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ARTICLE

The Impact of Insulin-like Growth Factor-1, Growth Hormone, and Oxidative Stress in the Experimental Model of Juvenile and Adult Hypothyroid Retinal Degeneration

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Abstract

Background: The pathophysiological mechanisms involved in hypothyroid retinal damage are still being debated. **Materials and methods:** Ninety-six pups of albino rats were equally divided into control, hypothyroid model and thyroid supplement groups. Each group was separated into juvenile and adult subgroups that were sacrificed at day 20 and 60 postnatally, respectively. Pups in the hypothyroid group were born to mothers who received 0.05 mg Carbimazole/day/pregnant female orally during gestation and at 20 days of lactation in juvenile subgroup. The adult subgroup received the hypothyroid agent until day 60 postnatally. The thyroid supplement group received antithyroid agent, then were treated with L-thyroxine orally (10 ug / kg body weight) for one month. **Results:** A significant reduction in body weight, temperature, heart rate, levels of Triiodothyronine (T3), Tetra-iodothyronine T4), growth hormone (GH) and Insulin-like growth factor (1GF-1), and antioxidant enzymes and an increase in systolic pressure and lipid peroxidation (LP) products were evident in hypothyroid subgroups associated with a decreasing thickness and degeneration of retina l layers. Thyroid supplementation group showed a partial normalization of the measured mediators accompanied by recovery of structural changes especially in the juvenile subgroup. **Conclusions**: Thyroid hormone has a vital role in normal retinal growth. GH, IGF-1 and oxidative stress are also significant mediators of retinal degeneration.

Keywords: Hypothyroidism, retinal degeneration, IGF-1, GH, oxidative stress and animals.

Abbreviations: T3: triiodothyronine, T4: Tetra-iodothyronine, GH: growth hormone, IGF-1: Insulin- like growth factor 1, LP: lipid peroxidation products

Introduction

The thyroid hormone axis has an important role in the

physiological functions and the development of the CNS, including the eye (1,2). Retinal degeneration continues to be a significant cause of blindness worldwide. It is possible that further reductions in the prevalence and severity of blindness caused by retinal degeneration will be made only with advancements in its prevention, rather than treatment once it occurs.

Insulin-like growth factor (IGF)-I and growth hormone (GH) are major regulators of normal growth and activity of retina in mice and humans (3,4). Besides, IGF-1 is a critical modulator of thyroid activity. Infants born prematurely (<27 weeks) are more likely to have low thyroxine (T_4) levels, indicating an abnormal hypothalamus-pituitary-thyroid axis function (5). These considerations raise the possibility that hypothyroidism may be involved in retinal degeneration.

Growth factors have been found in vitreous fluid, in which they regulate retinal function and provide markers of ocular degeneration. The presence of GH in the human retina and vitreous fluid suggests that GH may have roles in visual function and may be involved in the pathophysiological theories of ocular degeneration (6). Free radicals have been known to damage cellular components. Also, changes in the levels of lipid peroxidation and antioxidant enzyme activities in different tissues of hypothyroid rats are the primary cause for functional disorders of these tissues (7).

Multiple pathophysiological mechanisms may be involved in explanation of hypothyroid retinal degeneration. However, our knowledge of the exact mechanisms remains unclear. We hypothesized that hypothyroidism would induce retinal degeneration, and that supplementation with thyroxine can ameliorate this condition. The rationale behind this research was to focus on the role of IGF-1, GH and oxidative stress as contributors to hypothyroid retinal damage.

To test our hypothesis, an animal model (white albino rats) was established. The animals were divided into three equal groups: control, hypothyroid model and thyroid supplement. Each group was separated into juvenile and adult subgroups. To test the efficacy of the Carbimazole in the induction of hypothyroid model, number of living pups, body temperature, body weight, heart rate, systolic blood pressure, and serum levels of T3 and T4 were also evaluated.

Materials and Methods

Animals and Maintenance

During the experiments, the animals were treated in accordance with the published rules for animal laboratory care, and the protocol has been approved by the Committee of Assuit University, School of Medicine, Assuit, Egypt. A total of ninety six newly born male rats were used and obtained from Assuit University Animal Facility. They were housed in the Animal Facility with room temperature maintained at 65–75 _F, relative humidity of 50–70% and an airflow rate of 15 exchanges/h. In addition, a time-controlled system provided a 12:12-hour light-dark cycle. All animals were given food and water ad libitum.

Induction of hypothyroid model

After a seven-day acclimatization period, experimentally native, infection-free, adult female Wister-albino rats weighing 200-250 grams, were placed in individual wire mesh cages and were mated with adult male rats for copulation. After confirming pregnancies by daily morning examination until finding of vaginal plug, the pregnant rats were placed in nesting cages and remained undisturbed until delivery.

Thirty gravid rats (dams) were then randomly assigned into two groups. One of them was the hypothyroid group (n=25) that received anti-thyroid agent Carbimazole (Neomercazole, Sigma Chemical, Co., St Louis, MO,) 0.05 mg/kg body weight, orally by gastric tube from day 16 of gestation (date of retinal development) till delivery and continue for 20 days of lactation (8). The other group, control (n= 5) received only the vehicle in the same dose and route as hypothyroid group.

The pups of control mothers (n= 32) were divided into two equal subgroups, juvenile and adult that were sacrificed at day 20 and 60 postnatal respectively. While the pups of hypothyroid mothers were separated into two major groups, hypothyroid model and thyroid supplement, each group contained 32. Hypothyroid model group alienated into juvenile subgroup, their mothers received the antithyroid drug during gestation and 20 days lactation and adult subgroups, the pups themselves continuously receiving the hypothyroid agent orally until day 60 postnatal.

Thyroid Supplementation

The offspring of the thyroid supplement group were similarly separated into two equal subgroups, juvenile and adult, that received the same regimen as the hypothyroid model groups, then they were treated by L. thyroxine (Galaxo

Wellcome Company) 10 ug / kg body weight orally by gastric for one month (9).

Physiological Assays

The animals of each group were evaluated for number of living pups, body weight (gm) of the pups, systolic blood pressure, heart rate, body temperature then they were anesthetized by an IP injection of chloral hydrate, 40 mg/Kg body wt., (chloral hydrate was used once to induce anesthesia and was used in all groups, even the control group, to balance its suppression effect on thyroid function) and blood samples were taken immediately before decapitation. Their serum and vitreous fluid was evaluated for levels of T3 and T4, IGF1, GH and LP, SOD, catalase and GSH-Px (U/mg Hb).

Blood pressure: Systolic blood pressure was measured for all pups at the end of the experiment with a pneumatic tail-cuff device (NARCO, Biosystems, Inc., Houston, Texas) after the animal had been prewarmed for 30 min. in a metal chamber maintained at approximately 30° C. Three consecutive measurements were made at the same time of day. Mean systolic blood pressure value from three measurements was recorded as the pressure value for each animal.

Recording of heart rate with electrocardiogram

The pups were anesthetized, then needles of electrocardiograph were inserted in the skin of four limbs. The needle were connected to ECG recorder and counted the heart rate (ECG Cardiofax Nih Onkohn Kohden; Kogyo Co.Ltd , Japan).

Recording of body temperature

Body temperature was measured by insertion of a rectal thermistor probe (Cole-Parmer Instrument Company, Chicago, IL). The probe was 3.3 mm in diameter, and was lubricated with petroleum jelly. Temperature measurement was conducted on a table. Each rat was held at the base of its tail as the probe was inserted 2.5 cm into the rectum. The probe was removed after the animal's temperature stabilized 30–60 sec later. The thermistor was accurate to 0.1°C (10).

Laboratory Measurements

Blood samples were obtained from the jugular vein of anaesthetized animals immediately before sacrifice. Samples were collected in both types of tubes with and without EDTA, and were centrifuged at 3000 r.p.m. for 20 min to separate serum, plasma and RBCs. Erythrocytes were washed with saline then haemolysed with an equal volume of distilled water. The plasma, serum and haemolysed RBCs were preserved at - 20^{0}C for assy.

Estimation of T3, T4, GH and IGF1

Enzyme-linked imunosorbent assays (ELISA) were performed for measuring concentrations total triiodothyronine, total thyroxine, GH, IGF-1 in serum; also GH and IGF-1 were measured in vitreous fluid using Biosurce Europe commercial kits with monoclonal antibodies against each substance and following the instruction supplied with each kit. The apparatus used was Ansoth 2000, manufactured in Austria.

Estimation of Lipid Peroxidation

Lipid peroxidation products levels were measured in plasma according to Aruoma, et al (11).

Estimation of SOD in plasma and catalase activity in hemolysates was done according to Misra and Fridovich (12) and Luck (13) respectively.

Estimation of antioxidant enzyme glutathione peroxidase (GSH-PX): level in RBCs was made following the method described by Hafeman, et al. (14). The values were then divided on hae-moglobin concentration/l. Haemoglobin concentration was determined by the following method described by Crosby, et al. (15), by using Complete Hemoglobin reagent kits; (Merckotest, E. Merk, Darmstadt, Germany).

Histological and Morphometric analysis

Both eyes from each pup were enucleated and placed in ice-cold phosphate-buffered saline (PBS; pH 7.4). Enucleation was performed with the use of iris forceps and scissors for separation of the eyes from the surrounding connective tissue, nerves, and muscles. The eyes were dried on sterile gauze, and the vitreous fluid was aspirated with a 0.5-mL insulin syringe and placed on ice in sterile tubes (Eppendorf). The eyes were then fixed in 4% gluteraldehyde and prepared for light and electron microscopic examination.

The thickness of different layers of the retina was measured in toluidine blue stained sections, and cell count of the ganglion cell layer (linear cell density in number per unit length of the retina) was done. The measurements were performed using Image Analyzer (Leica Q 500).

Statistical Analysis

Data are expressed as mean ± SE for all parameters. The data were analyzed using GraphPad Prism data analysis program (GraphPad Software, Inc., San Diego, CA, USA).

For comparison of statistical significance between different groups Student Newman-Keuls t-test for paired data were used. For multiple comparisons, one-way analysis of variance (ONE- WAY-ANOVA) test followed by the least Significant Difference (LST). Correlations were assessed using Spearman's non-parametric correlation coefficient ϱ as described by Knapp and Miller (16). A value of $P \leq 0.05$ was considered statistically significant.

Results

Presentation of the results

The anti-thyroid drug Carbimazole (Neomercazole) successfully induced hypothyroid retinal degeneration in rats. Administration of L. thyroxine ameliorates to some extent theses effects. A summary of the results is shown in Tables 1-3, Histogram 1-6 and Figures 1-15. These are outlined below highlighting the pathological mechanisms:

Effects of anti-thyroid agent

Intake of anti-thyroid agent was associated with reduction of number of living pups, body weight, temperature and heart rate while increase in systolic blood pressure of both subgroups when compared with their corresponding age-matched controls. Treatment with L-thyroxine for one month significantly ameliorated these changes especially in juvenile subgroups as shown in Table 1.

Relation to serum levels of T4 and T3

Estimation of serum levels of both T4 and T3 showed a significant decrease in both subgroups of hypothyroid model rats when compared with their control euthyroid subgroups. The reduction was obvious in adult subgroups of hypothyroid model. Treatment with L. thyroxine significantly increased the levels of T4 and T3 in both subgroups (Table 2).

Relation to lipid peroxidation and antioxidants (SOD, CAT and GSH-Px)

Compared with the control euthyroid group, LP products significantly increased while the levels of measured antioxidants decreased in hypothyroid model subgroups. An administration of L. thyroxine was associated with a significant improvement in the plasma level of LP and antioxidants when compared with hypothyroid model subgroups, and the response was manifested more in the juvenile thyroid supplement subgroup (Table 2).

Relation to GH and IGF-1 levels in serum and vitreous

Table 1: Comparison mean values of survival rate, body temperature, blood pressure, heart rate in control, hypothyroid model and thyroid supplement rat groups.

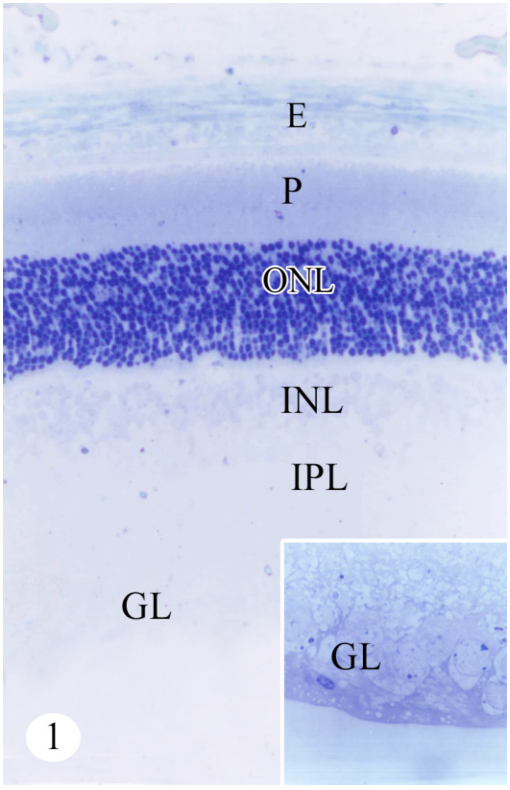
Values represent mean ± SD and were analyzed by a Student–3Newman–Keuls t-test. , Subgroup I and II of rats, Sacrificed at day 20 (juvenile subgroup) and 60 (adult subgroup) postnatal. (n=16 in each subgroup) , (*) Significance versus control group. (€) Significance of values in thyroid supplement subgroup versus its corresponding hypothyroidism subgroup, ***/ (€)(€)(€) significant difference at P<0.001.

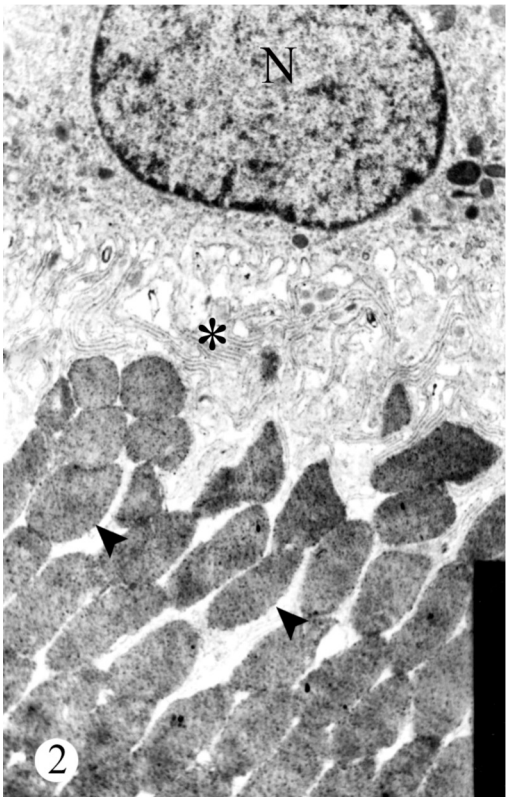
Groups/ Items	Group A Control (Euthyroid)		Group B (hypothyroid model)		Group C Thyroid H supplement	
	Subgroup I	Subgroup II	Subgroup I	Subgroup II	Subgroup I	Subgroup II
Survival rate	14/16	15/16	10/16	12/16	13/16	13/16
No. of living rats	14	15	10	12	13	13
Body weight (gm)	82.5±5.7	138.3±18.3	53.2±6.93 ***	104.9±9.3 ***	74.54±9.3 €€€	132.5±12.87 €€€
Body temp. ^{0}C	37.01±0.25	37.16±0.32	36.19±0.47 ***	35.98±0.34 ***	36.7±0.28 €€	36.42±0.35€€
BP (mmHg)	114±3.0	116±2.9	133±2.27 ***	148±5.4 ***	121±4.1 €€€	126±3.96 €€€
Heart rate (beats/m)	225.5±6.88	219 ±6.95	197.6±6.52 ***	184±4.9 ***	206±8.8 €	196± 8.2 €€€

Table 2: Alterations of levels of T4, T3, lipid peroxidation products LP, SOD, Catalase and glutathione peroxidase (GSH-PX) in hypothyroid model and thyroid supplement groups.

Values represent mean ± SD and were analyzed by a Student–3Newman–Keuls t-test. Subgroup I and II, Sacrificed at day 20 (juvenile subgroup) and 60 (adult subgroup) postnatal. (n=16 in each subgroup) , (*) Significance versus control group. (€) Significance of values in thyroid supplement subgroup versus its corresponding hypothyroidism subgroup, ***/ (€)(€)(€) significant difference at P<0.001, 8/ € significant difference at P<0.05, **/ €€ significant difference at P<0.01.

Groups/ Items	Group A Control (Euthyroid)		Group B (hypothyroid model)		Group C Thyroid H supplement	
	Subgroup I (n= 14)	Subgroup II (n=15)	Subgroup I (n= 10)	Subgroup II (n= 12)	Subgroup I (n=13)	Subgroup II (n=13)
SerumT4 (µg/dl)	13.2±0.83	12.76±0.68	6.1±0.1.5 ***	4.4±0.55 ***	8.6±0.75 €€€	7.9±0.76 €€€
Serum T3 (ng/l)	0.9±0.1	0.68±0.12	0.07±0,02 ***	0.08±0.4 ***	0.63±0.15 €€€	0.55±0.11 €€€
LP (nmol/ml)	2.37±0.45	2.4±0.47	4.96±0.5 ***	5.2±0.49 ***	4.37±0.46 €€	4.7±0.54 €
SOD (nmol/ml)	4.89±0.37	5.03±0.41	2.52±0.45 ***	2.97±0.6 ***	3.74±0.58 €€€	3.33±0.69 €
Catalse(U/mg Hb)	0.58±0.05	0.62±0.04	0.42± 0.05 ***	0.36±0.04 ***	0.53±0.04 €€€	0.44±0.05 €€€
GSH-Px(U/mg Hb)	58.3±0.8	58.5±0.7	53.2±2.1 ***	51.6±1.4 ***	55.1±0.9 €€	53.1±1.4€





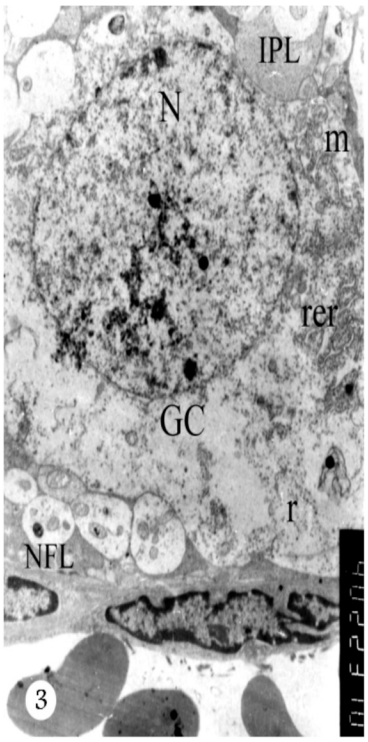
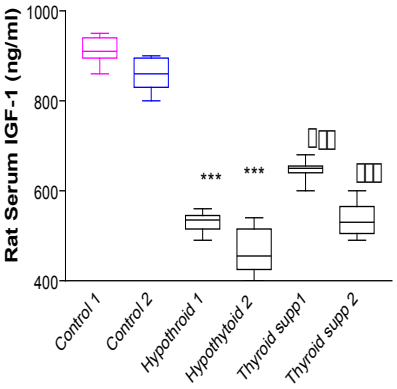


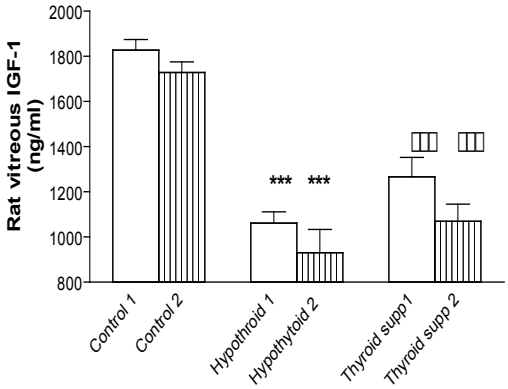
Fig. 1, 2 and 3: A semithin section of the juvenile retina of control albino rat that similar to that of control adult subgroup: showing; epithelial layer (E), photoreceptor layer (P), outer nuclear layer (ONL), inner nuclear layer (INL), inner plexiform layer (IPL) and ganglion cell layer (GCL). (Toluidine blue X 200, X1000). Electron microscopic examination showing; a part of epithelial and photoreceptor layers, structures of the photoreceptors outer segment (x 5000) (Fig. 2), inner plexiform layer (IPL) and ganglionic cell layer can be seen in figure 3 (x4000).

tistical analysis showed a significant increase ($P < 0.001$) GH and IGF-1 in the juvenile subgroup of controls when compared with the adult subgroup. Intake of anti-thyroid agents significantly decreased GH and IGF-1 in serum and vitreous of both hypothyroid subgroups in comparison to their control subgroups. Treatment with L. thyroxine for one month significantly restored their levels, especially in the juvenile subgroup (Histograms 1, 2).

Correlation and Regression Analysis

In both hypothyroid rat model subgroups, there was a significant positive correlation between serum T4 and serum GH, IGF-1 and antioxidants (SOD, CAT and GSH-Px)





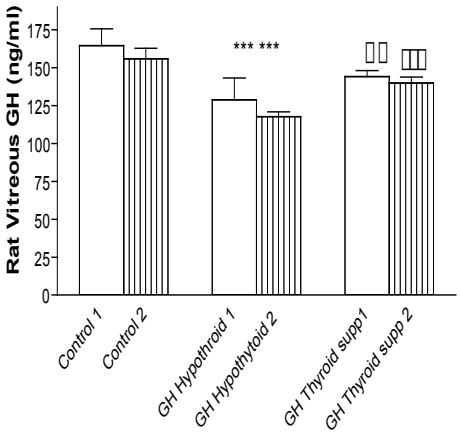
Histogram 1: Serum and vitreous IGF-1(mean ± SD from individual rats were analyzed by a Student–3Newman–Keuls t-test. Levels of IGF-1 were decreased in all (Carbimazole) treated rats in a dose of 0.05 mg mg/day/pregnant female during gestation and lactation, subgroups I and II, Sacrificed at day 20 (juvenile subgroup) and 60 (adult subgroup) postnatal respectively. The IGF-1 increases more in thyroid supplement subgroup 1. (n=16 in each subgroup) , (*) Significance versus control group. (€) Significance of values in thyroid supplement subgroup versus its corresponding hypothyroidism subgroup, ***/ significant difference at P<0.001.

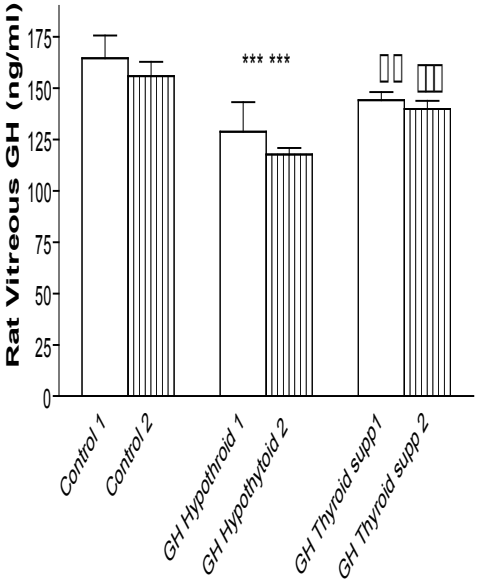
fluid

Estimation of vitreous levels of both GH and IGF-1 in both euthyroid subgroups revealed a two-fold increase in levels in vitreous fluid in comparison to levels in serum. Also, sta-

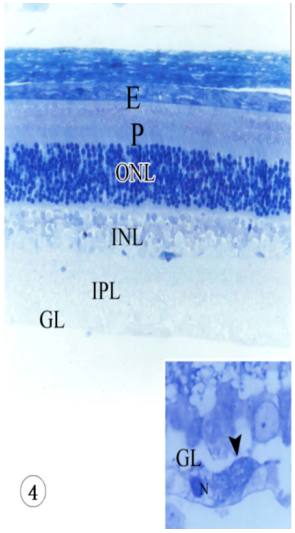
while a negative relation with LP products was noted (Histograms 3-6).

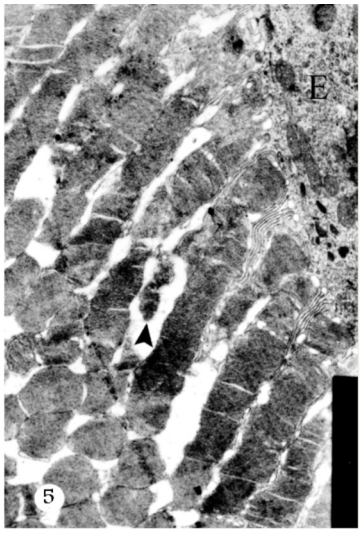
Histological results





Histogram 2: Serum and vitreous GH (mean ± SD from individual rats were analyzed by a Student–3Newman–Keuls t-test. Levels of GH were decreased in all (Carbimazole) treated rats in a dose of 0.05 mg /day/pregnant female during gestation and lactation, subgroups I and II, Sacrificed at day 20 (juvinealal subgroup) and 60 (adult subgroup) postnatal respectively. The GH increases more in thyroid supplement subgroup 1. (n=16 in each subgroup), (*) Significance versus control group. (€) Significance of values in thyroid supplement subgroup versus its corresponding hypothyroidism subgroup, ***/ significant difference at P<0.001.





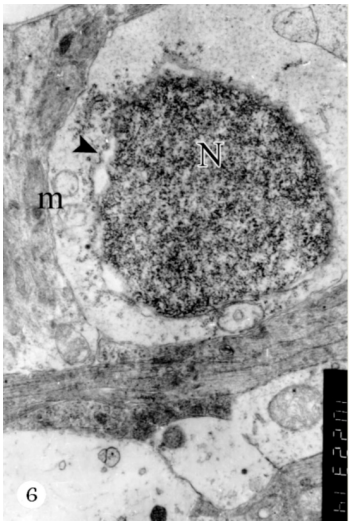


Fig. 4, 5 and 6: A semithin section of the juvenile retina of hypothyroid subgroup: showing reduction of the thickness of most layer, disturbed architecture of GCL with shrunken and degeneration of some ganglion cells were noticed (X 200, X 1000). Electron microscopic examination showing: distortion, degeneration of the outer segment of photoreceptors. Bleb –like protrusion (arrow) of the surrounding plasma membrane is noticed (x 5000) (Fig. 5). Degenerated ganglion cell with dense granular nucleus (N) surrounded by rarefied cytoplasm, dilated nuclear envelope (arrowhead) and mitochondria with destructed cristae can also be noticed (m) in figure 6 (X10000).

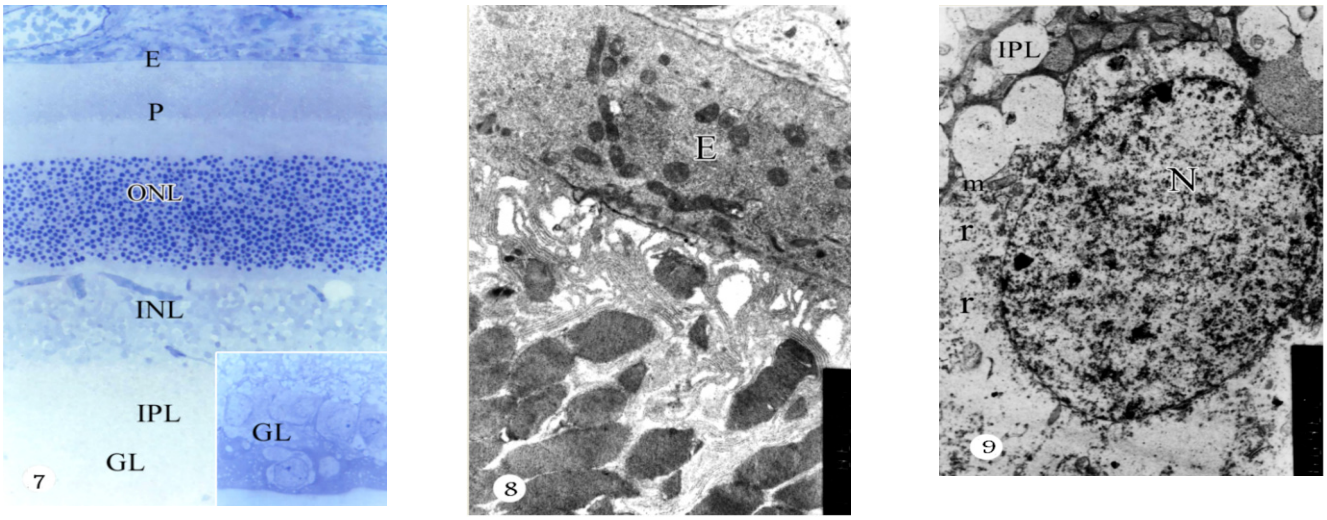


Fig.7, 8 and 9: A semithin section in the juvenile retina of thyroid supplement albino rat showing recovery of the thickness of most layers which appear similar to that of the control subgroup. A magnified part of GCL showing; normal appearance of ganglion cells (Toluidine blue X 200, X1000). Electron microscopic examination showing recovery of the lamellar structures of the outer segment of the photoreceptors (x 5000) (Fig. 8), normal ganglion cell with large rounded nucleus (N) and the cytoplasm contains ribosomes (r) and small sized mitochondria (m) X 000) (Fig. 9).

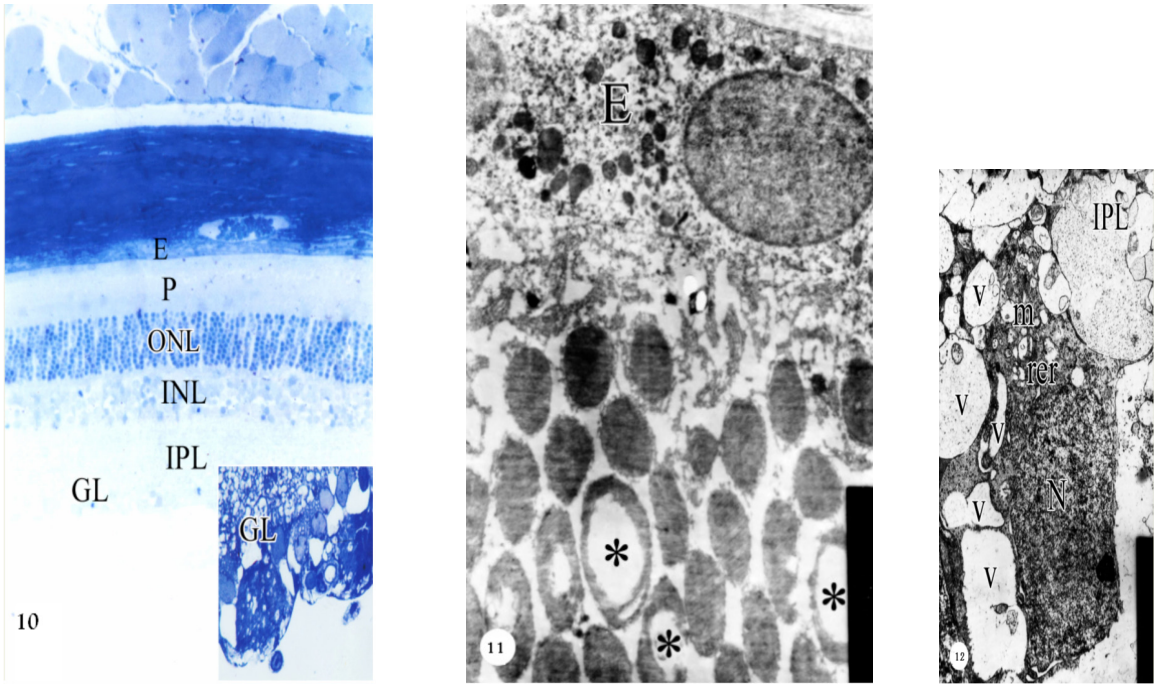
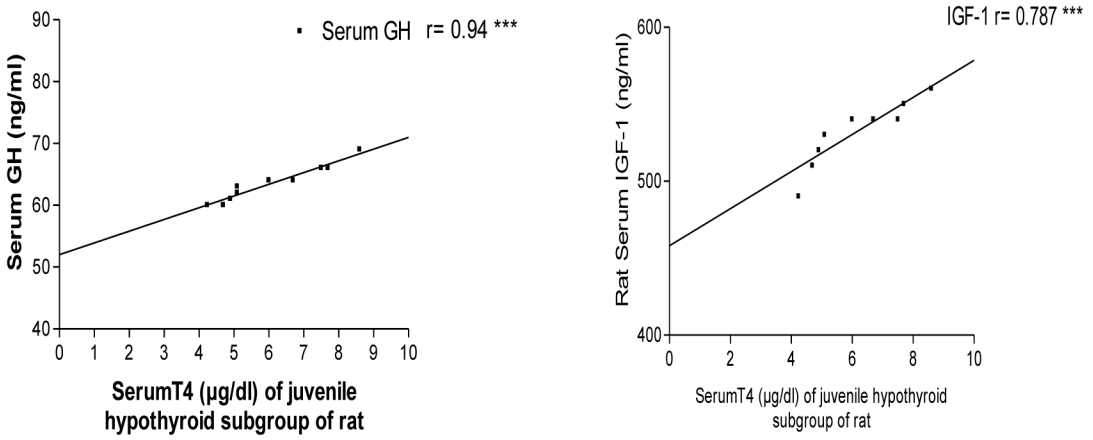
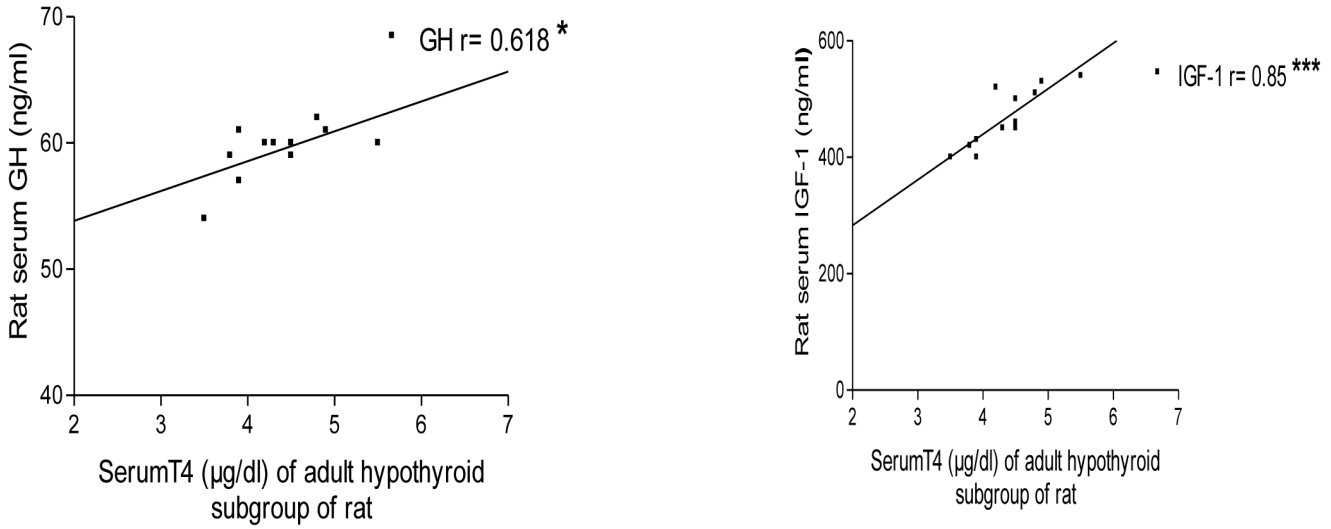


Fig. 10, 11, 12: A semithin section in adult retina of hypothyroid subgroup showing; reduction of the thickness of most layer (Toluidine blue X 200). Distortion, degeneration and decrease in the density of the outer segment of photoreceptors are observed. Central degeneration in the lamellae is noticed (*) (X000) (Fig. 14). Degenerated amacrine cells (AM) with rarefied cytoplasm is observed. Some nuclei (N) appear dense with dilated nuclear envelope. No cell organelles can be seen (X000) (Fig. 15).



Histogram 3.Correlation and linear regression of serum T4 with GH and IGF-1 in hypothyroid rat model (Juvenile subgroup) sacrificed at day 20 postnatal. A positive, significant relationship was noted between serum T 4 and GH and IGF-1.



Histogram 4: Correlation and linear regression of serum T4 with GH and IGF-1 in hypothyroid rat model (adult subgroup) sacrificed at day 60 postnatal. A positive, significant relationship was noted between serum T 4 and GH and IGF-1.

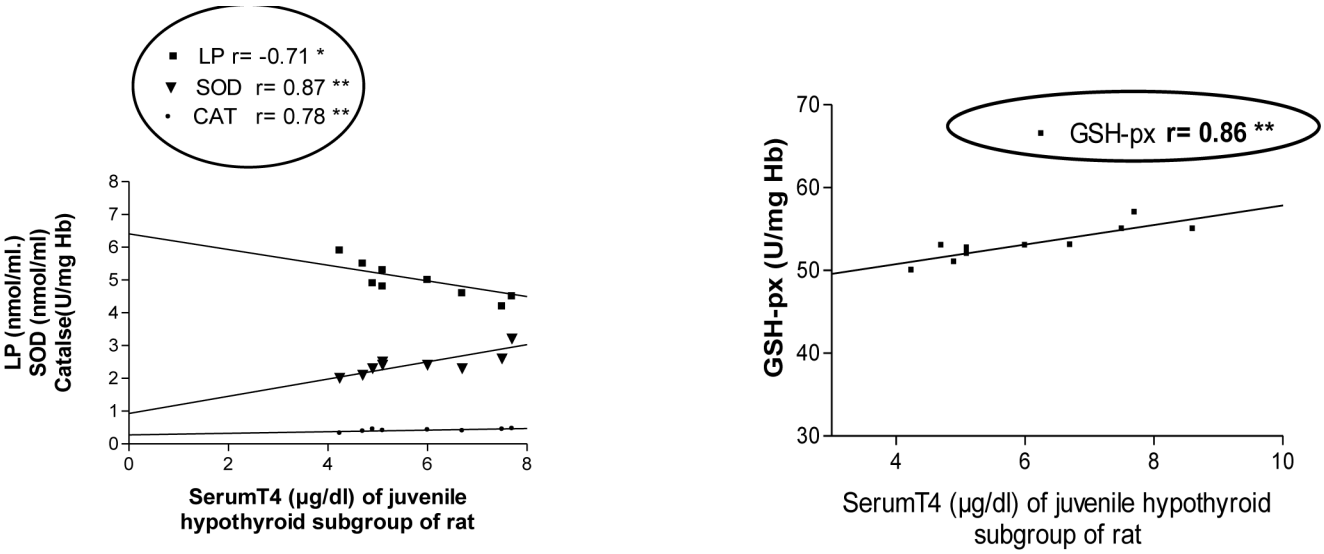
In the juvenile hypothyroid subgroup, the main finding was a reduction in the thickness of different retinal layers (Fig. 4, 5 and 6), which attributed to the reduction in the photoreceptor cell layer, inner nuclear layer and ganglion cell layer in comparison to the corresponding control subgroup (Fig. 1, 2 and 3). Electron microscopic examination showed degenerative changes with distortion and vacuolation of most layers. Distortions of the mitochondria were also observed.

Juvenile thyroid supplement subgroup (Fig. 7, 8, 9), light microscopic examination revealed a significant increase in the thickness of all cell layers in comparison to the hypo-

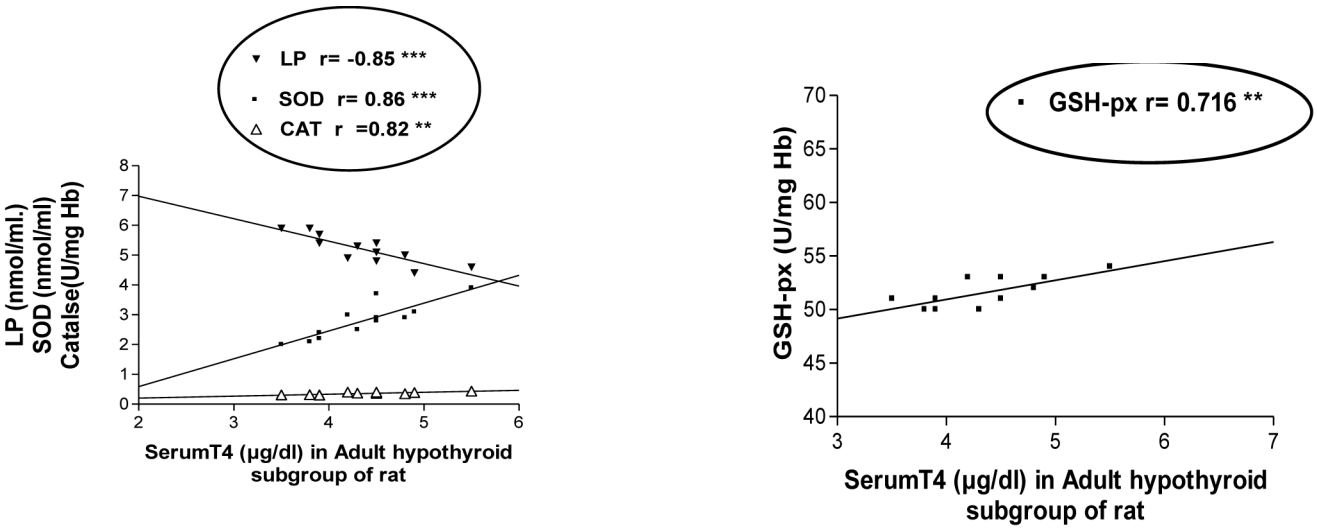
Table 3: Alteration of thickness of the retina and its layers (mm) in hypothyroid model and thyroid supplement groups. The data was represented as mean ± SD.

Values represent mean ± SD and were analyzed by a Student–3Newman–Keuls t-test. Subgroup I and II, Sacrificed at day 20 (juvenile subgroup) and day 60 (adult subgroup) postnatal. (*) Significance versus control group. (€) Significance of values in thyroid supplement subgroup versus its corresponding hypothyroidism subgroup, ***/ (€)(€)(€) significant difference at P<0.001, */ € significant difference at P<0.05, **/ €€ significant difference at P<0.01.

	Group A Control (Euthyroid)		Group B (Hypothyroid model)		Group C Thyroid H supplement	
	Subgroup I (n= 14)	Subgroup II (n=15)	Subgroup I (n= 10)	Subgroup II (n= 12)	Subgroup I (n=13)	Subgroup II (n=13)
Total retinal thickness (RT)	0.3 0± 0.01	0.36 ± 0.04	0.18 ± 0.06 ***	0.15 ± 0.06 ***	0.31 ± 0.003 €€€	0.19 ± 0.1
Photoreceptor layer (P)	0.06 ± 0.009	0.15 ± 0.02	0.04 ± 0.005 ***	0.034 ± 0.008 ***	0.12 ± 0.007 €€€	0.040 ± 0.005 €
Outer nuclear layer (ONL)	0.07 ± 0.004	0.076 ± 0.005	0.05 ± 0.005 ***	0.043 ± 0.008 ***	0.071 ± 0.005 €€€	0.054 ± 0.011 €€
Inner nuclear layer (INL)	0.05 ± 0.006	0.058 ± 0.003	0.03 ± 0.006 ***	0.023 ± 0.01 ***	0.056 ± 0.007 €€€	0.031 ± 0.004 €
Ganglionic cell layer (GL)	0.03 ± 0.004	0.04 ± 0.011	0.02 ± 0.005 ***	0.020 ± 0.001 ***	0.038 ± 0.008 €€€	0.024 ± 0.003 €€€



Histogram 5: Correlation and linear regression of serum T4 with LP and antioxidants (SOD, CAT and GSH-px) of juvenile hypothyroid subgroup of rat. A positive, significant relationship was observed between serum T 4 with SOD, CAT, GSH-px and negative with LP.



Histogram 6: Correlation and linear regression of serum T4 with LP and Antioxidants (SOD, CAT and GSH-px) of adult hypothyroid subgroup of rat. A positive, significant relationship was noted between serum T 4 with SOD, CAT, GSH-px and negative with LP.

thyroid model. Normal ganglion cells with marked recov-

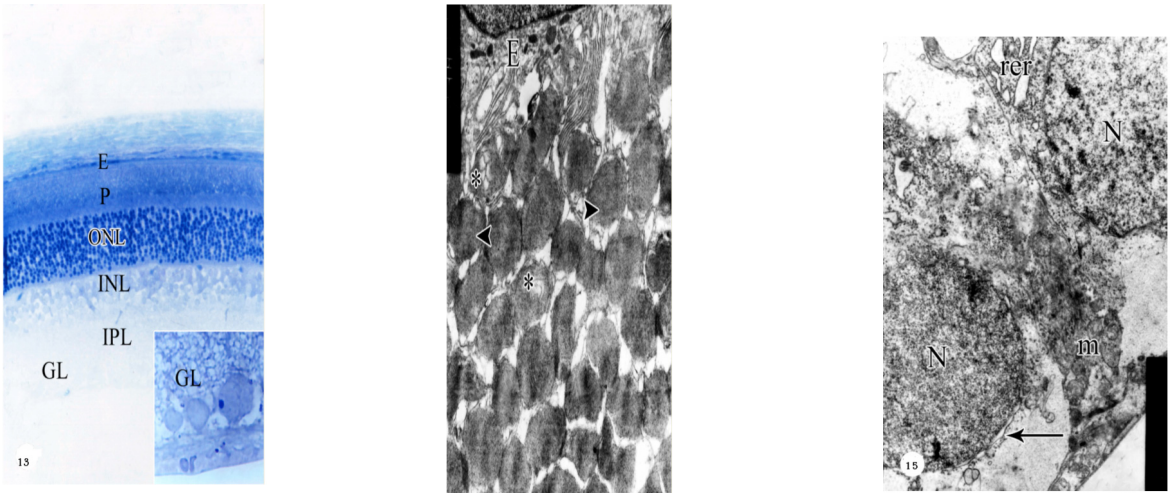


Fig.13, 14 and 15: A semithin section of the retina of adult thyroid supplement subgroup of rat showing: reduction of the thickness of most layers is still present. Inset: A magnified part of GL showing; ganglion cells with minimal recovery. (Toluidine blue X200, X 1000). Electron microscopic examination showing: partial recovery of the epithelial and the outer segment of photoreceptor layers. Central degeneration of disc membrane is observed (*). Some of them are converted into an amorphous electron-dense mass (arrow-head) (X000) (Fig. 17). Two ganglion cells with rarefied cytoplasm and small degenerated mitochondria (m) were still notice (X000) (Fig. 18).

ery in most layers were noticed and they became similar to that of the control.

Adult hypothyroid subgroup (Fig. 13, 14 and 15) showed marked reductions in the mean thickness of different retinal layers, with a noticeable degeneration when compared to the corresponding control subgroup that had the same characters as the adult juvenile subgroup (Fig.1, 2 and 3). Adult thyroid supplement subgroup (Fig. 16, 17, 18), showed a minimal improvement in the retinal layers in comparison to the adult hypothyroid subgroup .3-5-Mor-

phometric results (Table 3). The morphometric results revealed a significant improvement in all retinal layers after supplementation with l-thyroxine when compared with the hypothyroid subgroups (Table 3)

Discussion

Thyroid hormone is a critical regulator for normal growth and physiological function of retinal receptors, especially cones (17). However, the pathophysiological mechanisms of hypothyroid retinal degeneration and the involvement of GH, IGF-1 and oxidative stress in this case is still being debated. In this investigation, we hypothesized that thyroid supplement can ameliorate the experimental model of hypothyroid retinal degeneration. To accomplish our goals, an animal study was established that consisted of a control group, a hypothyroid model, and a Levothyroxine-treated group.

Suppression effects of hypothyroidism on the number of living pups and body weight in the current study concur with previous reports (18,19). This growth retardation can be explained by increasing competition for food or the declining of serum GH and IGF-1 (20). Unfortunately, it is impossible to ascertain the cause of death of pups as often the mothers eat their dead pups. Thyroid supplementation restores the body weight in the juvenile subgroup (21).

The reduction of body temperature and heart rate, the increase of systolic blood pressure in hypothyroid rat model subgroups, and the amelioration that happened after TH supplementation are in line with the previously published work (22-24). These effects are supported by many observations as thyroid hormones regulate metabolism through hypothalamo-pituitary neuroendocrine feedback loops. In turn, decreased T_3 output reduces metabolism and energy usage (25) and T4 administration rapidly increases the resting metabolic rate of hypothyroid rats and improves their survival (26). It has been thoroughly established that neonates with congenital hypothyroidism had a significant reduction of fetal heart rate, and this finding might be an early sign of fetal hypothyroidism (27). Levothyroxine treatment of maternal hypothyroid patients improves heart rate reduction (28).

Deprivation of thyroid hormone in the juvenile hypothyroid subgroup seemed to interfere with the normal course of development of the retina, decrease in the total thickness of all retinal layers, reduction of free ribosomes, distortion of mitochondria and induction of ultrastructural degeneration. The previous findings are in accord with results of others (29) who found that congenital hypothyroid rats had significantly smaller and thinner retinas, with fewer dividing progenitor cells. In addition, morphometric results of hypothyroid subgroups revealed a significant reduction of total retinal thickness and all retinal layers. These findings can be clarified by the following: thyroid hormone acts directly at the transcription level by binding to nuclear receptors to control a number of physiological and developmental processes (17,30). Also, hypothyroidism may depress ventilation leading to hypoxemia. It may also decrease blood vessel density, which in turn decreases delivery of oxygen and nutrients to tissues (31).

Supplementation with L-thyroxine showed amelioration of most degenerative and morphometric changes. Additionally, photoreceptors and ganglion cells restored their normal organization in agreement with the findings of Pinazo-Durán, et al (2). This improvement was obvious in the juvenile subgroup that received the L-thyroxine early, but there was marked resistance to cell recovery in the adult subgroup, which received the L-thyroxine after achieving adolescence.

In the present study, the two-fold increase in the vitreal levels of GH and IGF-1 than that found in the serum levels was evident in both juvenile and adult control subgroups, and this reveals their potential roles in the normal visual growth as proposed previously (32). Moreover, Arnold, et al. (33), elucidated that the aqueous and vitreous humor fluids are potential sites of synthesis of IGF-binding proteins, which can explain their high concentration of GH and IGF-1. Therefore, the vitreous fluid acts as a reservoir for stabilizing visual physiology. Low GH concentrations in the vitreous of diabetic patients may correlate with retinal neuro-degeneration, and may provide a marker to follow its progression (34).

Retinal degeneration noted after thyroid deprivation may be mediated by the recorded reduction in the serum and vitreous GH and IGF-1 as well as increased serum lipid peroxidation products and decreased antioxidants (SOD, CAT and GSH-px) in both hypothyroid subgroups. In addition, the deviation in the levels of GH, IGF-1, LP and antioxidants has been normalized partially after L- thyroxine supplementation, especially in the juvenile subgroup.

The decline in the serum and vitreous GH after medical thyroidectomy by carbimazole is at variance with the previously published results (6, 9, 34), as vitreal growth factors regulate retinal functions, act as neuro-protective factors

for retinal ganglion cells, and provide markers of ocular degeneration as in the case of hypothyroidism (35,20). Also, GH may act by induction of the IGF-I system or directly through its receptor (36).
It has been suggested that hypothyroid retinal degeneration may also be mediated by lower-than-normal IGF-1 in serum and vitreous fluid that is in agreement with previous work (18,37), as IGF-1 is a potent growth promoter of retinal endothelial cells and retinal pericytein. Also it is critical for normal retinal vascular growth and function (3).

In addition, a significant positive relationship between serum T4 and serum GH and IGF-1 was evident in both hypothyroid subgroups of rats, which proves the association between hypothyroid retinal degeneration and suppression of GH and IGF-1, as they constitute the vital modulators of thyroid activity (3). Furthermore, premature infants are more likely to have low T_4 levels and GH, indicating an abnormal hypothalamus-pituitary-thyroid axis function (38). Moreover, hypothyroidism is associated with significant reductions of IGF-1 and IGF-1 binding protein -3 (39). The most frequently quoted mechanism supports the relation of TH with GH and IGF-1, that GH either directly or through IGF-1 increases peripheral T4 to T3 deiodination (40). It is also noted that Akin, et al. [41], reported that GH-IGF axis was affected in patients with subclinical hypothyroidism, and that T4 replacement therapy could prevent abnormalities related to GH-IGF axis. Moreover, Joanna, et al. [42], proved that in children with neglected congenital hypothyroidism, even after long period of hypothyroidism, T4 replacement improved the growth rate, leading to a partial recovery of GH-IGF-I axis as GH and IGF-1 receptors are downstream of thyroid receptor alpha.

Alteration of plasma levels of LP and antioxidants (increase of LP products and decrease of SOD, CAT and GSH-px) in both hypothyroid model subgroups that was evident in this study harmonizes with other investigations. Cano-Europa, et al (43), found that hypothyroidism was associated with oxidative stress (increased levels of malondialdehyd, and decreased levels of CAT) that contributed to retinal degeneration (7,44).

The present study showed that thyroid supplementation for one month can re-establish the oxidant changes induced after in taking of carbimazole, as it significantly decreased the levels of LP products and increased the antioxidants (SOD, CAT and GSH-px), especially in the juvenile subgroup of rats. However, the pathophysiological consequences of the decelerated antioxidant levels in case of hypothyroidism are not yet elucidated. This biochemical change in the level of free radical and antioxidants is thought to be a physiological adaptation and response to hypothyroidism, as a chronic state of hypothyroidism is characterized by degeneration in the redox potential. This leads to free radical chain reactions and metabolic suppression of antioxidant capacity (44). Thus, it is likely that retinal cells may be damaged by prolonged oxidative stress that far exceeds the capacity of the tissue to synthesize antioxidants.

The previous results are supported by a significantly positive relationship between serum T4 and antioxidants (SOD, CAT and GSH-Px) while having a negative relation with LP products in both hypothyroid subgroups. This finding proves the potential role of oxidative stress as a mediator for retinal degeneration in the hypothyroid rat.

In conclusion, this work indicates that Carbimazole was successful in inducing a hypothyroid retinal degeneration model in rats. This was associated with: (i) reducing the number of living pups, body weight, body temperature, heart rate and increase in systolic blood pressure, (ii) dramatic decrease in the blood levels of T and T4 with decreased thickness and cell numbers of all retinal layers. The pathophysiology of retinal degeneration is mediated by suppression of T4, GH, IGF-1 and increased oxidative stress. Also, L-thyroxine administration can blunt the metabolic pathologies and morphological changes induced by anti-thyroid agents. Obligatory L thyroxine supplementation therapy in the juvenile stage has been recommended due to its dramatic response, and a trial for clinical application is mandatory.
This study recommends blood testing for hypothyroid patients' T4, GH, IGF-1 and antioxidant systems in order to monitor the progression of pathology and to prompt the consideration of medical care.

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