)

Case No.	Triglyceride mg/dl		Cholesterol mg/dl		Ca+ mg/dl		Albumin g/dl		Globulin g/dl		Total protein g/dl	
	before	after	before	after	before	after	before	after	before	after	before	after
3	150	150	190	190	8.7	8.7	5.2	5.2	3.2	3.2	7.5	7.5
4	155	155	188	188	6.3	6.3	4.9	4.9	4.5	4.5	6.9	6.9
6	90	90	260	260	8	8	4.8	4.8	5.8	5.8	5.4	5.4
7	80	80	240	240	5.3	5.3	8.3	8.3	6.3	6.3	7.2	7.2
8	70	70	24	24	5.7	5.7	3.8	3.8	2.9	2.9	8.7	8.7
9	60	60	190	190	8.7	8.7	5.2	5.2	3	3	5.4	5.4

Table (4): Hematological values after one day post irradiation (1st group)

Case No.	R.B.C count		ESR mm/hr		Hb g/l		Platelet count X 10^9%		W.B.C count X 10^9%		Fibrinogen mg/dl		PCV L/L	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after
1	Normal		30	30	160	159	140	140	8.8	8.6	270	270	0.44	0.44
2	Normal		40	40	168	170	210	210	7.8	7.8	281	280	0.50	0.50
5	Normal		38	38	188	188	130	130	8.3	8.3	420	420	0.52	0.52
10	Normal		55	55	133	133	25	25	6.0	6.0	330	330	0.36	0.36

Table (5): Hematological values after 7 days post irradiation (2ed group)

Case No.	R.B.C count		ESR mm/hr		Hb g/l		Platelet count X10^9%		W.B.C count X10^9%		Fibrinogen mg/dl		PCV L/L	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after
3	Normal		22	22	132	132	200	200	8.0	8.0	200	200	0.64	0.64
4	Normal		33	39	172	172	120	120	5.0	5.0	320	320	0.42	0.42
6	Normal		44	44	152	152	122	122	6.0	6.0	250	250	0.40	0.40
7	Normal		43	43	167	167	133	133	7.8	7.8	410	410	0.33	0.33
8	Normal		32	32	167	167	140	140	8.7	8.7	480	480	0.50	0.50
9	Normal		50	50	146	146	210	210	6.2	6.2	330	330	0.44	0.44

In the immunological tests, in the first group, there was a slight increase in the immunoglobulin values except in case No. 1 in IgM value and case No. 10 in IgG value , in both cases there is slight decrease in the values as shown in Table (6). In the second group, there was a high increase in the immunoglobulins values as shown in Table (7). The diffusions of the blood serum in Ig plates are shown in Figures (1),(2),and(3).

Table (6): Immunoglobulin values one day post irradiation(first group).

Case No.	IgG gm/dl		IgM gm/dl		IgA gm/dl	
	Before	After	Before	After	Before	After
1	1469.4	1917.5	70.4	65.5	223.2	338.8
2	1417.5	1653.9	125.5	290	276.9	303.4
5	1377.1	2377.1	125.5	158.7	226.6	338.2
10	1377.1	1360.6	150.3	158.7	119.5	372.6

Table (7): Immunoglobulin values seven day post irradiation(second group)

Case No.	IgG gm/dl		IgM gm/dl		IgA gm/dl	
	Before	After	Before	After	Before	After
3	888	1904.5	96.7	156.7	292.7	338.9
4	1304.5	1533.9	85.9	102.3	272.6	608.5
6	1533.9	1840.6	96.7	125	255.4	278.3
7	701	2592.8	85.9	273.6	142.4	376.9
8	1304.5	3048.1	85.9	273.6	142.4	376.9
9	1304.5	3488.8	65.5	290	16.6	222.4

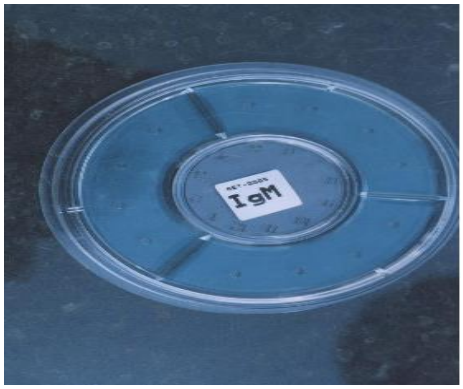

Fig.(1): Diffusion of serum in IgM plate.

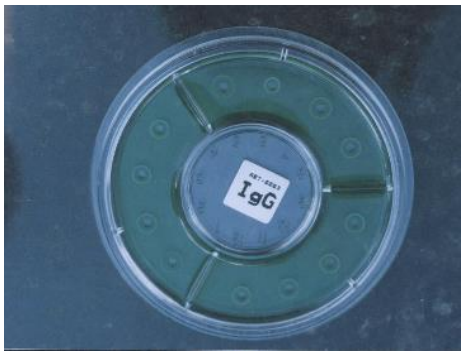

Fig.(2): Diffusion of serum in IgG plate

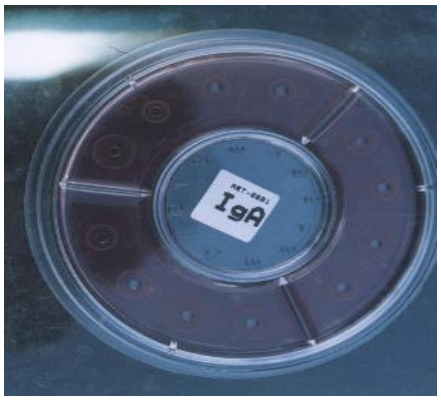

Fig.(3) Diffusion of serum in IgA plate

Discussion and conclusions

The pathogenesis of diabetic retinopathy consists of structural changes in the retinal capillary wall and rheological changes resulting in closure of capillaries and leakage from the diseased vessels wall.The treatment of diabetic retinopathy was achieved by using high energy frequency doubled Nd-YAG laser. The mechamism of the treatment is based on photothermal effects. Photothermal treatment affects on the retinal capillary wall and closure of capillaries. This treatment can prevent the permanent bleeding. With decreasing leakage following focal laser treatment exudates in the fovea will slowly resolve but will leave behind an RPE scar with associated poor visual acuity(Hansr,et,al 2001). In recent years, there are several reports that find the same finding of the immunological tests of this study, but they differ in the regions of human's body on which laser is applied, like "biostimulation of bone marrow cells with a diode soft laser".

The aim of this study was to determine the effect of continuous wave diode laser irradiation on osteoblasts-derived mesenchymal cells (Haas, 2000).. Others studies are by Nicolau and colleagues (2002) from Brazil demonstrated the positive effect of low level laser thearpy on the stimulation of bone with promotion of bone remodulation at injury sites without changes in bone architecture, increased bone volume and increased osteoblasts surface through increased resorption and formation of bone with higher opposition rate. A positive effect on bony implants has been demonstrated by Dörtbudak (2002) and Guzzardella (2003).

The effect of the laser irradiation on osteblastic cells has been reported by Yamamoto(2001) and Guzzardella (2002). All these studies have the same results in which immunoglobulin were increased after laser treatment. Other studies in the same field of low laser radiation were used for the radiation of red blood cells in the presence or absence of epinephrine The red blood cells deformability was increased by epinephrine (Haritou , 1994).

This study showed no clear changes in the values of biochemical and hemtologyical tests after irradiation with laser, probably because main organs that affecting these tests or the elements that are involved in these tests have not been irradiated directly. The main changes were in immunological values that show increase in immunoglobulin levels in the second group. Because all lasert- induced biological effects depend on the parameters of irradiation.

The results suggest that the laser irradiation may play two important roles in immunological changes. One is stimulation of cellular proliferation , and stimulation of cellular differentiation that is responsible for different types of immunoglobulin.The other suggestion is that the laser is acting as a triggering factor which induces systematic effects through circulation, laser interaction with living cells and systemic effects through circulating blood.

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التغييرات المناعية و الدموية و الكيموحيوية في دم المرضى المصابين بآفة شبكة العين السكري بعد المعالجة بواسطة ليزرالنيديميوم ياك

علي شكر محمود **فرح عبد الجليل الزهاوي**

معهد الليزر للدراسات العليا ، جامعة بغداد ، بغداد ، العراق

الخلاصة

أن آفة شبكة العين السكري هي من المشاكل المهمة التي تحدث في مرضى داء السكري التي من الممكن معالجتها بأستخدام الليزرالنيديميوم ياك. .أن الهدف من هذه الدراسة هو تقييم التغيرات في القيم الدموية و الكيموحيوية والمناعية التي قد تحدث بعد أجراء المعالجة بالليزر. أخذت نماذج دم من عشر مرضى مصابين بآفة شبكة العين السكري قبل و بعد المعالجة بالليزر حيث أخذت النماذج بعد يوم واحد من المعالجة (من أربعة مرضى/مجموعة أولى) و بعد سبعة أيام من المعالجة (من ستة مرضى/مجموعة ثانية) حيث تبين أنه لا يوجد تغيير في قيم الفحوص الدموية و الكيموحيوية و قد يكون السبب بأن الاعضاء المسؤولة عن هذه القيم لم تشعع بصورة مباشرة..وأن التغييرات قد حدثت في قيم الفحوص المناعية حيث أزدادت زيادة قليلة في المجموعة الاولى ماعدا في الحالة الاولى حيث كان هنالك أنخفاض قليل في نسبة IgM والحالة العاشرة حيث كان هنالك أنخفاض قليل في نسبة IgG وأزدادت زيادة أكثر في المجموعة الثانيةوقد يكون السبب هو تحفيز تكاثر الخلايا والتحفيز التميزي للخلايا المسؤولة عن المناعة.