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Effects of Levothyroxine Replacement Therapy on Oxidative Stress in Hypothyroid Patients

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Abstract
Objective: Free radical-mediated oxidative stress has been implicated in the pathogenesis of thyroid disorders. Hypothyroidism is a common thyroid disorder. The aim of this study was to investigate the influence of levothyroxine (LT_4) therapy on oxidative stress marker Malondialdehyde (MDA) level in hypothyroid patients.
Design: This Randomized clinical trial was conducted to compare MDA level in hypothyroidism before and after treatment by LT_4.
Methods: 30 patients with newly diagnosed hypothyroidism and 30 healthy age matched subjects of both sexes as the control group were enrolled in this study. 30 hypothyroid patients were reevaluated for MDA level after 6 month of LT_4 therapy. Fasting blood samples were taken at the initiation and after 6 months of therapy were analyzed for MDA level.
Results: MDA level increased in patients with hypothyroidism. The MDA level was significantly higher in newly diagnosed hypothyroid patients ($p<0.001$). After 6 months treatment with LT_4 decreased MDA levels significantly.
Conclusion: Our results reveal an increased generation of reactive oxygen species in patients with hypothyroidism. These findings indicate that thyroid hormones have a strong impact on oxidative stress and treatment of hypothyroidism with LT_4 can limit oxidative stress.

Key Words: *Hypothyroidism, Oxidative Stress, Reactive oxygen species, Levothyroxine.*

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I. Introduction

Oxidative stress (OS) results as the imbalance between antioxidative protective mechanisms and the rate of production of reactive oxygen species (ROS).[1] It not only leads to lipid peroxidation and oxidative DNA damage but also interferes with physiologic adaptation and intracellular signal transduction. The resulting change in the intracellular redox status leads to the activation of protein kinase, for example, tyrosin kinase, protein kinase C, and the mitogen activated protein kinase cascade leading to altered cellular functions.[2] Under normal physiological conditions, a widespread antioxidant defense system protects the body against the adverse effects of ROS generation. Generation ROS occurs as a consequence of the oxidative cell metabolisms. When oxidants are produced beyond the capability of neutralization by antioxidants, cellular component are damaged **(Fig.1)**. Several relatively stable products including aldehyde compounds are produce that can be measured in plasma as an indirect index of free radical activity. Malondialdehyde (MDA) is the most common measured index of oxidative stress in human studies.[4]

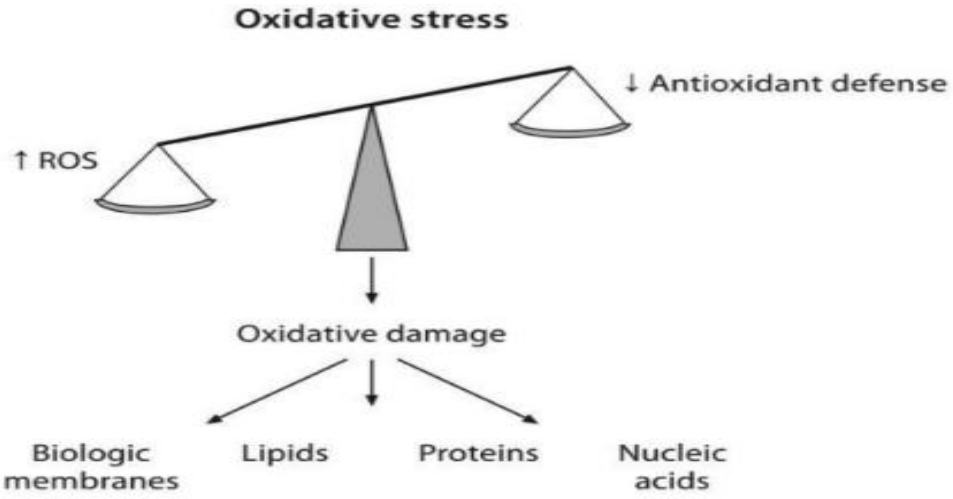

Figure 1: Mechanism of oxidative damage of biological membranes and molecules like lipids, proteins and nucleic acids [3]

OS plays an important role in the development of diseases such as thyroid disorders (hypothyroidism and hyperthyroidism), atherosclerosis, coronary heart diseases, autoimmune diseases and neurodegenerative diseases. Thyroid diseases affect approximately 5% of the population in various forms.[4,5] The thyroid hormones (THs) are important regulators of gene expression. THs regulate cell development, homeostasis, differentiation, growth and metabolism by interaction with thyroid hormone recepors.[6] Thyroid hormones are necessary for the normal growth of our body and one of the most important involved hormonal factors in the regulation of the basic metabolic rate of target organs such as the liver, heart, kidney and brain.[7]

Hypothyroidism is a clinical syndrome characterized by the deficiency of thyroid hormones and increased serum thyroid-stimulating hormone (TSH).[8] The most common causes of hypothyroidism are autoimmune destruction of the thyroid gland, thyroid surgery and radioiodine therapy. Thyroid hormones regulate both basal and adaptive metabolic rate and also play important physiological roles in differentiation, growth and metabolism.[9-11] Hypothyroidism induced dysfunction of the respiratory chain in the cell mitochondria leads to accelerated production of free radicals. The most common form of TH replacement therapy for hypothyroid patients is levothyroxine (LT_4) and is used to suppress TSH. Thus, TH remains in normal range and oxidative tissue damage decrease after therapy.[13,14]

So, in the present study, MDA levels were determined as indices of OS in hypothyroid patients to indirectly assess the role of oxidative tissue damage in the pathogenesis of thyroid disorder as well as to see the changes in MDA level following treatment.

II. Materials And Methods

This randomized clinical trial was conducted in the Department of Pharmacology & Therapeutics, Rajshahi Medical College in collaboration with the Institute of Nuclear Medicine and Allied Sciences (INMAS), Rajshahi between July 2018 to June 2019 to compare serum MDA level between newly diagnosed hypothyroid patients and after levothyroxine (LT_4) therapy. The study population comprised of 30 apparently healthy subjects and 30 newly diagnosed hypothyroid patients. MDA level is measured at the initiation of diagnosis and after 6 months of LT_4 therapy. The following eligibility criteria were employed to select the study population.

Inclusion criteria:

I. Newly diagnosed hypothyroid patients in the age group of 18 -50 years.
II. Control group comprising of normal healthy individuals of 18-50 years of both sexes.

Exclusion criteria:

I. Patients with serious co-morbid diseases (DM, MI, HTN etc.).
II. History of using drugs such as lipid lowering drugs, glucocorticoids, oral contraceptives or vitamin supplements.
III. Female patients with pregnancy and taking oral contraceptive pills.

Blood collection and sample preparation:

Ten (10) ml of blood was withdrawn from patients of hypothyroidism and healthy controls after overnight fasting with dry disposable syringe and needle, under aseptic conditions. Venipuncture of antecubital vein was

performed and the blood collected into sterile, dry and acid washed vial. The blood samples were incubated at 37℃ temperature for 25-30 minutes for proper clot formation and these blood samples were then centrifuged at 3000 rpm for 10 minutes for serum separation. This serum sample was used for biochemical assay.

Malondialdehyde (MDA): Malondialdehyde (MDA) was measured by thiobarbituric acid –reactive substances (TBARS).

Thiobarbituric acid –reactive substances (TBARS) assay:

Principle:

MDA is frequently measured in plasma Thiobarbituric acid –reactive substances(TBARS) assay. Here, TBA reacts with MDA to form pink 2:1, TBA: MDA adduct, which is extracted by N-butanol and absorbed maximally at 530 nm. This colored complex measured by colorimeter.

Procedure:

Thiobarbituric acid method by colorimetry:

According to Satoh (1978) serum MDA level was determined by following method:

MDA in serum was precipitated with protein by adding 5 volumes of 20% trichloroacetic acid (TCA) to 0.2 ml of plasma in the test tube. After centrifugation at 3500 rpm for 10 min the supernatant was decanted. Then 2.0 ml of 0.1N sulfuric acid and 1.0 ml of TBA reagent (0.2% TBA in 2M sodium sulfate) was added to this precipitate and the coupling of MDA with TBA was carried out heating in boiling water bath for 30 min. After cooling in cold water the resulting chromogen was extracted with 2.5 ml of n-butanol by vigorous shaking. This was followed by centrifugation at 3000 rpm for 10 min. A standard of MDA was treated similarly. The optical density (OD) of n-butanol extract of plasma and MDA standard was measured at 530 nm against a butanol blank.[15]

Serum MDA value = $\frac{O.D\ of\ unknown}{O.D\ of\ standard}$ **× 10 µmol/l.**

III. Result

The findings of the present study, intended to compare the level of MDA among newly diagnosed hypothyroid, after LT_4-treatment and normal healthy individuals with 30 subjects in both groups, are presented below:

The mean serum MDA level of hypothyroid patients was significantly higher than that of LT_4-treated hypothyroid and the normal healthy subjects. The MDA of healthy control groups was the lowest among the three groups ($p < 0.001$) (Table I). The serum TSH level of newly hypothyroid group was highest compared to that of after LT_4 therapy and normal healthy control ($p < 0.001$), while the mean serum T_4 level was the lowest in hypothyroid patients compared to that in other two groups ($p = 0.193$) (Table I).

The serum TSH and MDA exhibits a significantly linear correlation ($r = 0.606$, $p < 0.001$) **(Fig.2)**. On the other hand, serum T_4 bears a significantly negative correlation with serum MDA level suggesting that the higher the serum T_4 level the lower is the serum MDA level ($r = -0.500$, $p < 0.001$**) (Fig. 3).**

Table I. Comparison of MDA, TSH and T_4 level in hypothyroid patients after treatment

Variables	Group			p-value
	Newly diagnosed Hypothyroid (n = 30)	After LT_4-Therapy	Control (n = 30)	
Serum MDA (1.5-4µmol/L)	10.0 ± 2.13	5.88 ± 1.90	2.66 ± 0.61	<0.001
TSH (0.3-5 mIU/L)	26.8 ± 20.9	5.07 ± 9.18	2.53 ± 1.55	<0.001
T_4 (8.56–25.6 µg/dl)	5.25 ± 3.83	13.5 ± 20.4	8.95 ± 3.15	0.193

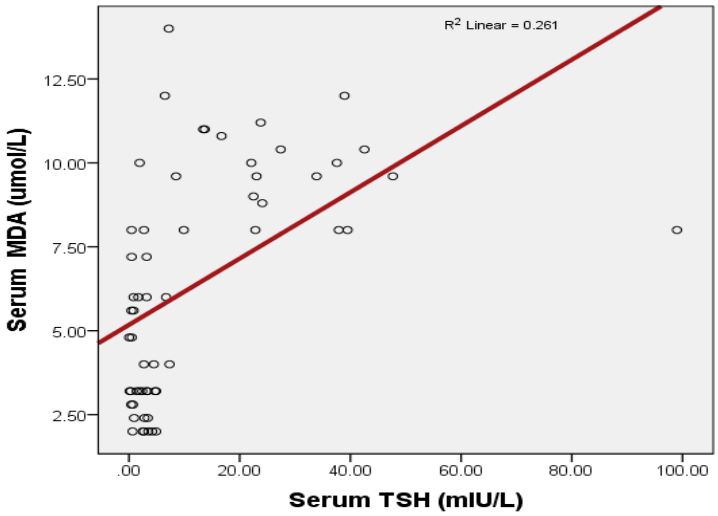

Fig. 2: Correlation between serum TSH and MDA

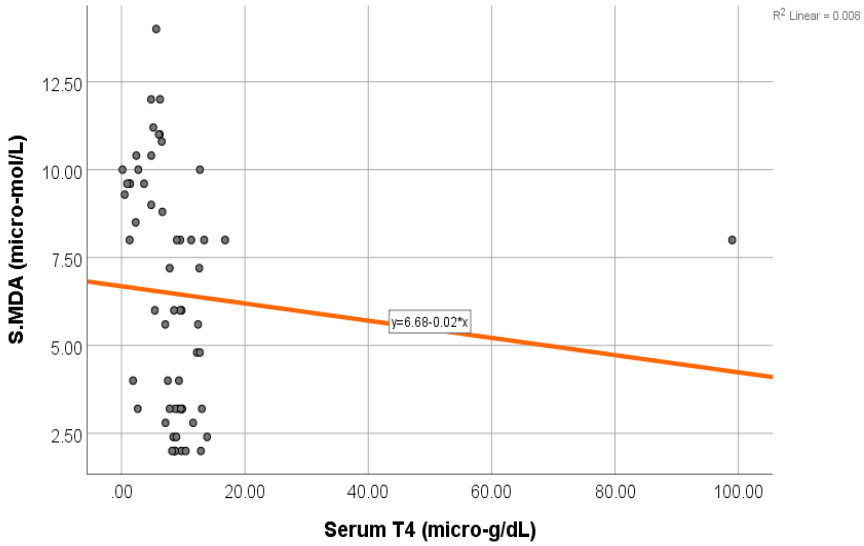

Fig. 3: Correlation between serum T_4 and MDA

IV. Discussion

In present study out of 60 subjects there were 30 newly hypothyroid patients, treated with LT_4 for 6 months and 30 normal healthy individuals as controls. Mean of MDA levels of normal individuals, hypothyroid patients and LT_4 treated hypothyroid patients were 2.66±0.61 µmol/l, 10.0±2.13 µmol/l and 5.88±1.90 µmol/l respectively. The mean serum total MDA level of hypothyroid group was significantly higher than that of LT_4-treated hypothyroid group which was again higher than that of normal healthy individuals. Mean of serum TSH levels of normal individuals, hypothyroid patients and LT_4 treated hypothyroid patients were 2.53±1.55 mIU/l, 26.8 ± 20.9 mIU/l and 5.07 ±9 mIU/l respectively. It reflects that LT_4 significantly decreased serum TSH level and improved disease process. The serum TSH and MDA exhibits a significantly positive correlation indicating that higher the TSH level higher is the serum MDA level **(Figure 2).** In contrast, the serum T_4 bears a significantly negative correlation with serum MDA level suggesting that higher the serum T_4 level lower is the serum MDA level **(Figure 3).**

Mutlu and associates (2013)[2] conducted a study to evaluate the effect of levothyroxine replacement therapy on lipid profile and oxidative stress parameters in patients with hypothyroid. At the baseline they measured serum MDA level and observed that it was higher in hypothyroid patients compared to euthyroid group. After LT_4 therapy, statistically significantly decrease in serum MDA with simultaneous increase in T_4 level. This was the agreement of our study.

Masullo and colleagues (2018)[8] recently performed a study on levothyroxine replacement in hypothyroid patients, which improves the oxidative status of the patients. They found a positive effect of LT_4

treatment on oxidative status on patients of primary hypothyroidism. There was significantly decreased level of MDA and increase catalase (CAT) activity. This also correlates with our present study.

Bascol *et al* (2007)[9] studied that 'oxidative stress and enzymatic antioxidant status in patients with hypothyroidism before and after treatment'. In their study MDA level before treatment was found higher in patients with hypothyroidism than the controls and significantly decreased after 6 months treatment with LT_4. In present study we also found mean serum MDA level of hypothyroid patients was higher than that of LT_4 treated hypothyroid group and the normal healthy control group. The MDA of healthy control group was the lowest among the three groups.

A study performed by Singh and Singh (2013)[11] on Indian Punjabi populations reflects that MDA level was determined as an index of lipid peroxidation in hypothyroidism patients to indirectly assess the role of oxidative tissue damage in the pathogenesis of hypothyroidism before and after treatment with LT_4. They found significantly decreased MDA level in LT_4 treated hypothyroid patient in comparison to newly diagnosed hypothyroids.

Chakraborti *et al* (2016)[12] conducted a study in attempt to find the role of antioxidant supplementation on oxidative stress in hypothyroid patients. They observed that MDA level was high in newly diagnosed hypothyroid patients. After treatment by LT_4 MDA level was reduced to a significant extent. Result of our current study reached to the same conclusions. Role of addition of selenium as antioxidant remained inconclusive in their study.

Observations of our current study have some clinical importance. Serum MDA level of the hypothyroid patients correlate with the T_4 and TSH value. These biochemical markers can be used to assess the prognosis of hypothyroid patients and the outcome of treatment with LT_4. Addition of antioxidant can play a pivotal role in the better outcome in hypothyroid patients with traditional LT_4 therapy. Well designed clinical trials are required to clarify the effect of antioxidants supplementation in hypothyroid patients.

V. Conclusion

From the findings of the study it can be concluded that that MDA level is increased in newly diagnosed hypothyroid patients. The study revealed that treatment with LT_4 therapy in hypothyroidism decreased MDA levels significantly thereby improves the disease process by decreasing oxidative stress. Supplementation with antioxidants with traditional treatment of hypothyroid patients should be undertaken in further researches.

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