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## RESEARCH ARTICLE

### **Oxidative Stress and Antioxidative Defence Mechanism in Patients with Hypothyroidism.**

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#### **Abstract**

Free radical mediated oxidative stress has been implicated in the etio-pathogenesis of several autoimmune disorders. Hypothyroidism in humans is widely believed to impair health. The biochemical factors mediating decline in health, however, are poorly elucidated. Pathological consequences of hypothyroidism point to a high potential for antioxidant imbalance. The study population consisted of 50 subjects divided into two groups: 25 people with hypothyroidism and 25 age-matched healthy participants. This study examined the levels of total tri-iodothyronine (T3), total thyroxine (T4), thyroid stimulating hormone, (TSH), and some enzymatic antioxidant status. The mean TSH level was significantly higher in hypothyroid patients than in control group. On the other hand, the levels of T3 and T4 were significantly lower in hypothyroid patients compared to control group. However, the activities of catalase (CAT), and reduced glutathione (GSH) were significantly lower in hypothyroid patients than in healthy group. These results confirm the hypothesis that people with hypothyroidism have reduced anti-oxidative defense.

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#### **Introduction:-**

The thyroid hormones, triiodothyronine (T3) and its prohormone, thyroxine (T4), are tyrosine-based hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism. T3 and T4 are partially composed of iodine (see molecular model). A deficiency of iodine leads to decreased production of T3 and T4, enlarges the thyroid tissue and will cause the disease known as simple goitre. The major form of thyroid hormone in the blood is thyroxine (T4), which has a longer half-life than T3<sup>1</sup>. In humans, the ratio of T4 to T3 released into the blood is between 14:1 and 20:1.

Thyroid hormones are among the most important humoral factors involved in setting the basal metabolic rate on long term basis in target tissues such as liver, heart, kidney and brain<sup>2</sup>

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<sup>3</sup>.

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.<sup>4</sup>

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.<sup>5</sup>

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<sup>6</sup>. Under physiological conditions, ROS generation is controlled by a large number of anti-free radical systems which acts as protective mechanisms. These systems consist anti oxidant 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.<sup>7</sup>

Hypothyroidism-associated oxidative stress is the consequence of both increased production of free radicals and reduced capacity of the anti-oxidative defense.<sup>8</sup>

Metabolic disorder from autoimmune-based hypothyroidism can also increase oxidative stress.<sup>9</sup>

Hypothyroidism-induced dysfunction of the respiratory chain in the mitochondria lead to accelerated production of free radicals (i.e., superoxide anion, hydrogen peroxide, and hydroxyl radicals as well as lipid peroxides), which consequently leads to oxidative stress (OS).<sup>10</sup>

## **Materials And Methods:-**

### **Study Population:-**

The study population consisted of 50 subjects (age and sex-matched) divided into two groups: hypothyroid patients (n=25) and healthy control subjects (n=30). All the participants were enrolled from out-patient department of Medicine, NIMS Medical College and Hospital. All the relevant demographic data and clinical history were obtained by verifying patient records. Exclusion criteria involved, surgical patients, pregnant women, ICU admitted patients, chronic hypertensive patients, Children, Urinary tract infections, Emotional or physical stress, Strenuous exercise.

Blood samples were collected by venous puncture in plain tubes and the plasma was separated by centrifugation 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 for 15 minutes.

### **Hormonal Assays:-**

The levels of serum thyroid stimulating hormone (TSH), total triiodothyronine (T3), and total thyroxine (T4) were measured by using enzyme immuno assay (ELISA) methods.

### **Estimation Of Glutathione Reductase:-**

Reduced glutathione (GSH) content was determined by the method of Ellman's<sup>11</sup> 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.

### **Catalase Assay**

Catalase (CAT) was assayed colorimetrically at 620nm and expressed as  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  consumed  $\text{min}/\text{mg}/\text{Hb}$ <sup>12</sup>. 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  $\text{H}_2\text{O}_2$ . 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).

### **Statistical Analysis:-**

Statistical analysis was done, using IBM SPSS 20 for Windows software Microsoft Excel 2007 and scientific calculator.

The normal range of hormones was calculated from the data which was obtained from the blood samples from normal healthy subjects. The Student's 't' test was used to compare the results of the normal healthy controls and the hypothyroidism patients. The results were expressed as Mean  $\pm$  Standard Deviation (SD). The comparison of T<sub>3</sub>, T<sub>4</sub>, TSH, GSH, CAT between control and study group was analyzed using unpaired "t"-test. Statistical significance was considered to be significant at a p value of <0.05.

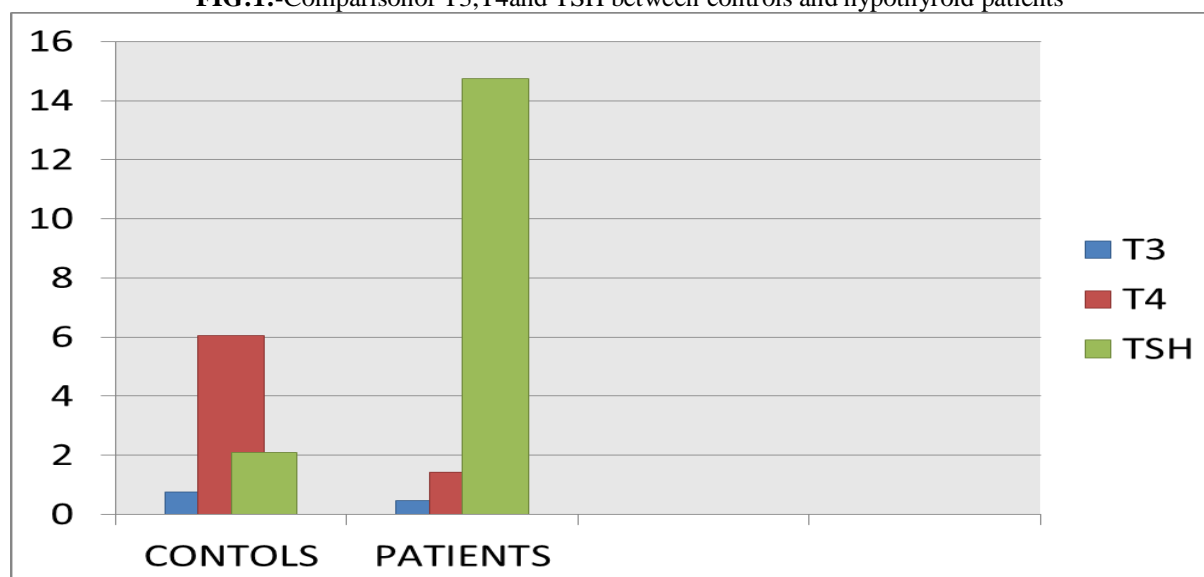
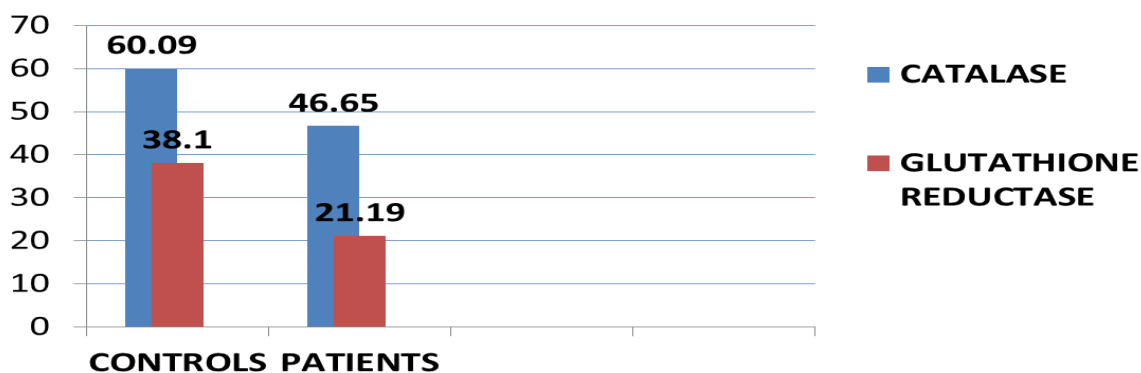
**Observations And Result:-**

The results of this study is shown in the following table1. The levels of TSH of hypothyroid patients show significant increase ( $P<0.01$ ) in a comparison with healthy control. Hypothyroid patients also had significantly lower ( $P<0.01$ ) levels of T4 and T3. For studying the deleterious consequence of hypothyroidism on antioxidant status, the activities of enzymatic antioxidants (CAT) and non enzymatic antioxidants were measured. The activities of the enzymatic antioxidants CAT and GSH were significantly lower ( $P<0.01$ ) in hypothyroid patients when compared with healthy subjects.

**Table:1:-**Comparison of T3,T4,TSH ,CAT,GSH between controls and hypothyroid patients

Parameter	Control subjects	Hypothyroid patients	eulavp
T3(ng/ml)	0.76±0.240	0.46±0.10	<0.001
T4(µg/dL)	6.04±1.09	1.42±0.62	<0.001
TSH(mIU/ml)	2.09±1.14	14.75±4.06	<0.001
CAT(U/mg Hb)	60.09±9.89	46.65±8.39	<0.001
GSH (mg/dL)	38.10±6.52	21.19±3.82	<0.001

Values are Mean±Standard deviation

**FIG:1:-**Comparison of T3,T4 and TSH between controls and hypothyroid patients**FIG:2:-**Comparison of CAT and GSH between Controls and Hypothyroid patients**Discussion:-**

Pasupathi. P., et al.concluded that a very high production of ROS and oxidative stress in patients with hypothyroidism, with enhanced lipid peroxidation and concomitant failure of antioxidant defense mechanisms.

Physical signs and symptoms in people with hypothyroidism are less reliable and there is a need for serum testing to determine the appropriate dosage of replacement thyroid hormones. Our purpose in this study was to provide evidence for, and to recommend, blood testing for hypothyroid patients' antioxidant system in order to monitor the progression of pathology and to prompt the consideration of medical care.<sup>13</sup>

Resh et al., found that hypothyroidism was associated with enhanced oxidative stress and lipid peroxidation, and supposed that this might lead to the development and progression of atherosclerosis<sup>14</sup>. Both glutathione peroxidase and catalase are major defenses against harmful effects of ROS in cells, and in cultured thyrocytes, both have a high capacity to degrade exogenous hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>15</sup>

Antioxidant deficiencies may lead to a failure to effectively combat extrinsic factors (i.e., weather, diet, drugs, and physical exercise) and intrinsic factors (i.e., injuries, weakness, and fatigue involved in oxidative stress. An extensive body of evidence now exists confirming that antioxidants are involved in the cellular defense against oxidative stress in a variety of pathological conditions.

It has been suggested that hypothyroidism lead to oxidative stress and to a reduction of antioxidant defenses. In addition, previous experimental studies have reported that hypothyroidism is characterized by endothelial dysfunction of blood vessels.<sup>16</sup>

Thus, under normal conditions, the protective effect of thyroid hormone against oxidative stress can be explained by the function of antioxidants as a defense system. However a chronic state of hypothyroidism is characterized by impairments in the redox potential. This lead to free radical chain reactions and to metabolic suppression on antioxidant capacity. Results from this study support the suggestion that the hypothyroidism of patients with intellectual disability in some way is linked to the low levels of the major antioxidant molecules found in these patients. The depletion of antioxidants observed in hypothyroid individuals may reflected the increased free radical production in the electron transport chain in the mitochondrial inner membrane.

The increase of free radicals is not compensated, as one would expect, by a decrease of antioxidants. A high oxidative state in hypothyroid people has metabolic and biochemical characteristics such as increased mitochondrial enzyme activity.

Thus, it is likely that patient's cells are damaged by prolonged oxidative stress that far exceeds the capacity of the patient's organs to synthesize antioxidant molecules or to synthesize them from extra cellular sources<sup>6</sup>

### **Conclusion:-**

In conclusion, the present study suggests a very high production of ROS and oxidative stress in patients with hypothyroidism, with enhanced lipid peroxidation and concomitant failure of antioxidant defense mechanism. Physical signs and symptoms in people with hypothyroidism are less reliable and there is a need for serum testing to determine the appropriate dosage of replacement thyroid hormones.

The purpose in this study was to provide evidence for, and to recommend, blood testing for hypothyroid patient's antioxidant system in order to monitor the progression of pathology and to prompt the consideration of medical care

### **References:-**

1. Irizarry, Lisandro (23 April 2014). "Thyroid Hormone Toxicity". Medscape. WedMD LLC. Retrieved 2 May 2014.
2. Guerrero, A.; Pamplona, R., Postero-otin, M.; Barja, G., and Lopez-Torres M. (1999). Effect of thyroid status on lipid composition and peroxidation in mouse liver. *Free. Rad. Biol. Med.*, 26: 73-80.
3. Hauck, J.S. and Bartke, A. (2000). Effects of growth hormone on hypothalamic catalase and Cu/Zn superoxide dismutase. *Free. Rad. Biol. Med.*, 28: 970-979
4. Halliwell, B., and Gutteridge, J.M.C. (1990). Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol.*, 186: 1-85
5. Yilmaz, S.; Ozan, S., Benzer, F.; and Canatan, H. (2003). Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. *Cell. Biochem. Funct.*, 21(4): 325-330.

6. Komosinska-Vassev, K.; Olczyk, K.; Kucharz, E.J.; Marciz, C.; and Kotulska, A (2000). Free radical activity and anti oxidants defense mechanisms in patients with hyperthyroidism due to Grave's disease during therapy. *Clinical chimica Acta.*, 300: 107-117.
7. Kehrer, J.P. (1993). Free radicals as mediators of tissue injury and disease. *Crit. Rev.; Toxicol.*, 23: 21-48
8. Sarandol, E.; Tas, S.; Dirican, M.; and Serdar, Z. (2005). Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: Effect of vitamin E supplementation. *Cell. Biochem. Funct.* 23(1): 1-8.
9. Carmeli, E; Bachar, A.; Bachad, S.; Mora, M.; and Merrick, J.(2008). Anti oxidant status in the serum of persons with intellectual disability and hypothyroidism: A pilot study. *Res. Development. Disab.*, 29: 431-438
10. Venditti, P., Balestrier, M., Dimeo, S. and Deleo, T. (1997). Effect of thyroid state on lipid peroxidation, antioxidants defenses, and susceptibility to oxidative stress in rat tissues. *J. Endocrin.*, 155(1): 151-157.
11. Ellman, G.L. (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, 82(1): 70-77.
12. Sinha, A.K. (1972). Colorimetric assay of catalase. *Anal. Biochem.*, 47(2): 389-394.
13. Pashupati P and Latha R(2008).Free radicals activity and antioxidant defence mechanism in patients with hypothyroidism.*Thyroid Science* 3(12),CLS 1-6.
14. Resh, U., Helsel, G., Tatzber, F., and Sinzinger, H. (2002). Anti-oxidant status in thyroid dysfunction. *Clin. Chem. Lab. Med.*, 40: 1132-1134.
15. Bjorkman, U. and Ekholm, R. (1995). Hydrogen peroxide degradation and glutathione peroxidase activity in culture of thyroid cells. *Mol. Cell Endocrinol.*, 111: 99-107
16. Taddei, S.; Caraccio, N.; and Viridis, A. (2003). Impaired endothelium dependent vasodilation in subclinical hypothyroidism: Beneficial effect of levothyroxine therapy. *J.C.E.M.*, 88(8): 3731-3737.