

LEVOTHYROXINE REPLACEMENT THERAPY WITH VITAMIN C SUPPLEMENTATION PREVENTS OXIDATIVE STRESS IN HYPOTHYROID PATIENTS

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ABSTRACT

In hypothyroidism, large doses of levothyroxine are required to achieve therapeutic endpoint, preliminary observations indicated that hypoacidic gastric environment is associated with reduction in levothyroxine bioavailability and co-administration of vitamin C might enhance absorption. Pathological consequences of hypothyroidism point to high potential for antioxidant imbalance. We therefore assessed effect of vitamin C supplementation with Levothyroxine replacement therapy on thyroid status and oxidative stress in hypothyroid patients. The study population consisted of 80 subjects divided into 4 groups. 20 patients into Normal untreated Healthy Subjects, 20 patients into untreated hypothyroid patients, 20 patients into hypothyroid Patients treated only with Levothyroxine and 20 patients with Levothyroxine+ Vitamin C 1000mg. This study examined the levels of some enzymatic antioxidant status. The antioxidant status was significantly lower in hypothyroid patients than in healthy group. After 12weeks of supplementation of vitamin C with levothyroxine replacement therapy there was significant elevation of catalase, GR activities and decrease in MDA, GPx and GSH levels in treated groups. It can be concluded from the above study that Supplementation with Vitamin C decreases the oxidative stress and increases antioxidant status in hypothyroid patients. Hence the Antioxidant therapy and antioxidant diet should be advised along with thyroid hormone replacement therapy to diminish further complications.

KEYWORDS: Oxidative stress, Vit C supplementation, levothyroxine.

INTRODUCTION

Levothyroxine sodium is commonly prescribed for the treatment of hypothyroidism and thyroid neoplasia. As a result of hypofunction or absence of the thyroid gland, the level of serum thyroid stimulating hormone (TSH) is elevated because of the absence of the regulatory negative feedback mechanism. Patients with hypothyroidism are supplemented with synthetic thyroxine (i.e. levothyroxine, LT4) in oral doses to achieve physiological T4 and TSH serum levels. The mean treatment dosage of LT4 is 1.6ug/kg body weight/day.^[1]

The absorption of levothyroxine is approximately 50-100 % after oral administration^[2-6], but there is considerable inter-individual and intra-individual variability.^[7] On occasions, when extraordinarily large doses of LT4 are required to achieve a therapeutic endpoint, clinicians should suspect either some interference with absorption

or non-compliance. Of note in this regard are the several factors relating to LT4 absorption in the stomach, including the role of gastric acid secretion, timing of food ingestion, gastric pH impairment, and the effect of the latter on facilitating LT4 absorption in the gut.^[8] Dissolution of LT4 is a crucial step in its oral absorption and bioavailability^[9], and tablets of LT4 need intragastric acid pH in order to achieve an adequate dissolution. Decreased dissolution of LT4 with higher gastric pH as described by Pabla et al confirmed the relationship of LT4 absorption to alterations in gastric pH and the importance of variable dissolution of LT4 on the bioavailability of LT4.^[9]

Conceivably, even physiological variations in intragastric pH might determine differences in LT4 tablets dissolution, and therefore LT4 absorption. Preliminary observations indicated that co administration of acidic compounds—as officinal HCl— might enhance absorption of LT4. Similarly, a favorable effect of taking

certain drugs with orange juice has been described; thus, in *H. pylori* and HIV-positive hypochlorhydric subjects, delavirdine absorption increased by 57 % with orange juice administration.^[10] Although controversial, in certain cases it has been found that vitamin C improves iron absorption.^[11]

Thyroid hormones are among the most important humoral factors involved in setting the basal metabolic rate on a long term basis in target tissues such as liver, heart, kidney and brain.^[12] Oxygen free radical can develop during several steps of normal metabolic events. Although free radicals have the potential to damage the organism, their generation is inevitable for some metabolic process. The main endogenous sources of free radicals are the microsomal membrane electron transport chain, reaction of oxidant enzymes, and auto-oxidation reactions.^[13,14]

Both hydrogen peroxide and superoxide anion produce highly reactive hydroxyl radicals through the Haber-Weiss reaction. The hydroxyl radical can initiate lipid peroxidation, which is a free radical chain reaction leading to damage of membrane structure and function.^[15] Variations in the levels of thyroid hormones can be one of the main physiological modulators of *in vivo* cellular oxidative stress due to their known effects on mitochondrial respiration. In particular, it has been suggested that the increases in reactive oxygen species induced by a deficiency of thyroid hormones can lead to an oxidative stress conditions in the liver and in the heart and some skeletal muscles with a consequent lipid peroxidative response.^[14]

Reactive oxygen species (ROS) including partially reduced forms of oxygen, i.e. super-oxide anion, hydrogen peroxide, and hydroxyl radical, as well as organic counter parts such as lipid peroxides, are produced as natural consequences of oxidative cell metabolism.^[16] Under physiological conditions, ROS generation is controlled by a large number of anti-free radical systems which acts as protective mechanisms. These systems consist of antioxidant enzymes such as super-oxide dismutase, catalase, glutathione peroxidase and glutathione reductase as well as non-enzymatic anti-oxidants, among which the most important are vitamins C and E, carotenoids, and glutathione. Disturbance of the prooxidant antioxidant balance results from the increased production of ROS, inactivation of detoxification systems, or excessive consumption of anti-oxidants. The disturbance is a causative factor in oxidative damage of cellular structures and molecules such as lipids, proteins, and nucleic acids.^[17]

Hence in this study it is further envisaged to evaluate the effect of vitamin C supplementation on oxidative stress status in these patients.

MATERIALS AND METHODS

Study population

The study population consisted of 80 subjects divided into 4 groups. 20 patients into Normal untreated Healthy Subjects, 20 patients into untreated hypothyroid patients, 20 patients into hypothyroid Patients treated only with 50µg levothyroxine and 20 patients with 50µg Levothyroxine + Vitamin C 1000mg. All the patients and controls were recruited from Malla Reddy teaching hospital during January to June of 2016.

The patients were diagnosed by physicians on the basis of detailed clinical history, clinical examination, and other relevant biochemical investigations. Patients who have been diagnosed with Hypothyroidism, of either sex and of age greater than 18years were included for the study. Patients with co-morbid conditions, who have undergone thyroidectomy, Pregnant, lactating women and who are not willing to cooperate were excluded from the study. Informed consent was obtained from each participant in the study. The study was cleared by institutional ethical committee.

Blood Collection and hemolysate preparation

Blood samples were collected by venous puncture in plain tubes and the plasma was separated by centrifugation at 1000g for 15 minutes after centrifugation, the Buffy coat was removed and the packed cells were washed three times with physiological saline. A known volume of the erythrocytes was lysed with hypotonic phosphate buffer (pH 7.5). The hemolysate was separated by centrifugation at 2500g for 15 minutes.

Estimation of reduced glutathione

Reduced glutathione (GSH) content was determined by the method of Ellman's (Ellman, 1959). Plasma, 1.0ml, was treated with 0.5ml of Ellman's reagent (19.8 mg of 5,5-dithiobisnitro-benzoic acid [DTNB] in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH8.0). The absorbance was read at 412nm.

Assay of catalase

Catalase (CAT) was assayed colorimetrically at 620nm and expressed as µmol of H₂O₂ consumed min/mg/Hb as described by (Shina, 1972). The reaction mixture (1.5ml) contained 1.0ml of 0.01 mole pH 7.0 phosphate buffer, 0.1 ml of hemolysate, and 0.4 ml of 2mole H₂O₂. The reaction was stopped by addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

Assay of glutathione reductase

The 10,000 g supernatant of 5% tissue homogenate was used for estimation of GR activity by the method of Carlberg and Mannervik, 1985. The assay system consisted of phosphate buffer (0.2M, pH 7.0, containing 2mM EDTA), 20 mM GSSG, 2 mM NADPH and supernatant. The enzyme activity was quantitated at 25°C

by measuring the disappearance of NADPH at 340 nm. The enzyme activity was expressed as nmole of NADPH oxidized per minute per mg of protein.

Assay of malondialdehyde

To the 0.2 ml of experimental sample, 0.2ml of 8.1 % SLS, 1.5 ml of 20 % Acetic acid (p^H 3.5) and 1.5 ml of 0.8 % aqueous solution of TBA was added and made the volume up to 4 ml with double distilled water. Then heat the mixture at 95°C for 60 min in water bath on hot plate to develop light pink color. The mixture was allowed to cool and measure the absorbance spectrophotometrically at 532 nm using U.V – Visible spectrophotometer.

Assay of Glutathione peroxidase

The reaction mixture consisted of 0.02ml of 0.8mM EDTA, 0.1ml of 10mM Sodium azide, 0.1 ml of 2.5mM H_2O_2 , 0.2ml of homogenate and was arrested by adding 0.5ml of 10% of TCA and the tubes were centrifuged at 2000 rpm for 15 min. 3 ml of 0.3mM of Disodium hydrogen phosphate and 1 ml of 0.04% DTNB were added to the supernatant and develops colour was detected at 420 nm immediately. Glutathione peroxidase (GP_x) activity was expressed as μ mole of the oxidized glutathione / min / mg protein.

Statistical analysis

All data were expressed as mean \pm SEM. The statistical significance was evaluated by student's t-test and one way ANOVA using SPSS and graph pad prism 7.0.

RESULTS

The study subjects were grouped into four groups, 20 patients into Normal untreated Healthy Subjects, 20 patients into untreated hypothyroid patients, 20 patients into hypothyroid Patients treated only with 50 μ g levothyroxine and 20 patients with 50 μ g Levothyroxine + Vitamin C 1000mg (Figure 1).

Out of 80 patients 71 are female and 9 are male as shown in figure 2. From the figure we can observe that female patients are more affected with hypothyroidism than male patients.

After treatment with 1000mg of ascorbic acid there was a drop in estimated MDA, GSH, GPx levels and CAT, GR levels increased. The alleviation of oxidative stress and antioxidant defense reached statistical significance with $P < 0.001$ [Table 1].

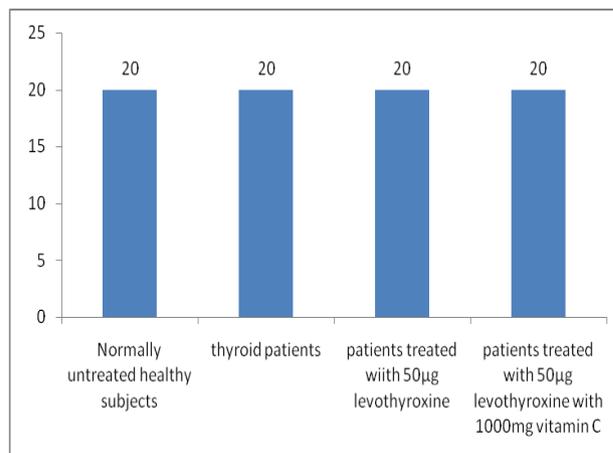


Figure 1: Illustrates out of 80 patients 20 are Normal untreated healthy subjects, 20 are Thyroid patients, 20 are Patients treated with 50 μ g of Levothyroxine and 20 are patients treated with 50 μ g of Levothyroxine with 1000mg Vitamin C.

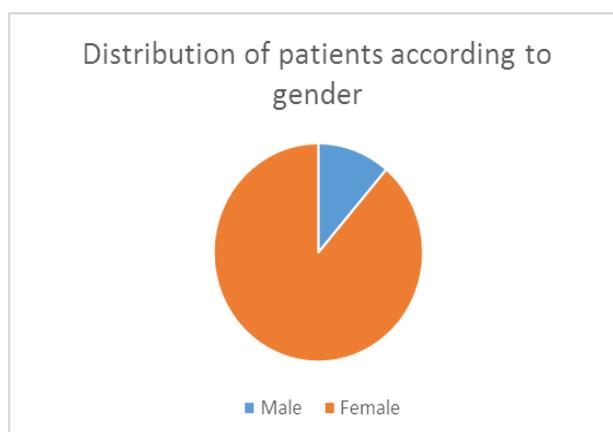


Figure 2: Illustrates out of 80 patients 71 are female and 9 are male.

Table 1: This table indicates mean \pm standard error of mean values before and after administration of 1000mg of Ascorbic acid along with levothyroxine replacement therapy.

| | MDA(M \pm SEM) (nm/g) | | CAT(M \pm SEM) (k/min) | | GSH(M \pm SEM) (mcg/ml) | | GR(M \pm SEM) (nmol/min/mg of protein) | | GPX(M \pm SEM) (μ g/ml) | |
|---|-------------------------|--------------------|--------------------------|--------------------|---------------------------|-------------------|--|--------------------|--------------------------------|--------------------|
| | Before | After | Before | After | Before | After | Before | After | Before | After |
| Normal control | 5.94 \pm 0.127 | 6.880 \pm 0.1943 | 17.34 \pm 0.562 | 18.467 \pm 0.52 | 3.91 \pm 0.215 | 4.100 \pm 0.208 | 3.270 \pm 0.215 | 4.100 \pm 0.208 | 9.50 \pm 0.402 | 9.736 \pm 0.382 |
| Positive control | 12.60 \pm 0.472 | 12.63 \pm 0.527 | 9.94 \pm 0.221 | 9.403 \pm 0.493 | 9.316 \pm 0.584 | 8.52 \pm 0.496 | 1.183 \pm 0.092 | 1.926 \pm 0.397 | 12.89 \pm 0.306 | 13.12 \pm 0.342 |
| Patients treated with 50 μ g levothyroxine | 11.32 \pm 0.219 | 8.933 \pm 0.208 | 8.406 \pm 0.435 | 12.503 \pm 0.532 | 9.683 \pm 0.164 | 7.543 \pm 0.569 | 1.390 \pm 0.313 | 2.726 \pm 0.4153 | 11.073 \pm 0.743 | 10.913 \pm 0.756 |
| Patients treated with 50mcg levothyroxine with vitamin c 1000mg | 10.33 \pm 0.296 | 6.239 \pm 0.218 | 16.46 \pm 0.834 | 17.02 \pm 0.373 | 8.606 \pm 0.409 | 4.921 \pm 0.375 | 2.42 \pm 0.330 | 4.921 \pm 0.375 | 10.59 \pm 0.263 | 9.273 \pm 0.409 |

DISCUSSION

Hypothyroidism causes immunosuppression that may lead to oxidative stress. TSH at a higher concentration may induce secretion of inflammatory cytokines and decrease the antioxidant status. Hypothyroidism-associated oxidative stress is the consequence of both increased production of free radicals and reduced capacity of the anti-oxidative defense. Variations in the levels of thyroid hormones can be one of the main physiological modulators of *in vivo* cellular oxidative stress due to their known effects on mitochondrial respiration. In particular, it has been suggested that the increase in reactive oxygen species induced by a deficiency of thyroid hormones can lead to an oxidative stress condition in the liver and in the heart and some skeletal muscles with a consequent lipid peroxidative response. Metabolic disorder from autoimmune-based hypothyroidism can also increase oxidative stress. Hypothyroidism undeniably can be risk factor for increased oxidative stress; can eventually lead to many other complications. Antioxidant therapy and antioxidant diet should be advised along with thyroid hormone replacement therapy to diminish further complications.

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Conflicts of Interest

No conflicts of interest have been declared.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical Standards of the Institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or Comparable ethical standards.

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