

Reverse effect of laser treatment on hyperthyroidism in female rabbits

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Abstract:

This experiment was conducted to study the effect of Laser treatment on LH, FSH, Progesterone and Cortisol and the relationship between them and after physiological functions in adult female rabbits serum affected with hyperthyroidism. Eighteen adult female rabbits at eight months and weighing (1.2 - 1.8 Kg) were used in this experiment. At the end of the two weeks of adaptation, the rabbits were divided equally into three groups. The first group was given distilled water and used as a control. The second and third groups were administered orally with L- thyroxine at a dose of 200µg/Kg body weight daily for twenty days. After the induction of hyperthyroidism, the third group was exposed to laser treatment (40J / cm²) at the thyroid gland region for fifteen successive days for ten minutes on each side of the gland. Where as the second (hyperthyroid) group was left without laser treatment. Blood samples were collected from ear vein and LH, FSH, Progesterone and cortisol levels in serum were assayed. On the other hand food, water intake, body temperature and weight of all animals were estimated. The result show significant increase in these hormones in the second group with an increase food, water intake and body temperature as compared with control. The relationship between these hormones and thyroid gland was discussed. Laser treatment to the hyperthyroid gland seems to normalize these hormones.

Keyword: - Female rabbit, laser, hyperthyroid, hormones

التأثير العكسي للعلاج بأشعة الليزر على تضخم الغدة الدرقية في إناث الأرانب

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الخلاصة:

صمم هذا البحث لدراسة تأثير العلاج بالليزر على الهرمون اللوتيني، هرمون محفز الجريبات، البروجستيرون والكورتيزول والعلاقة بين الهرمونات ووظائف فسلجية أخرى، في الأرانب المصابة بتضخم الغدة الدرقية. تم استخدام 18 أرنب بالغ من الإناث بعمر ثمانية أشهر ووزن (1.2 - 1.8 كغم). تركت أسبوعين كفترة تأقلم ثم قسمت إلى ثلاثة مجاميع متساوية. المجموعة الأولى جرعت بالماء العادي أما المجموعة الثانية والثالثة جرعت بهرمون الدرقين بجرعة 200 مايكروغرام/كغم من وزن الجسم يوميا ولمدة 20 يوماً. بعد ظهور علامات تضخم الغدة تعرضت المجموعة الثالثة لأشعة الليزر بجرعة (

40جول / سم²) على منطقة الغدة الدرقية لمدة خمسة عشر يوماً متتابة وبواقع 10 دقائق لكل جهة من الغدة بينما تركت المجموعة الثانية بدون علاج بالليزر. تم سحب الدم من الوريد الاذني الحافي واستخدم المصل لقياس هرمونات الكونادوتروبين (الهرمون اللوتيني وهرمون محفز الجريبات) وكذلك هرمون البروجستيرون والكورتيزول. من الناحية الأخرى تم حساب كمية العلف والماء المستهلك، درجة حرارة ووزن الجسم للحيوانات. اوضحت النتائج ارتفاع معنوي بمستوى هذه الهرمونات في الحيوانات المصابة بتضخم الغدة الدرقية مع زيادة كمية العلف والماء ودرجة حرارة الجسم ونوقشت العلاقة بين الهرمونات والغدة الدرقية. كما تبين ان العلاج بالليزر أعاد الهرمونات الى مستواها الطبيعي في الحيوانات المصابة بتضخم الغدة الدرقية.

Introduction:

Thyroid gland is the largest of the endocrine organs. It's composed of two lateral lobes joined by an isthmus. It's located in front of the neck. The follicular cells produce two amino acid hormones, triiodothyronine (T3) and tetraiodothyronine (T4) or thyroxine (1).

Thyrotoxicosis is the hypermetabolic condition associated with elevated levels of free T3 and T4. Hyperthyroidism includes diseases that are a result of thyrotoxicosis. Overactive thyroid gland is diagnosed by a high level of T3 and T4 in blood with a low level of TSH in case of hyperthyroidism (2).

The secretion of thyroid hormones is very well established by the hypothalamo-pituitary-thyroid axis. Moreover, the physicians and physiologists have long hypothesized connection between hypothyroidism and adrenocortical dysfunction (3,4).

However, the interaction of pituitary-thyroid and pituitary-adrenal function have been studied (5,6). The influences of thyroid hormones on adrenocortical function have been demonstrated. Chronic administration of T3 at high

concentration increases plasma and adrenal corticosterone levels in male rats as well as the induction of hypertrophy in adrenal gland (7). Moreover, ACTH action on increasing plasma free corticoid is exaggerated in hyperthyroid rats (6).

On the otherhand, the effect of thyroid hormones on other hormonal release and action are not very well established. Some authors (8) had reported that normal reproduction behavior depend on normal level of thyroid hormones. This adequate functioning of both maternal and fetal thyroid glands are very important for brain development.

Moreover, thyroid hormones have been found to regulate the synthesis and secretion of several pituitary hormones. Thyroid hormones can stimulate the transcription of growth hormone m RNA and growth hormone synthesis in rat pituitary tumor cells (9). They also can negatively regulate TSH and TRH transcription (10 and 11). Thyroid hormones have been found to have a direct effect on the transcription of prolactin m RNA (12).

Since the last century laser had been considered the treatment of

choice for many difficult pain management challenges and is becoming a common practice in medicine. It had been used for eye treatment, autoimmune thyroiditis, menopausal bleeding, fallopian tube opening and other uses (13). Our findings revealed a clear depression in serum thyroid hormones in normal and hyperthyroid rabbits after laser exposure to thyroid gland (14, 15).

The available literature lacks the effect of laser treatment on endocrine glands and the related hormones from one side and the relationship between these hormones in some thyroid diseases from the other side. Thus this study was designed to investigate the effect of laser treatment on LH, FSH, progesterone and cortisol and the relationship between these hormones in hyperthyroid adult female rabbits. The effect of laser treatment on these hormones and on food, water intake, body temperature and weight were studied.

Materials and Methods:

A total of 18 adult female rabbits of about eight months old and weighing (1.2 - 1.8 Kg). Were used in this experiment as it was reported that female has more incidence for thyroid dysfunction than male.

They were housed in a clean plastic cage in conditioned room (23-26C⁰) and a light: night cycle of about 12:12 hours. All cages were provided with an easily cleaned floor with an opening for urine and faeces drainage. The animals were left for

two weeks for adaptation with the experimental conditions. After that the animals were divided equally into 3 groups. Standard pellet diet and clean fresh water were provided daily along the experimental period.

Hyperthyroidism induction:

Levothyroxine (L- thyroxin) tablets of 100µg (Menarin : international/Italy) was administered orally to the second and third group at dose of 200µg/ Kg body weight daily for 20 weeks to induce hyperthyroidism. The control group was administered with distilled water.

Clinical signs and symptoms were noticed carefully on the animals during this period and hyperthyroidism was diagnosed from the following features:-

Goiter which is clear from the swelling at the neck region , constipation, depression, muscle weakness, breathing difficulty, tachycardia, hypersensitivity, high body temperature and bulging eyes.

At the end of this period, the third group was exposed to laser diode treatment (40J / cm²) for fifteen successive days at the thyroid region [the location was noticed anatomically (14)] for 20 minutes and the second group was left without treatment. Blood samples were collected from the marginal ear vein of all animals and serum was isolated for the following parameters (16):-

1- Luteinizing Hormone (LH): The immunoradiometric assay of LH

(1 μ /L) is a sandwich-type assay. It was measured by special LH Kit.

2- Follicle Stimulation Hormone (FSH): Its level was estimated in serum (1 μ /L) by special FSH Kit.

3- Progesterone (nmol/L): The radioimmunoassay of progesterone in serum is a competition assay was carried out by special Kit.

4- Cortisol (nmol/L): Determination by radioimmunoassay method by special Kit.

The mean daily food and water intake were estimated for the two weeks of the experiment. Body temperature and weight were also recorded for each animal separately.

All data were subjected to statistical analysis by using two way analysis of variance (ANOVA) and are expressed as Mean $\pm$ SE (17).

Results:

The results illustrated below shows that hyperthyroid rabbits have significantly high ($P<0.05$) LH, FSH, Progesterone and Cortisol hormones level than control rabbits (table 1). The mean values of LH were 7.80 $\pm$ 0.45, 4.38 $\pm$ 0.27 and values of FSH were 8.46 $\pm$ 0.95, 6.38 $\pm$ 0.25 for the hyperthyroid and

control groups respectively. At the mean time, the level of progesterone hormone was 5.05 $\pm$ 0.39, 2.46 $\pm$ 0.17 and values of Cortisol was 2.70 $\pm$ 0.12, 2.10 $\pm$ 0.63 for the hyperthyroid and control groups respectively. There is a significant decrease in LH, FSH, progesterone and cortisol level after laser treatment as compared with non treated group. However, the relationship between these four hormones in each animal of the control and hyperthyroid groups was shown in fig (1) and (2) respectively.

Table (2) demonstrate that hyperthyroid rabbits showed a significant increase in food and water intake for the two weeks as compared with control. Laser treatment to hyperthyroid rabbits reduces these parameters significantly ($P>0.05$). A significant increase in body temperature of hyperthyroid rabbits with concomitant decrease after laser treatment were shown (table 3). On the other hand, this table shows non significant increase ($P<0.05$) in body weights in rabbits as compared with control.

Table (1)- shows the level of LH, FSH, (IU/L) and Progesterone, Cortisol (nmol/L) in serum of control, laser non treated and treated adult hyperthyroid female rabbits.

Group Parameter	control	Hyperthyroid	Hyperthyroid + Laser treatment
LH (1U/L)	4.38 ^B ± 0.10	7.80 ^A ± 0.05	4.73 ^B ± 0.11
FSH (1U/L)	6.38 ^B ± 0.10	8.46 ^A ± 0.15	6.65 ^B ± 0.10
Progesterone(nmol/L)	2.46 ^B ± 0.07	5.05 ^A ± 0.09	2.58 ^B ± 0.01
Cortisol (nmol/L)	2.10 ^B ± 0.03	2.70 ^A ± 0.02	2.21 ^B ± 0.02

Values represent mean ±SE of control and hyperthyroid groups (n=6).

Different Capital letters denote significant difference between groups (p>0.05).

Table (2) – Food intake (gm) and water intake (ml) in control, laser non treated and treated hyperthyroid adult female rabbits.

Group Week	Food Intake (gm)			Water Intake (ml)		
	Control	Hyperthyroid	Hyperthyroid + Laser treatment	Control	Hyperthyroid	Hyperthyroid + Laser treatment
1	Bb 25.4 ± 1.70	Aa 56.0 ± 1.20	ABb 42.0 ± 1.60	Bb 82.8 ± 1.80	Ab 137.0 ± 4.20	Bb 88.0 ± 4.50
2	Cb 31.0 ± 1.09	Aa 60.0 ± 1.07	Bb 47.8 ± 1.70	Ba 146.0 ± 3.10	Aa 196.8 ± 4.70	Ba 142.0 ± 4.40

Values represent mean ±SE of control and hyperthyroid groups (n=6).

Different Capital letters denote significant difference between groups (p>0.05).

Different small letters denote significant difference within groups (p>0.05).

Table (3) – Body temperature ($^{\circ}\text{C}$) and Body weight (gm) in control, laser non treated and treated hyperthyroid adult female rabbits.

Group Week	Body temperature ($^{\circ}\text{C}$)			Body weight (gm)		
	Control	Hyperthyroid	Hyperthyroid + Laser treatment	Control	Hyperthyroid	Hyperthyroid + Laser treatment
1	Bb 38.3 $\pm$ 0.09	Aa 39.2 $\pm$ 0.05	ABb 38.6 $\pm$ 0.05	Aa 960 $\pm$ 6.9	Ab 975 $\pm$ 7.1	Aa 950 $\pm$ 8.8
2	Bb 38.7 $\pm$ 0.06	Aa 39.2 $\pm$ 0.02	ABb 38.9 $\pm$ 0.04	Aa 990 $\pm$ 7.6	Aa 1025 $\pm$ 9.0	Aa 1130 $\pm$ 8.1

Values represent mean $\pm$ SE of control and hyperthyroid groups (n=6).

Different Capital letters denote significant difference between groups ($p>0.05$).

Different small letters denote significant difference within groups ($p>0.05$).

Discussion:

The results of this investigation reveal a significant increase in LH, FSH, Progesterone and Cortisol level in serum of hyperthyroid female rabbits. Thyroid hormones participate in regulating metabolic processes, neurologic development, muscular contraction, hormonal synthesis and secretion (1) at the mean time, the circulating levels of thyroid hormones have a major effect on the central regulation of (HPA) hypothalmo-pituitary-adrenal axis (7).

There has been adramatic increase in the incidence of thyroid neoplasma and other focal proliferative lesions in human and animals since the late 1970s (4).

Hyperthyroidism is being one of the two common endocrine diseases (diabetes mellitus being the first one). On the other hand, females are more susceptible to thyroid diseases than males (3,6). Recently, thyroid disorders has been connected 80 % with ovarian cancer and concluded to be due to the hormonal disturbances and inflammation caused by autoimmune hyperthyroidism (18).

Although, the role of thyroid hormones in reproduction is not very well established and why hyperthyroid patients reveal oestrous disturbances and delay in ovulation is not been answered yet. We could explain the results from our

physiological knowledgment that calcium may be responsible for the stimulation in the hormonal secretion of our hyperthyroid female rabbits.

However, hyperthyroid patients often show disturbances in calcium homeostasis with diffuse chief cell hyperplasia in the parathyroid glands (4). At the mean time, increased calcium has been reported in hyperthyroid rabbits beside the increasing of plasma T3 and T4 levels (14). This was suggested to be mainly due to increase calcium absorption by intestine or calcium resorption from bone.

As calcium is required for synthesis of nucleic acid, proteins and activation of some enzymes (9). Thus, increasing LH and FSH in our experiment maybe attributed to calcium elevation in blood or the direct effect of thyroid hormones on the hypothalamo-pituitary axis (10,11,12).

Another possibility for the hormonal increase in the present study is that chronic administration of T4 might increase the c AMP production in the ovary and adrenal cortex in response to LH, FSH and ACTH respectively and thus, increasing the production of Progesterone, Estrogen and Cortisol respectively (18). Our recent study revealed a decrease in the precursor of these hormones (Cortisol) in serum of hyperthyroid rabbits mainly to compensate the metabolic demand (14). Cortisol increased in

hyperthyroid and decreased in hypothyroid rats (7).

The c AMP has been found to be increased in the Zona Fasciculata-Reticularis (ZFR) cells of adrenal cortex after chronic thyroxine injection in rats (7).

However, the hyperthyroid rabbits in this study showed an increase energy expenditure which is associated with increasing body temperature, food and water consumption. Increasing in resting metabolism rate (RMR) is accompanied by a parallel active tissues such as liver, skeletal muscles and heart (1).

On the other hand, treatment by hyperthyroid rabbits with laser seems to accelerate the regeneration of thyroid gland from any damage caused by excessive thyroid hormones (19). Therefore, the level of other hormones in blood is normalized and restored in their hyperthyroid rabbits. This is associated by decreasing their body temperature, food and water intake. Laser therapy produce a decrease in T3&T4 levels with an increase in TSH level not in hyperthyroid rabbits only but in normal rabbits also (15).

In conclusion, first of all our results proved the relationship and connection between hyperthyroidism and sexual hormones in female. Thyroid disorder function could be restored and retreated by laser and thus normalize the reproductive related hormones. It is very difficult to discuss these changes since the

main action of laser is not very well studied. We need more work and facilities to prove this evidence. At the mean time, studying the effect of laser treatment on some reproductive glands and hormones is very necessary.

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