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Comparison of 1, 25-Dihydroxyvitamin D3 and Glutathione Reductase Levels in Thyroid Gland Patients

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ABSTRACT

Background: Thyroid gland stands for a part of endocrine system in a human body, and it works to manufacture, store, and secrete hormones in the blood in order to reach the cells of the body, and has many important functions in the human bodyheart and blood vessels, metabolism, pregnancy, skeletal muscles and thyroid disorders. Thyroid gland diseases represent a global health issue. The imbalance in a regulation of thyroid hormones can instigate as well numerous disorders. Aim:This study aimed to find a direct relationship between DHVD3 and GR levels with thyroid hormones, which can lead to thyroid disorders that are directly affected by the levels of these hormones. Methods:The study was conducted between September 2018 to July 2019 in the Specialized Center for Endocrinology and Diabetes in Baghdad province / Iraq. : Serum triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH) and glutathione reductase (GR) and 1, 25 -Dihydroxyvitamin D3 (DHVD3) activities also, BMI, HbA1C were determined for 100 subjects, including 80 patients divided into two groups. Group I (20 hypothyroidism, 20 subclinical hypothyroidism), Group II (20 hyperthyroidism, 20 subclinical hyperthyroidism) and 20 healthy subjects as control groups.Results: showed a significant increase compared with control group in HbA1c in hypothyroidism and subclinical hypothyroidism but other student groups unchangedIt could also be observed a significant decrease in vitamin D and GR in hypothyroidism and subclinical hyperthyroidism but with a significant increase in hyperthyroidism and subclinical hypothyroidism.Conclusion:in correlation between vit D3 deficiency and thyroid dysfunction was also found with Glutathione Reductase (GR) as a result of oxidative stress which indicated these parameters effect on thyroid dysfunction.
Keywords: Hyperthyroidism, hypothyroidism, 1, 25 -Dihydroxyvitamin D3 (DHVD3) and Glutathione Reductase

INTRODUCTION

Thyroid gland illnesses stand for a global health problem. Where imbalance in a regulation of thyroid hormones can result in countless syndromes that differ from a minor goiter to life-threatening diseases, like a thyroid dysfunction, hyperthyroidism and hypothyroidism may influence the circulatory system through influencing the outputs of the heart and contraction heart, blood pressure (Walter F. Boron, Emile L. Boulpaep, 2012; Hall, 2011; Sho, 2011).

The hyperthyroidism, when the thyroid produces too much thyroid hormone. The creation of thyroid hormones can be controlled by thyroid-stimulating hormone (TSH) that is prepared by a pituitary gland (Cury et al., 2013; Devereaux & Tewelde, 2014; Nicki, Brian, & Stuart, 2010). Subclinical hyperthyroidism can be defined based on a low or undetectable serum thyroid-stimulating hormone level, with normal free thyroxine (FT4) in addition to free triiodothyronine (FT3) levels (Rieben et al., 2016). Hypothyroidism occurs as a thyroid gland doesn't supply sufficient thyroid hormones to agree with the requirements of human body(Ke, Hu, Yang, & Tong, 2015; McAninch & Bianco, 2016). Subclinical hypothyroidism stands for an initial, minor variety of hypothyroidism, where a body does not make sufficient thyroid hormones. It is known as subclinical for the reason that just serum level of thyroid stimulating hormone based on a front of pituitary gland is somewhat higher than normal. The thyroid hormones produced by the thyroid gland are still within the normal laboratory range (Bekkering et al., 2019).

1,25-dihydroxyvitamin D3 (Calcitriol) stands for powerful variety of vitamin D, and it is necessary for calcium absorbing and mineralization of bone. It stands for a steroid which is ingested in a diet or formed in a skin in an existence of ultraviolet light. It can be transformed in a liver into 25-hydroxyvitamin D3. After that, it is hydroxylated once more in a proximal tubular epithelial cells of a kidney through 1 α hydroxylase enzyme into an active metabolite DHVD3. Parathyroid hormone motivates calcitriol creation in a kidney through raising 1-α hydroxylase synthesis. It has numerous significant functions in a body. It upholds serum calcium levels through raising calcium absorbing in a gastrointestinal tract. It stimulates healthful bone creation based on osteoid tissue calcification. It as well straightforwardly constrains parathyroid gland action through lessening synthesis and release of parathyroid hormone (Goeman et al., 2014; Moorthy, Becker, Boehm, & Djamali, 2017; Pike & Meyer, 2012).

Glutathione reductase (GR) is one of a chain of enzymes which serves to maintain glutathione in the reduced form. It reinstates intracellular GSH through decreasing GSSG in an existence of FAD and NADPH, this stands for water-soluble vitamin riboflavin derivative. Riboflavin stands for the cofactor of GR(Deponte, 2013). It characterizes the one gene product, but has detected in connection with mitochondria, nuclei, cytoplasm and with sub-nuclear structures. Glutathione has important role in upholding suitable function and averting oxidative stress in human cells (Couto, Wood, & Barber, 2016).

It can be the scavenger for hydroxyl radicals, singlet oxygen, and numerous electrophiles. Lowered glutathione decreases an oxidized arrangement of an enzyme

glutathione peroxidase. So, this, consecutively, decreases hydrogen peroxide, the hazardously reactive species contained by a cell. Furthermore, it has important role a metabolic rate and xenobiotics clearance. It stands for a cofactor in definite detoxifying enzymes, contributes in transporting, and regenerating antioxidants like E and C Vitamins to their reactive arrangements. A ratio GSSG /GSH in a cell stands for the significant feature in appropriately upholding the oxidative balance of a cell, namely, it is important that a cell upholds great levels of a lowered glutathione and minor oxidized glutathione disulfide. This contracted balance has upheld through glutathione reductase, that catalyzes the GSSG decrease to GSH (Couto, Wood, & Barber, 2016; Deponte, 2013; Fleisher, Shearer, Frew, Schroeder Jr, & Weyand, 2013; Gill et al., 2013). Therefore,this study aimed to find a direct relationship between DHVD3 and GR levels with thyroid hormones, which can lead to thyroid disorders that are directly affected by the levels of these hormones

MATERIALS AND METHOD

This study conducted on 100 subjects, including 80 patients divided into two groups. Group I (20 hypothyroidism, 20 subclinical hypothyroidism), Group II (20 hyperthyroidism, 20 subclinical hyperthyroidism) and 20 healthy subjects as control groups. In the control group, a complete medical history was taken into consideration to any associated medical problems, and subjected to full clinical investigation. None of the subjects showed any clinical symptoms or signs of thyroid dysfunction. table 1 summarize the study design of the study.

Table 1: Patient groups

Group I		Group II	
Hypothyroidism	Subclinical Hypothyroidism	Hyperthyroidism patients	Subclinical Hyperthyroidism
20	20	20	20

All subjects are under a range of (30-55) years with body mass index ranged from 25 to 29 kg/m2.

Clinical samples and Consent to participate: Ten milliliters of venous blood was drawn from All patients of all groups and were placed in athat placed in a plane tube, left for (15 min) at room temperature, then centrifuged (at 3500 rpm from 10 min). Serum that obtained stored at (-20 ˚C) unless used immediately. Whole blood was used for HbA1c determination. Blood samples were collected from healthy controls and patients after 12-14 hours of fasting. The study was conducted between September 2018 to July 2019 in the Specialized Center for Endocrinology and Diabetes in Baghdad province / Iraq.All participants agreed to participate in this study and all samples were collected with the consent of patients or their relatives.

Biochemical assay: Determination of BMI

Body mass index was a value resulting from a mass (weight) in kilograms and height in meter of a person. The body mass is divided by a square of a body height. Based on the following equation.

$$BMI = \frac{Weight\ in\ Kilograms}{(Height\ in\ Meters)^2}$$

Determining Glycated Hemoglobin (HbA1c): A hemolyzed whole blood was associated with a feebly binding cation-exchange resin. The non-glycosylated hemoglobin (HbA0) was associated with resin, leaving (HbA1) free to be removed by a resin separator. The HbA1 percentage has measured as a result of evaluating absorbance magnitudes under 415nm by UV-Visible spectrophotometer (Cecil, England)for HbA1 fraction with total Hb fraction, to compute the ratio of absorbance (R) This ratio was compared with glycohemoglobin standard obtained from the same procedure. The kit was gotten from Stanbio (USA), to determine the glyco-hemoglobin that generated gradually and irreversibly in the erythrocyte during its 120-day life cycle.

Determination of DHVD3 Levels in Blood Serum: This ELISA kit is based on Sandwich-ELISA principle. A micro ELISA plate supplied in this kit was pre-coated with specific antibody to DHVD3. Criteria or tasters have been inserted to a micro ELISA plate wells and joined with a specific antibody. At that point, a biotinylated detecting specific antibody for DHVD3 and Avidin-Horseradish Peroxidase (HRP) conjugate have added sequentially to every micro plate well and incubated. Free components have been washed away. A substrate solution has inserted to every well. Only those wells that contain DHVD3, biotinylated detection antibody and AvidinHRP conjugate will be in blue color. An enzyme-substrate reacting has ended by adding stop solution and the color turns yellow. The optical density (OD) has determined spectrophotometrically under wavelength of 450nm ± 2nm (by using Shimadzu UV-Vis Spectrophotometer (UV-1650Pc). The OD magnitude has been proportional to the concentration of DHVD3. A concentration of DHVD3 in the samples can be computed by comparing OD for samples with standard curve. The enzyme-linked immunosorbent assay (ELISA) kit was bought from (Elabscience, China) and used to define the levels of DHVD3. ELISA sandwich format is used and executed according to the manufacturer directions.

Determination of GR Levels in Blood Serum: This ELISA kit has been based on Sandwich-ELISA standard. A micro-ELISA plate supplied in this kit was pre-coated with specific antibody to Human GR. Testers are inserted to a micro-ELISA plate wells and joined with a specific antibody. At that point, a biotinylated detecting specific antibody for Human GR and Avidin-Horseradish Peroxidase (HRP) conjugate have been inserted sequentially to every micro plate well and incubated. Free components have been washed away. A substrate solution has inserted to every well. Just those wells that have biotinylated detection antibody, Human GR as well as Avidin-HRP conjugate can look like blue in color. The enzyme-substrate reacting has terminated through inserting stop solution and the color becomes yellow. The optical density (OD) has measured spectrophotometrically under wavelength of 450nm ± 2nm(by using Shimadzu UV-Vis Spectrophotometer (UV-1650Pc). The OD magnitude has been proportional to the Human GR concentration. A concentration of Human GR in the samples can be calculated by comparing the OD of the samples with standard curve. The enzyme-linked immunosorbent assay (ELISA) kit was bought from (Elabscience, China) and used to define the levels of GR. ELISA sandwich format is used and executed according to themanufacturerdirections

Determination of TSH, FT4 and FT3 Levels in Blood Serum: The enzyme-linked fluorescent immuneassay (ELFA) kit was bought from (bioMerieux SA-France) and used to define the levels of TSH, FT4 and FT3 ELFAmethodology is that it can easily be automated. ELFA principal format is used and executed according to the manufacturer directions

Statistical Analysis: Data has translated into electronic database structure. Statistical analysis has done by means of Student's F-test from one-way ANOVA in Excel 2010 to compare between the measured parameters in patients and control groups. Also, the measurement includes the means and standard error mean. A level of significant of less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Table 2 shows the general characteristics of Group I and control group such as BMI, HbA1c, TSH, FT3, FT4, DHVD3 and GR, this study showed significant increase in HbA1c levels in hypothyroid and subclinical hyperthyroid patients when compared to controls. Thyroid hormones use antagonistic actions and insulin agonistic in dissimilar organs. Nevertheless, this happens in a well balance required for typical glucose metabolic rate. Shortfall or extra of thyroid hormones can stop this balance leading to changes in carbohydrate metabolism (Brenta, 2011). Elevation of HbA1c has been as well demonstrated in a study of 45 hypothyroid patients in which HbA1c has been bigger in comparison to control subjects (5.54± 0.43% versus 5.34±0.31% in hypothyroid patients and controls correspondingly; p<0.001) (Glenda Courtney-Martin , Ronald O. Ball , Paul B. Pencharz & Rajavel Elango, 2016).Permissive influence of T3 is seen in gluconeogenic and glycogenolytic consequences of epinephrine and glucagon.

Table 2. BMI, HbA1c, TSH, FT3, FT4, DHVD3 and GR level in the Group Iand control groups

Parameters	Control group (Mean ± SEM)	Group I	
		Hypothyroidism Patients (Mean ± SEM)	Subclinical Hyporthyroidism Patients (Mean ± SEM)
BMI (Kg/m2)	29.03±0.34	29.48±0.84	28.98±0.24
HbA1C (%)	4.81±0.13	10.13±0.64	9.17±0.51
TSH (mU/L)	2.150±0.26	26.650±2.62	8.921±0.90
FT3 (ng/dL)	3.346±0.15	1.088±0.06	2.360±0.18
FT4 (pg/dL)	1.203±0.09	0.482±0.05	1. 24±0.10
DHVD3(ng/mL)	0.334± 0.005	0.222±0.007	0.334±0.006
GR (ng/mL)	4.573±0.17	2.783±0.16	4.479±0.21

Table 3: BMI, HbA1c, TSH, FT3, FT4, DHVD3 and GR in Group IIand control groups

Parameters	Control group (Mean ± SEM)	Group II	
		Hyperthyroidism Patients (Mean ± SEM)	Subclinical Hyperthyroidism Patients (Mean ± SEM)
BMI (Kg/m2)	29.03±0.34	26.36±0.53	27.27±0.39
HbA1C (%)	4.81±0.13	9.17±0.51	4.97±016
TSH (mU/L)	2.150±0.26	0.139±0.02	0.252±0.02
FT3 (ng/dL)	3.346±0.15	6.613±0.47	1.929±0.19
FT4 (pg/dL)	1.203±0.09	5.114±0.66	1.192±0.04
DHVD3(ng/mL)	0.334± 0.005	0.358±0.015	0.253±0.007
GR (ng/mL)	4.573±0.17	5.309± 0.16	2.619±0.23

Other hepatic gluconeogenic enzymes that absolutely controlled through thyroid hormones contain phosphoenolpyruvate carboxykinase (PEPCK), the enzyme that catalyzes a rate-controlling step (Park, Jerden, & Bahouth, 1995). Despite thyroid hormones promoting metabolism of carbohydrates, there is false high or low levels of HbA1c in patients with altered thyroid status. Several factors other than glycemic status can influence HbA1c levels, including life span of red blood cells (RBC) and conditions affecting RBC turnover. Erythrocytes turnover is increased in thyrotoxic states whereas hypothyroidism has the opposite effect (Organization, 2001). The observed correlation in this paper has as well reported by other paper that significantly correlated TSH in addition to HbA1c (r =0.46, p <0.05) (Billic-Komarica, Beciragic, & Junuzovic, 2012). We observed vit-D deficiency in this study in patients with hypothyroidism, these results agree with study included vit-D and vit-B12. Also, there has been negative correlation among anti-TPO levels and vit-D deficiency. We conclude that vit-D deficiency has contribution with pathogenesis of hypothyroidism. There has been a negative correlation among vitamin D levels, vitamin B12 and anti-thyroid peroxidase antibodies for those who suffer from autoimmune hypothyroidism (Mahaboob Fayaz S , Ravikumar Y. S , Manthappa M , Mahesh MG , Aditya Nadella & Sai Vivek V. 2015). The association between thyroid and vit-D deficiency as well as autoimmunity remains unclear. Nevertheless, we think that vit-D is in association with anti- inflammatory in addition to immunomodulatory influences. Vit-D has a noteworthy role in modulating immune system, improving the innate immune response and employing the inhibitory action on an adaptive immune system (Azrielant & Shoenfeld, 2017). There has been no significant change in vitamin D level between females with hypothyroidism and healthful controls (Musa et al., 2017). The paper establishes the fact that 1,25DHCC may stand for a significant study about

altering thyroid hormones and TSH levels in subclinical hypothyroidism. Vitamin D insufficiency is associated with subclinical hypothyroidism (Sudha, Hegde, Manjrekar, & Kumarchandra, 2013).

A study has detected a positive relationship among thyroid hormones unnecessary and lipid peroxides are correlated through linear regression that evidently recommend inducing oxidative stress. Like this effect is feasibly in relation with an enhanced metabolic rate produced by thyroid hormone administration, causing accelerated produced ROS (Petrulea, Duncea, & Muresa, 2009). In contrast, hyperthyroid patients had similar median HbA1c value in comparison to controls at the baseline. We propose that this could possibly be due to promotion of glaciation by malondialdehyde, which is produced by lipid peroxidation induced by excessive thyroid hormones. This phenomenon might have counterbalanced an increased RBC turnover seen in hyperthyroid patients. Median HbA1c value did not change significantly after treatment of hyperthyroidism despite significant change in reticulocyte count (Bhattacharjee, et al., 2017). Significant baseline or post treatment change was not observed in hyperthyroid patients. Our study suggests that HbA1c data should be interpreted with caution in patients with hypothyroidism (Angeline Jeyakumar & Vidhya Shinde,2019). The level of FBS and HbAlc is greater in hyperthyroid group than typical controls acted as variations of carbohydrate metabolism (H. B. Kim et al., 2001). In another study significant (p = 0.002) increase in the mean value for hemoglobin A1C were found in the hyperthyroid group which is similar to the finding in this study (Ford, Lim, & Crooke, 1987). Similar findings found HbA1c levels significantly greater in hyperthyroid group than hypothyroid and euthyroid groups (p<0.001)(Tam et al., 2015). Experimentally investigations and epidemiological data have revealed that hyperthyroidism has in association with the increased production of free radicals and lipid peroxidation levels (fernandez, barrientos, kipreos,valenzuela & videla, 1985). A cell has various substances able to scavenge the free radicals, keeping them from risky effects. Based on the enzymatic antioxidants, there are groups of glutathione reeducates (GR) (Messarah, Boulakoud, Boumendjel, Abdennour, & El Feki, 2007). Based on the highly recognized enzymatic antioxidants, we observe superoxide dismutase (SOD), glutathione peroxydase (GPx), glutathione reductase (GR) and catalase (CAT) GPx in the enzyme having selenium ion as cofactor (Weydert & Cullen, 2010). Thyroid hormones uphold the oxidant/antioxidant equilibrium for protecting a cell. Advanced levels of thyroid hormones have been known for accelerating metabolic reacting, increasing oxygen consuming due to oxidative reactions with increased free radical production. Thyroid hormones cause increased free radicals and stimulate the antioxidant enzymes. As energy requires increasing in hyperthyroidism, it is detected that oxidants are accumulated in a cell. Consequently, thyroid hormones create a hazard of oxidant stress for cells (Dariyerli, Toplan, Akyolcu, Hatemi, & Yigit, 2004). No changes have noted in GR activities and GGT activities among HT patients and healthful matched controls (Rostami, Aghasi, Mohammadi, & Nourooz-Zadeh, 2013). In the liver, hypothyroidism caused by activation of SOD, GPx has reduced activity of GR and reduced ratio GR/GPx. All together, the levels of full, oxidized, and decreased glutathione have increased, but the ratio GSH/GSSG in addition to enzymes activities has included purine nucleotide metabolic ratio (and their ratio 5'N/AD + AMPD) reduced. Based on these data, a functional association of a glutathione redox system can be suggested not just with antioxidant enzymes, but as well with the enzymes activity including immune status and purine nucleotide metabolism (Tapbergenov, Sovetov, Bekbosynova, & Bolysbekova, 2015).

Table 4. Correlation of D3 levels with some studied parameters in all patient and control groups

Group	**Control group**		**Hyperthyroidism group**		**Subclinical Hyperthyroidism group**		**Hypothyroidism group**		**Subclinical Hypothyroidism group**	
Parameters	**r-value**	**p**-value*	**r-value***	**p**-value	**r-value**	**p**-value	**r-value**	**p**-value	**r-value**	**p**-value
D3 VS GR	- 0.094	S	+ 0.518	S	+ 0.651	S	+ 0.044	S	- 0.509	S
D3 VS TSH	- 0.356	S	- 0.111	NS	- 0.029	S	- 0.252	S	+ 0.189	S
D3 VS FT4	+0.304	S	+ 0.154	S	+ 0.507	S	- 0.293	S	- 0.544	S
D3 VS FT3	+0.240	S	+ 0.019	S	- 0.247	S	- 0.039	S	- 0.723	S
D3VSHbA1C	+0.350	S	- 0.233	S	- 0.214	S	+ 0.433	S	- 0.048	S
D3 VS BMI	+0.269	S	- 0.282	S	- 0.334	S	+ 0.368	S	- 0.136	S

*(S) significant ($p \leq 0.05$), (NS) non-significant (p >0.05), * p-value (probability) , r-value (correlation coefficient)

Table 5. Correlation of GR levels with some studied parameters in all patientand control groups

Group	**Control group**		**Hyperthyroidism group**		**Subclinical Hyperthyroidism group**		**Hypothyroidism group**		**Subclinical Hypothyroidism group**	
Parameters	r-value*	p-value*	r-value	p-value (S)	r-value	p-value	r-value	p-value	r-value	p-value
GR VS TSH	+0.147	S*	- 0.443	S*	- 0.102	S*	+0.288	S*	- 0.271	S*
GR VS FT4	- 0.331	S*	+ 0.096	NS*	+ 0.471	S*	+ 0.283	S*	- 0.107	S*
GR VS FT3	+0.023	S*	+ 0.106	S*	+ 0.081	S*	- 0.296	S*	+ 0.350	S*
GR VS HbA1C	+0.404	S*	+ 0.219	NS*	- 0.425	S*	- 0.035	S*	+ 0.321	S*
GR VS BMI	+0.364	S*	- 0.110	S*	+ 0.134	S*	+ 0.178	S*	- 0.452	S*

*(S) significant ($p \leq 0.05$), (NS) non-significant (p >0.05),*p-value (probability), *r-value (correlation coefficient)

Vitamin D has been identified for its principal starring role in bone and mineral homeostasis. It is lately reported that its shortage is related to numerous illnesses like cancer, cardiovascular disease, infection, and adiposity in addition to osteoporosis(Tetsuyuki Yasuda, et al., 2012). Remarkably, vitamin D has potent immunomodulatory sound influences and has significant roles in the autoimmune disease pathogenesis. One of the dual indicators may clarify the small vitamin D levels in patients with hypothyroidism. The 1st one, the small levels of vitamin D can be as a result of low absorption of vitamin D from the intestine. The 2nd one, a body cannot stimulate vitamin D appropriately. Notably, the vitamin D and thyroid hormone bind to comparable receptors known as steroid hormone receptors. Another gene in the Vitamin D receptor has given to predispose people to autoimmune thyroid disease involving Graves' disease and Hashimoto's thyroiditis (Friedman). Serum concentration of 25(OH)D stands for the finest sign of vitamin D level. It exhibits vitamin D that created cutaneous and that gotten from food and complements(Glenda Courtney-Martin , Ronald O. Ball , Paul B. Pencharz & Rajavel Elango, 2016). Earlier studies were detected that serum 25(OH)Dlevels did not vary significantly among females and males (Nizar, Battikhi, 2017). Nevertheless ,a Japanese research involving 200 euthyroid patients with Graves' illness specified that vitamin D lack in 40% for women and about 20% for men ($p < 0.005$)(YAMASHITA et al., 2001). Byron Richards in 2008 has investigated the influence of vitamin D lack on thyroid gland in experimental study, he stated that a lack of vitamin D possibly led to low thyroid hormones (Richards, 2008). Other reported papers have confirmed that patients with Graves's disease as well have minor Vitamin D levels (Yasuda et al., 2012). Vitamin D constrains the creation of Th1 polarizing cytokine (IL-12), thus incidentally moving a polarization of T cells from a Th1 toward a Th2 phenotype. In the CD4+ T cell response, vitamin D straightforwardly constrains producing Th1 cytokines (IL2 and IFN-c), and augments Th2 cytokine (IL-4) creation (Baeke, Takiishi, Korf, Gysemans, & Mathieu, 2010). Furthermore, current reported papers have indicated the association of vitamin D with numerous autoimmune illnesses. Vitamin D receptor (VDR) gene polymorphisms and vitamin D status have related to diverse autoimmune diseases(Naderi et al., 2008; Ponsonby et al., 2008). Conversely, the conducted study in Netherlands had depicted that Vitamin D shortage is not related to early phases of thyroid autoimmunity (Effraimidis, Badenhoop, Tijssen, & Wiersinga, 2012).

CONCLUSIONS

The results suggested correlation between vit D3 deficiency and thyroid dysfunction also with Glutathione Reductase (GR) as a result of oxidative stress. The paper has shown that thyroid patients must have systematic investigation for their glucose levels. Patients must have satisfactory treatment of the thyroid syndromes and hence must avoid themselves from adversative influences of hyperglycemia.

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