Role of Antioxidants on Thyroid Hormones in Wister Rats

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Abstract: ***It is estimated that huge amount of ROS, especially of H_2O_2, are produced in the thyroid under physiological conditions, justifying the statement that the thyroid gland is an organ of "oxidative nature". The present research work aimed to investigate the free radical scavenging activity, lipid peroxidation and antioxidant status in both hyperthyroid and hypothyroid patients.Adult male Wister rats, weighing around 150-200 gms used in this study. Rats were maintained in the animal care facilities. Under veterinary supervision, food and water were supplied ad labium to the animals. All rats were fed with normal diet (20% protein). Animal studies were performed in compliance with generally accepted guidelines governing such work. Rats have been administered with known amount of Vitamin C, Vitamin E and turmeric. Results showed increased levels of thyroxin in rats after 15 days. (Vit C-5.2 ± 1.2 NS,Vit E - 5.3 ± 0.5 NS and Turmeric-5.3 ± 0.87 NS)***

Keywords: Antioxidants, Thyroid hormones, Free radicals, Vit C, Vit E, Turmeric

1. Introduction

The thyroid gland is located in the neck. It is most noted for the two major hormones it produces. These hormones are triiodothyronine or T3 and thyroxine or T4. The thyroid produces these two hormones from iodine, an essential mineral, and tyrosine, an amino acid. The thyroid gland is the body's primary regulator of metabolism. Thyroid hormones are necessary for the normal development of body organs. A high concentration of thyroid hormones may change the metabolism of oxygen in the cells and stimulate the production of free radicals [1].The thyroid gland synthesizes more T4 than T3 (eleven times more). However, outside of the thyroid, T3 is produced from T4. Of these two hormones, T3 is the more bioactive compound. It is chiefly responsible for most of the metabolic effects of the thyroid hormones. Besides, T3 and T4, the thyroid can produce other compounds from iodine and tyrosine. T1 (monoiodothyronine) and T2 (diidothyronine) are examples of other iodinated compounds found in the thyroid. These two iodine complexes do not contribute to thyroid function. While T4 is chiefly converted to T3, it may also be converted to another triiodothyronine compound known as reverse T3.Thyroid disorders are medical conditions caused by wide variations (from the normal range) of thyroid hormone levels.

The free radicals, such as superoxide's, peroxidase and hydroxyl radicals are known to play an important role and have been identified as major contributors to cell and tissue damage in many disease conditions. In the thyroid gland, relatively high levels of H_2O_2 are generated particularly in response to thyrotropin[2].This H_2O_2 serves as substrate for the thyroperoxidase enzymes which catalyzes the synthesis of thyroid hormones, namely thyroxine and triiodothyronine. However, in hypothyroidism, hormone synthesis being low, the H_2O2 may get accumulated. Such H_2O_2 might destroy the thyroid gland cells if protective mechanism are deficient**[**3**]**.Antioxidants are intimately involved in the prevention of cellular damage- the common pathway for cancer, aging, and a variety of diseases. Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defense system of antioxidants. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. The clinical syndrome produced by excess hormone is usually referred to as thyrotoxicosis or as hyperthyroidism. Hyperthyroidism is characterized, by weight loss, weakness, dyspnea, palpitation, increased thrust or appetite, irritability, profuse sweating, sensitivity to heat or increased tolerance to cold, or tremor, occasionally prominence of the eyes and goiter. Hypothyroidism is present when there is insufficient secretion of thyroid hormones to meet the requirements of the body.

Ascorbic acid is involved in biologic oxidations. Apparently its link into the mitochondrial election transport chain is at the stage of cytochrome-C. Thus the oxidation of a metaboilite through one mole of ascorbic acid would yield one mole of ATP via oxidative phosphorylation. An involvement of ascorbic acid in biologic oxidations is a predictable function because of the readily reversible conversion of ascorbic to dehydroascorbic acid. Ascorbic acid may also be involved in the metabolism of tyrosine. This also appears to act as a regulator of cholesterol metabolism.

The primary function of the thyroid is the formation, storage, and secretion of thyroid hormones [4], [5].Thyroid hormone formation involves a coordinated series of steps controlled

by thyrotropin (TSH) but requiring insulin/insulin-like growth factor-1. This includes thyroglobulin (TG) synthesis and vectorial transport to the lumen of the thyroid follicles making up the gland, where TG is stored [4],[5].This involves concentrative iodide uptake by the sodium iodide symporter (NIS), as well as iodination of TG and coupling of TG iodotyrosine residues by the thyroid peroxidase (TPO) [4],[5].

Oxidative reactions occur in all tissues and organs; the thyroid gland constitutes such an organ, in which oxidative processes are indispensable for thyroid hormone synthesis. It is estimated that huge amount of ROS, especially of H_2O_2, are produced in the thyroid under physiological conditions, justifying the statement that the thyroid gland is an organ of "oxidative nature". Hydrogen peroxide is an essential factor for thyroid hormone biosynthesis. It is produced in the thyroid gland [6].Hydrogen peroxide acts as an electron acceptor at each step of thyroid hormone synthesis, namely at iodide oxidation and, next, at its organification, as well as at coupling reaction of iodotyrosines [7].It is essential for activity of thyroperoxidase (TPO) – the key enzyme for thyroid hormone synthesis.

Thyrotropin (TSH), the main secretory and growth stimulatory factor for the thyroid, is obviously involved in the production of H_2O_2 in that gland. The major meaning of that fact is that an increased production of H_2O_2, with subsequently enhanced formation of free radicals (especially $^{\bullet}OH$), takes place in any conditions accompanied by the increased blood TSH concentration. Thus, TSH stimulation results in goitre formation and, under certain conditions, in thyroid cancer initiation via the mechanism of, at least in part, oxidative stress. In agreement with this, oxidative stress creates required conditions for thyroid cell proliferation [8],[9].

The present study has been taken in the vision of role of Vit C, E and Turmeric as an antioxidants being a positive modulators of thyroxine. Vitamin C is one of the antioxidant vitamins. Therefore, it can promote thyroid health by reducing the oxidative stress placed on the gland either by foreign toxins and harmful free radicals or from the reactive oxygen species produced during the syntheses of thyroid hormones.

2. Methods

This research study has been performed at the Radiation Medicine Centre,BARC,Tata Memorial Centre,Parel,Mumbai,India. Adult Male Wister rats, weighing around 150-200 gms. were used in this study. Rats were maintained in the animal care facilities of the Radiation Medicine Centre, Bhabha Atomic Research Centre (B.A.R.C.) Parel, Mumbai. Under veterinary supervision food and water were supplied ad labium to the animals. All rats were fed with normal diet (20% protein). Animal studies were performed in compliance with generally accepted guidelines governing such work. Following chemicals has been used:

1) L. Ascorbic Acid (Vitamin 'C') $C_6H_8O_6$ obtained from the LOBA chemicals private limited Mumbai.
2) D1–α-TOCOPHEROL ACETATE (Vitamin 'E') $C_{31}H_{52}O_3$. Obtained from the LOBA CHEMICALS private limited Mumbai.
3) Turmeric extract: fine powder of Dry Thizomes of turmeric (Curcuma-Longa-L) was obtained from a local market and was subjected to cold ethanolic percolation. (Saiba Industries Pvt. Ltd. Bombay) 1% of this dry powder was mixed in normal diet (20% of proteins).
4) RIA Kits : The thyroid status of the treated animals was confirmed by serum thyroid hormone levels measured by radioimmuno assay (RIA) KITS supplied by the Board of radiation and isotope technology (BRIT) [Vashi], Mumbai, India.
5) Thyroid hormones free serum: Normal rats serum was pooled and thyroid hormones free serum was prepared.

Rats were divided into four different groups

Group I	- labeled as control
Group II	- treated as Vit. 'C' (250 mg Vit. C in diet)
Group III	- treated as Vit. 'E' (250 mg Vit.E in diet)
Group IV	- labeled as turmeric extract (1% turmeric extract in diet)

After 15 days of antioxidants doses, all rats were weighed and found healthy, under general anesthesia with Ether, Blood was taken from the heart in heparinized syringes from all the animals in separate test tubes (According to group). The Blood was centrifuged for 30 minutes at $4^{o}C$. The serum was separated for the Radioimmunoassay's.

3. Statistical Analyses Method

Statistical Analyses were performed by the standard method. All the results were expressed as mean ± standard deviation (S.D.). The mean of the two groups compared using the students't' test. 'P' value of less than 0.05 was considered to represent statistically significant change.

4. Results

Evaluation of Antioxidants effects on thyroid hormones in rats has been done. Fourty Wistar rats were used in this study and fed with antioxidant namely Vit. C, E and turmeric. After 15 days and 30 days of Vit.C feeding blood samples were collected and plasma was used for the radioimmunoassay of trioiodothyronine (T**3**) and thyroxine (T**4**) to evaluate the effect of antioxidants on thyroid hormones.

Table 1: Intraassay variation of T3 & T4 Radioimmunoassay

No. of Observations	Triiodothyronine T3 ng/d1	No. of Observations	Thyroxin T4µgm/d1
1.	114	1.	5.6
2.	114	2.	5.3
3.	117	3.	6.2
4.	129	4.	5.4
5.	115	5.	5.0
6.	107	6.	5.5
7.	116	7.	5.8
8.	113	8.	5.3
		9.	5.2
		10.	5.7
		11.	5.2
		12.	5.8
T3		**T4**	
Mean = 117 S.D. = 6.05 C.V. = 5.21%		Mean = 5.5 S.D. = 0. 3 C.V. = 6.23%	

Table 2: Triiodothyronine & Thyroxin Levels After 15 Days of Treatment with Vitamin C, Vitamin E, & Turmeric In Rats

SN	*Group N=10*	*TRIIODOTHYRONINE-T3 ng/d1*	*THYROXINE- T4 ng/d1*
1.	Control	49.2 ± 10.9	4.6 ± 0.8
2.	Vitamin 'C'	76.6 ± 15*	5.2 ± 1.2 NS
3.	Vitamin 'E'	76.6 ± 15*	5.3 ± 0.5 NS
4.	Turmeric	65.3 ± 17 NS	5.3 ± 0.87 NS

NS = Not significant.
* p < 0.01 vs. control

Table 3: Triiodothyroxine & Thyroxin Levels After 30 Days of Treatment With Vitamin 'C', Vitamin 'E' And Turmeric in Rats

SN	*Group N=10*	*TRIIODOTHYRONINE-T3 ng/d1*	*THYROXINE-T4 µg/d1*
1.	Control	60.4 ± 16.3	3.8 ± 0.54
2.	Vitamin 'C'	89.7 ± 8.0*	4.3 ± 0.84 NS
3.	Vitamin 'E'	93.7 ± 19*	4.7 ± 0.58 NS
4.	Turmeric	84.8 ± 11*	4.8 ± 0.76 **

NS = Not significant.
*p < 0.01 vs. control
**p < 0.02 vs. control

5. Discussions

The thyroid is frequently investigated using a radionuclide of iodine and in some patients with hyperthyroidism or thyroid cancer, thereby involved radioiodine (131-Iodine). In both these situations, the gland is exposed to internal radiation. In the past the thyroid was exposed to external radiation when head, neck and chest lesions were treated (the range of doses was usually 500-1000 rad; 5 to 10 Gy) for reasons which are now not considered legitimate. There is unequivocal evidence that thyroid are responsible for producing an increase in the incidence of both benign thyroid nodules and thyroid cancer. Both internal and external radiation, if the dose is great enough can cause hypothyroidism. Photons produce their effects on tissues indirectly. They interact with molecules most frequently with water. Since that is the main constituent of the body result in the release of e-from an atom. Whether the radiation to the thyroid is internal, as with radionuclides of iodine or external, as in the case of external radiation, the major effect on tissues is due to ionization. Therefore these forms of radiation are also called ionizing radiation. The radiation can have a direct effect on tissues. This occurs when the radiation per se damages a structure such as DNA (Deoxyribonucleic acid). Most often, the damage is indirect and usually related with FREE RADICALS, which have unpaired electrons in the outer shells.

These atoms of molecules are very active and can cause damage to adjacent molecules. Since H_2O is the most prevalent molecule, it is most likely to be ionized and form free radicals.

H_2O ----------> $H_2O^+ + e$
$H_2O + H_2O = H_2O^+ + OH^*$

Hall [10] states that 75% of damage to DNA from radiation is due to OH*. The nucleus is the most sensitive part of the molecule and severe damage results in cell death. Less severe damage produces a cell, which is unable to reproduce. Therefore, at the end of its life span, it dies and is not replaced. The radiation can shorter the life of the cell and cause premature death of a cell, which is also incapable of replacing itself. This type of damage in the thyroid results in Hypothyroidism.

Friedberg [11] failure to repair defects can result in mutations, which can cause the normal regulatory mechanisms to be lost and tumors, both benign and malignant, to result. Recent publications have shown that in the thyroid, hydrogen Peroxide (H_20_2) produced via superoxide (02-) by superoxide dismutase (S.O.D) is used by thyroid peroxidase to synthesize hormones. Superoxide dismutase (S.O.D) is deficient in endemic goitre tissues the prolonged exposure to reactive oxygen species (ROS) may contribute to the degeneration changes frequently seen in these tissues. Recent studies [12] shows the Role of ROS and antioxidant defense of thyroid tissue in hyperthyroidism and hypothyroid goiter. Thyroid hormones have been reported to exhibit antioxidant properties [13], [14]. In hypothyroidism, the synthesis and levels of thyroid hormones are very low. This fact along with the reduced SOD and elevated lipid peroxidase (L.P) has observed suggest the possible direct involvement of superoxide radicals and L.P. production in the development of goitrogen induced hypothyroid goiters. Propylthiouracil (PTU) itself may influence the antioxidant defense of thyroid tissues apart from its known interference with thyroid peroxidase.

To find out whether thyroid hormone influences antioxidant defense parameters of rat brain,several oxidative stress and antioxidant defense parameters of mitochondrial fraction (MF) and post-mitochondrial fractions (PMF) of cerebral cortex (CC) of adult rats were compared among euthyroid (control), hypothyroid [6-**n**-propylthiouracil (PTU)-challenged], and hyperthyroid (T_3-treatment to PTU-challenged rats) states. Oxidative stress parameters, such as thiobarbituric acid-reactive substances (TBA-RS) and protein carbonyl content (PC), in MF declined following PTU challenge in comparison to euthyroid rats. On the other hand, when PTU-challenged rats were treated with T_3, a significant increase in the level of oxidative stress parameters in MF was recorded. Hydrogen peroxide content of MF as well as PMF of CC was elevated by PTU-challenge and brought to normal level by subsequent treatment of T_3.

Results of the current investigation suggest that antioxidant defense parameters of adult rat brain are considerably influenced by thyroid states of the body [15].

Modification of Carcinogenesis by antioxidants in the initiation stage:In this stage antioxidants could modify carcinogenesis by:(a) Altering the metabolic activation of procarcinogens,(b) Altering detoxifying enzymes,(c) Direct interaction with the proximate carcinogenic species,(d) Trapping active oxygen species or(e) Influencing absorption of carcinogens from the gastrointestinal tract. The present study was undertaken to evaluate the effect of antioxidants on thyroid hormones. It was observed that the circulating levels of T3 were significantly increased in Vit.C. Hence our results show positive effects of antioxidants (Vit.C) on thyroid hormones levels, which could be due to direct involvement of antioxidants on thyroid gland or on deiodinase enzyme activity. This needs further detailed study to prove the exact pathway of the mechanism of action of antioxidants. We have demonstrated that the administration of antioxidants namely Vit. 'C',Vit. 'E' and turmeric significantly increased in the circulation levels of T4 and T3. The thyroid hormones responded to antioxidants indicating the importance of antioxidants for the prevention of occurrence of certain diseases in thyroid gland by protecting biological system against potentially harmful effects of processes or reactions that can causes excessive oxidations.

Hyperthyroid Graves' disease is one of the commonest autoimmune disorders, affecting about 1% of women. It is most frequent in the 4th decade of life. Factors that have an influence are cigarette use, pregnancy, estrogen use, and stressful life events. The hyperthyroidism is caused by thyroid-stimulating hormone (TSH) receptor stimulating autoantibodies that lead to excess thyroid hormone production and thyroid growth. Thyroid peroxidase autoantibodies are also frequently found and may be important in thyrocyte destruction and perpetuation of autoimmunity [16]. GD is characterized by a condition of increased oxidative stress, not only in the acute phase of the disease, but also in the state of euthyroidism, induced by anti-thyroid medications [17].

6. Conclusions

We have demonstrated that the administration of antioxidants namely Vit 'C',Vit 'E' and turmeric significantly increased in the circulating levels of T4 and T3.The thyroid hormones responded to antioxidants for the prevention of occurrence of certain diseases in thyroid gland by protecting biological system against potentially harmful effects of processes or reactions that can cause excessive oxidations. The study concluded with the authenticity of antioxidants positively transforming the thyroid levels. As a known fact that thyroid hormone plays a very important role in metabolism, growth and development of the body. The increased oxidative damage macromolecules in the thyroid occur in response to different exogenous pro-oxidants. Antithyroid drugs may also pertub excessive oxidative load. The contribution of this oxidative damage to the development of thyroid diseases, cancer included, should be considered. From this study antioxidants are strongly recommended to prevent thyroid disorders (hypo/hyperthyroidism and cancer).

7. Acknowledgement

The author would like to thank the teachers, guide, and co-guide, scientific officer of animal laboratory, physicians and staff members of the Radiation medicine centre for their valuable help during the research.

Conflicts of interests: Nil
Findings: Nil

References

[1] Kandasamy Babu, Indira A Jayaraaj: Effect of Abnormal thyroid hormone changes in lipid peroxidation and Antioxidant imbalance in Hypothyroid and Hyperthyroid patients; International Journal of Biological & Medical Research
[2] Corvilain B, Van Sande J, Laurent E & Dumont J E,The H_2O2 generating system modulates protein iodination and the activity of the pentose phosphate pathway in dog thyroid ;Endocrinology,128(1991)779
[3] Goyens P, Golstein J, Nsombola B, Vis H and Dumont J E, Selenium deficiency as a possible factor in the pathogenesis of myxoedematous endemic cretinisms ;Acta Endocrinol(Co pench), 114 (1987) 497.
[4] Robbins J, Rall J E, Gorden P. In: Metabolic Control and Disease. Bondy P K, Rosenberg L E, editors. Philadelphia: Saunders; 1980. pp. 1325–1426.
[5] Kohn L D, Saji M, Kosugi M, Ban T, Giuliani C, Hidaka A, Shimura H, Shimura Y, Okajima F. In: Thyroid Diseases: Basic Science, Pathology, Clinical and Laboratory Diagnosis. Troncone L, Shapiro B, Satta M A, Monaco F, editors. Boca Raton, FL: CRC Press; 1993. pp. 59–118).
[6] Rigutto S, Hoste C, Grasberger H, Milenkovic M, Communi D, Dumont JE, Corvilain B, Miot F, De Deken X: Activation of dual oxidases Duox1 and Duox2: differential regulation mediated by camp-dependent protein kinase and protein kinase C-dependent phosphorylation. *J Biol Chem* 2009, 284:6725-6734.
[7] Karbownik M, Lewiński A: The role of oxidative stress in physiological and pathological processes in the thyroid gland; possible involvement in pineal-thyroid interactions. *Neuro Endocrinol Lett* 2003, 24:293-303.].
[8] Poncin S, Gérard AC, Boucquey M, Senou M, Calderon PB, Knoops B, Lengelé B, Many MC, Colin IM: Oxidative stress in the thyroid gland: from harmlessness to hazard depending on the iodine content. ***Endocrinology* 2008,** 149:424-433.
[9] Poncin S, Van Eeckoudt S, Humblet K, Colin IM, Gérard AC: Oxidative stress: a required condition for thyroid cell proliferation. ***Am** J Pathol* 2010, **176:**1355-1363.)
[10] Hall E.J. (1978);Radiobiology for the Radiologist,2nd Edn.Harper & Row,Hagerston.
[11] Friederg,E.C(1985)DNA Repatr,W.H.Freeman,New York.
[12] Sadani,G.R and Nadkarni G.D.: Role of reactive oxygen species in induced hypothyroid goiters and hyperthyroidism in rats.Med. Sci,Research;1996:24,835-836.
[13] Bunyan,J.Green,J.Edwin,E.L. and Diplock,A 1961:Biochem. Biophys Acta 47,401-405

[14] Faure,MLiss,E.A. Videla,LA.1991,Chem. Boi.Interactions,77, 173-185
[15] Kajari Das,G.B.N. Chaiy; Thyroid Hormone Influences Antioxidant Defense System in Adult Rat Brain; Neurochemical Research :September 2004, Volume 29, Issue-9 pp 1755-1766
[16] Simon H.S. Pearce MD,FRCP : disease Graves' Disease; Autoimmune Diseases in Endocrinology, Contemporary Endocrinology 2008, pp 117-135
[17] Admoglu et al 2006; In fact, several studies outlined the association of GD with impaired antioxidant activity. Nutr Cancer.1992; 18(1):1-29.