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ORIGINAL ARTICLE

Effect of Levothyroxine on Oxidative stress and Lipid Profile in Hypothyroid Patients

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ABSTRACT

Background & Objective: Hypothyroidism is a common endocrine disorder and requires lifelong treatment. The present study was designed to evaluate the impact of levothyroxine (LT_4) on the oxidative stress and metabolic state by assessment of malondialdehyde (MDA) level along with lipid profile in hypothyroid patients.

Methods: This cross-sectional analytical study was conducted in the Department of Pharmacology & Therapeutics, Rajshahi Medical College in collaboration with the Institute of Nuclear Medicine & Allied Sciences (INMAS), Rajshahi between July 2018 to June 2019 to compare malondialdehyde (MDA) level and lipid profile among hypothyroid patients, normal individuals and levothyroxine treated hypothyroid patients. The study included 20 newly-diagnosed hypothyroid patients, 20 Levo-treated hypothyroid patients and 20 healthy controls of both sexes.

Result: The MDA level was significantly highest in newly diagnosed hypothyroid patients among the three groups ($p < 0.001$). The mean of MDA level of hypothyroid patients, LT_4-treated hypothyroid patients and normal healthy controls were 10.0 ± 2.13 µmol/l, 5.88 ± 1.90 µmol/l and 2.66 ± 0.61 µmol/l respectively. Significantly higher values of total cholesterol (TC) (198.5 ± 19.2 mg/dl), triglycerides (TG) (147.0 ± 14.5 mg/dl), low density lipoprotein-cholesterol (LDL-C) (133.9 ± 19.7 mg/dl), and a significantly decreased value of high density lipoprotein-cholesterol (HDL-C) levels (35.1 ± 3.67 mg/dl) were observed in newly-diagnosed hypothyroid patients as compared to those in Levo-treated hypothyroid patients and normal healthy individuals ($p = 0.009$).

Conclusion: The MDA level is significantly raised which suggests that people with hypothyroidism have high risk of free radical damage and risk of development of atherosclerosis, which may lead to coronary heart diseases (CHD). Treatment with LT_4 significantly improves oxidative stress and corrects lipid profile but not up to the normal level. Addition of lipid lowering drugs and supplementation with antioxidants to traditional LT_4 therapy can be tested to see further improvement in outcome parameters.

Key words: Levothyroxine, oxidative stress, lipid profile, hypothyroid patients etc.

INTRODUCTION:

Oxidative stress (OS), defined as the imbalance between oxidative processes and antioxidative protective mechanisms, is implicated in the pathophysiology of various diseases. It plays an important role in the development of diseases like atherosclerosis, coronary heart disease, thyroid disorders, autoimmune diseases & neurodegenerative diseases (Parkinson's, Alzheimer's), damage to the genetic material or the aging process. Hypothyroidism

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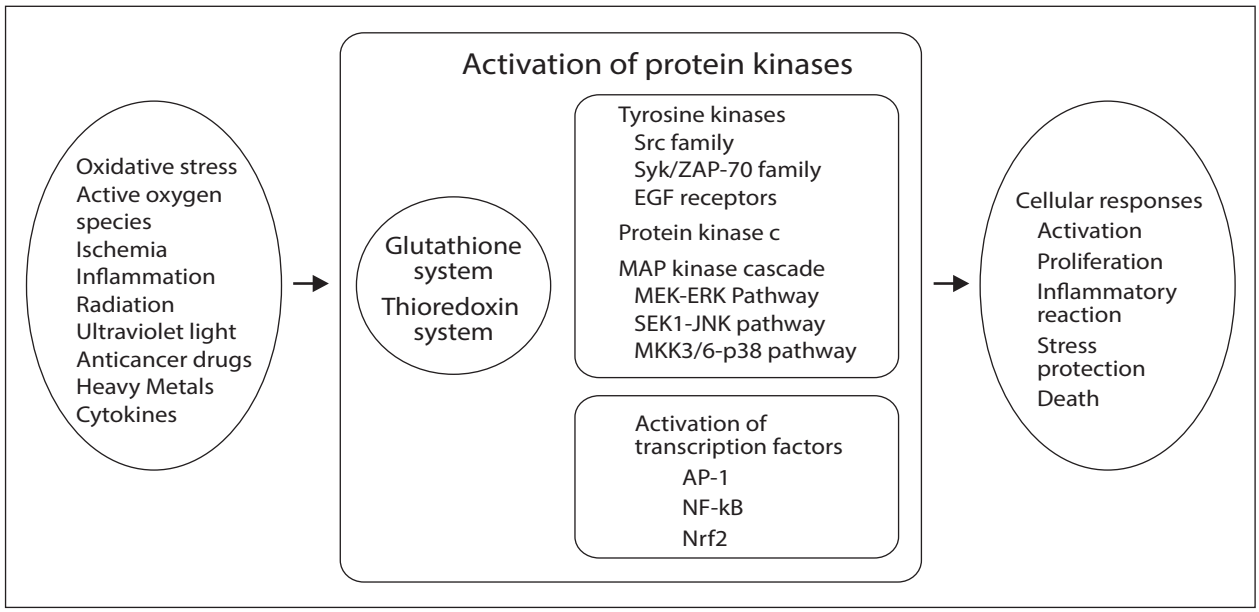

Fig.1: Oxidative stress and cellular responses (Source: Yoshikawa & Naito, 2002)[2]

is widely believed to impair normal health. The pathological consequences of hypothyroidism point to a high potential for oxidant and antioxidant imbalance. Thyroid diseases affect approximately 5% of the population in various forms. Thyroid hormones are most significant humeral factors involved in inducing the basal metabolic rate. Hypothyroidism induced dysfunction of the respiratory chain in the cell mitochondria leads to accelerated production of free radicals[1].

Oxidative stress results in an imbalance between the antioxidant defense systems and the rate of production of reactive oxygen species (ROS). 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 (Fig.1). When oxidants are produced beyond the capability of neutralization by antioxidants, cellular component are damaged, especially lipids those containing polyunsaturated fatty acids. This process, called lipid peroxidation, eventually yields several relatively stable products including aldehyde compounds 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.[2]

Monitoring of MDA levels in different biological systems can be used as an important indicator of lipid peroxidation both in vitro and in vivo for various health disorders like hypothyroidism. Determination of MDA in blood, plasma or tissue homogenates is one of the useful methods to predict the oxidative stress levels[3] and to assess prognosis of the hypothyroid patients following treatment.

The most common form of thyroid hormone (TH) replacement therapy for hypothyroid patients is synthetic T_4 hormone, which is generally known as levothyroxine (LT_4) and is used to suppress TSH. Thus, TH remains in normal range and oxidative tissue damage decreases. As a result, MDA level decreases but does not come at euthyroid state.[4] So, in the present study, malondialdehyde levels were determined as indices of lipid peroxidation in hypothyroidism patients to indirectly assess the role

of oxidative tissue damage in the pathogenesis of hypothyroidism as well as to see the changes in MDA level following treatment with levothyroxine. The study intends to test the hypothesis that malondialdehyde (MDA) level is increased and lipid profile is altered (Serum TC, LDL and TG increased and HDL-cholesterol decreased) in primary hypothyroid patients.

METHODS:

This cross-sectional analytical study was conducted in the Department of Pharmacology & Therapeutics, Rajshahi Medical College, Rajshahi in collaboration with the Institute of Nuclear Medicine and Allied Sciences (INMAS), Rajshahi over a period of 12 months between July 2018 to June 2019. A total of 40 hypothyroid patients (20 newly diagnosed primary hypothyroid and 20 receiving levothyroxine at least for 6 months) aged 18-40 years were consecutively included in the study. To compare the findings of these patients, 20 apparently healthy control subjects were included. Patients with serious comorbid diseases (DM, MI, HTN etc.), history of using drugs such as glucocorticoids, oral contraceptives or vitamin supplements were excluded from the study. Pregnant women were also refrained from participating in the study. Having obtained ethical clearance from Institutional Review Board (IRB) of Rajshahi Medical College, Rajshahi, data were collected from the recruited patients on variables of interest using a semi-structured questionnaire. The primary outcome variable was MDA, the level of which was compared among the three study groups to see the effect of LT_4 on oxidative stress. The secondary outcome variables were lipid profile which were also compared among the groups to see their levels as supportive evidences of response of hypothyroid patients to LT_4. Data were analyzed using SPSS (Statistical Package for Social Sciences), version 25.0. The test statistics used to analyze the data were descriptive statistics, ANOVA statistics, Chi-square ($\chi 2$) Test and Spearmen's correlation. Data presented on categorical scale were compared among the three groups using Chi-squared ($\chi 2$), while the data presented on continuous scale were compared among the groups using ANOVA statistics. The level of significance was set at 5% and p-value < 0.05 was considered significant.

RESULTS:

The findings of the present study, intended to compare the level of MDA and lipid profile among three groups of individuals (primary hypothyroid, LT_4-treated hypothyroid patients and normal healthy individuals) with 20 subjects in each group, are presented below:

The three groups were almost homogeneous in terms of age ($p = 0.705$). Females demonstrated their significant presence in the levothyroxine- treated and primary hypothyroid groups compared to normal healthy individuals ($p=0.017$) (Table I). One-quarter of the primary hypothyroid group was obese compared to none in the levothyroxine- treated hypothyroid group and 10% in the healthy control group. The mean BMI of primary hypothyroid group was considerably higher than that of levothyroxine-treated hypothyroid and normal healthy subjects, although the difference was not statistically significant ($p = 0.216$) (Table II).

The mean serum total cholesterol level of hypothyroid group was significantly higher than that of levothyroxine-treated hypothyroid group which was again higher than that of normal healthy individuals ($p < 0.001$). Likewise, the serum LDL & triglycerides of the hypothyroid group were significantly higher than those of levothyroxine- treated hypothyroid group which were again much higher than those of normal healthy control group ($p<0.001$ and $p<0.001$ respectively). The mean serum HDL was significantly lower in the newly diagnosed hypothyroid than that in the LT_4-treated hypothyroid subjects; the latter was again lower than that in the normal healthy subjects ($p=0.009$). 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 III). The serum TSH level of primary hypothyroid group was highest compared to that of levothyroxine-treated hypothyroid group 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 IV).

The serum TSH and MAD exhibits a significantly linear correlation (r = 0.606, p < 0.001) indicating that 60% of the variation in MDA can be explained by serum TSH (Fig.2). In contrast, the serum T_4 bears a significantly negative correlation with serum MAD 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: Distribution of patients by their baseline characteristics

Baseline characteristics	Group			*p-value
	Hypothyroid (n = 20)	LT_4-Treated Hypothyroid (n = 20)	Normal (n = 20)	
Age*(yrs)	34.1±9.2	36.7±10.8	34.5±11.3	0.705
Sex#				
Male	3(15.0)	1(5.0)	8(40.0)	0.017
Female	17(85.0)	19(95.0)	12(60.0)	

*Data were analyzed using **ANOVA statistics** and were presented **as mean ± SD**.
Data were analyzed using **Chi-square** ($\chi 2$) **Test**. Figures in the parentheses denote corresponding %.

Table II: Distribution of BMI among the study groups

BMI* (kg/m²)	Group			*p-value
	Hypothyroid (n = 20)	LT_4-Treated Hypothyroid (n = 20)	Normal (n = 20)	
<18.5 (underweight)	0(0.0)	0(0.0)	1(5.0)	
18.5-25.0 (normal)	8(40.0)	12(60.0)	10(50.0)	
25.0-30.0 (overweight)	7(35.0)	8(40.0)	7(35.0)	
≥ 30.0 (obese)	5(25.0)	0(0.0)	2(10.0)	
Mean ± SD	26.9 ± 3.4	24.2 ± 2.7	25.3 ± 3.3	0.216

Figures in the parentheses denote corresponding percentage
*Data were analyzed using **ANOVA statistics** and were presented **as mean ± SD**.

Table III: Comparison of lipid profile and MDA level among the study groups

Biochemical variables	Group			F-value p-value
	Hypothyroid (n = 20)	LT_4-Treated Hypothyroid (n = 20)	Normal (n = 20)	
Serum TC*(mg/dl))	198.5 ± 19.2	185.0 ± 30.0	165.0 ± 22.6	9.566, <0.001
Serum LDL (mg/dl)	133.9 ± 19.7	125.7 ± 34.7	85.2 ± 35.2	14.381, <0.001
Serum HDL (mg/dl)	35.1 ± 3.67	37.3 ± 9.35	49.7 ± 25.0	5.135, 0.009
Serum TG (mg/dl)	147.0 ± 14.5	124.0 ± 29.6	113.4 ± 26.1	9.985, <0.001
Serum MDA (µmol/L)	10.0 ± 2.13	5.88 ± 1.90	2.66 ± 0.61	9.133, <0.001

Figures in the parentheses denote corresponding percentage
*Data were analyzed using **ANOVA statistics** and were presented **as mean ± SD**.

Table IV: Comparison of TSH and T4 among study group

Thyroid hormone profile	Group			F-value p-value
	Hypothyroid (n = 20)	LT_4-Treated Hypothyroid (n = 20)	Normal (n = 20)	
TSH (mIU/L)	26.8 ± 20.9	5.07 ± 9.18	2.53 ± 1.55	15.23, <0.001
T_4 (µg/dl)	5.25 ± 3.83	13.5 ± 20.4	8.95 ± 3.15	1.63, 0.193

*Data were analyzed using ANOVA **statistics** and were presented as mean ± SD.

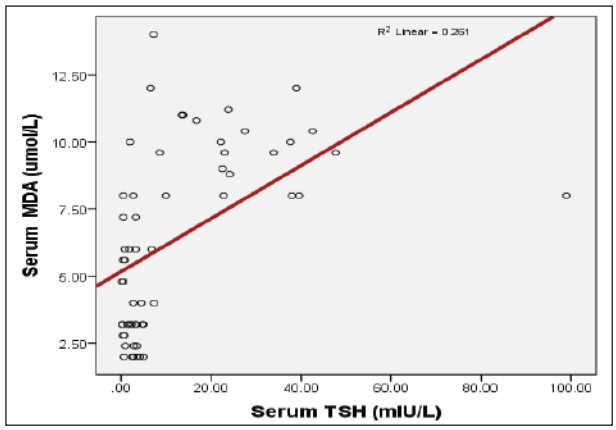

Fig. 2: Correlation between serum TSH and MDA

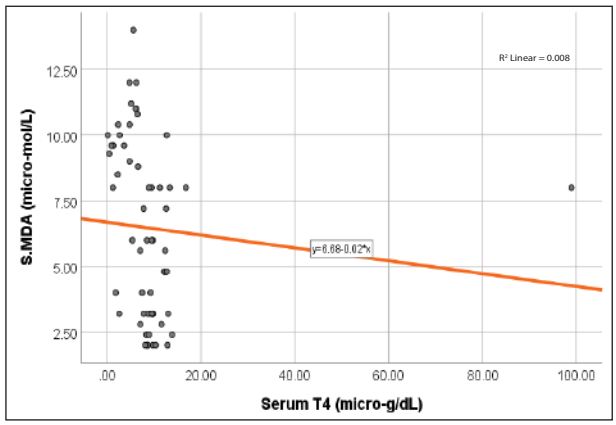

Fig. 3: Correlation between serum T4 and MDA

DISCUSSION:

Hypothyroidism is often accompanied by increased MDA level, in association with dyslipidemia which may promote atherosclerosis and may lead to coronary artery disease. Increased MDA levels is believed to be indicative of the involvement of free oxygen radicals in tissue damage. Although there is no clear evidence that hypothyroidism causes coronary artery disease, it increases the oxidation of plasma

ORIGINAL ARTICLE

cholesterol mainly because of an altered pattern of binding and due to the increased levels of cholesterol, which presents substrate for oxidative stress.

The present study demonstrated that malondialdehyde (MDA) level is increased and lipid profile is altered (Serum TC, LDL and TG increased and HDL-cholesterol decreased) in primary hypothyroid patients. The alteration of lipid profile is characterized by significantly higher levels of total cholesterol, triglycerides, LDL and reduced HDL-cholesterol compared to the control group hypothyroidism patients receiving levothyroxine and healthy controls. The findings clearly indicate that there is a relationship between hypothyroidism and dyslipidemia. The significantly higher prevalence of dyslipidaemia among the hypothyroid patients compared to that in healthy controls are in agreement with the literature reports that hypothyroidism is one the risk factor for the onset of CHD. Mutlu and associates[5] 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 the oxidative stress parameters and serum lipid levels and observed that they were higher in hypothyroid patients compared to euthyroid group. After LT_4 therapy, significant decrease in oxidative stress parameters and serum cholesterol with simultaneous increase in HDL-C level were observed. This is agreement with the present study.

In the present study the mean serum MDA level of hypothyroid patients was significantly higher than that of levothyroxine-treated hypothyroid group and the normal healthy control group. The MDA of healthy control groups was the lowest among the three groups. A study performed by Singh and Singh[1] on Indian Punjabi populations. Malondialdehyde levels were 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 levothyroxine. They found significantly decreased MDA level in LT_4 treated hypothyroid patients in comparison to newly diagnosed hypothyroids. They also found significant decrease in serum TC, TG and LDL-C levels with simultaneous increase in serum HDL-C. The significantly higher MDA levels found among hypothyroid patients, relative to the controls and hypothyroid patients under treatment appears to point towards increased lipid peroxidation as one possible pathomechanism for the increased cardiovascular risk in hypothyroid patients. The study reflects that hyperlipidaemia increases lipid peroxidation, which may be an additional factor contributing towards high cardiovascular risk in patients with hypothyroidism.[1]

The mean of MDA level of normal individuals, hypothyroid patients and LT_4 treated hypothyroid patients was 2.66 ± 0.61 µmol/l, 10.0 ± 2.13 µmol/l and 5.88 ± 1.90 µmol/l respectively. The result revealed that treatment with levothyroxine decreased MDA levels significantly but not up to the normal level. However, treatment with LT_4 can improve disease process by decreasing oxidative stress. Mean serum TSH level of normal individuals, hypothyroid patients and LT_4 treated hypothyroid patients was 2.53 ± 1.55 mIU/l, 26.8 ± 20.9 mIU/l and 5.07 ± 9 mIU/l respectively reflecting that treatment with LT_4 significantly decreases serum TSH level in hypothyroid patients thereby improving the disease process. The serum TSH and MDA exhibits a significantly positive correlation indicating that higher the TSH level the higher is the serum MDA level while the serum T_4 bears a significantly negative correlation with serum MDA level suggesting that higher the serum T_4 level, the lower is the serum MDA level. Masullo and colleagues[6] 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 the our present study.

Gluvic and associates[4] demonstrated the effect of levothyroxine replacement therapy on parameters of metabolic syndrome and atherosclerosis in hypothyroid patients. Results of their study showed that the LT_4 therapy significantly helps to correct the

risk factors like serum total cholesterol, TG and HDL-C of metabolic syndrome and atherosclerosis in hypothyroid patients, which are consistent with the findings of the present study. Bascol and colleagues[7] in their study of oxidative stress and enzymatic antioxidant status in patients with hypothyroidism found that MDA level before treatment was higher in patients with hypothyroidism than that in controls but significantly decreased after 6 months treatment with LT_4. In an attempt to find the role of antioxidant supplementation on oxidative stress in hypothyroid patients Chakraborti et al[8] conducted a study and observed that MDA level was high in newly diagnosed hypothyroid patients. After treatment by LT_4, MDA level was reduced to a great extent.

The findings of the current study have much clinical implication. Serum MDA level and lipid profile 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. Lipid profile of hypothyroid patients reflect the risk of development of cardiovascular diseases and atherosclerosis. Addition of lipid lowering agents and antioxidants can play a pivotal role in the better outcome in hypothyroid patients with conventional LT_4 therapy alone. Well-designed clinical trials are required to clarify the effect of lipid lowering agents and antioxidants supplementation in hypothyroid patients. Before concluding the study findings, the following limitations deserve mention.

Limitations:

• As the study was a cross-sectional study, it was not possible to observe the effects of LT^4 replacement therapy in the same patients over time.

• As the study was conducted in single center with small sample size, caution should be exercised to generalize the findings to reference population.

CONCLUSION:

From the findings of the study it can be concluded that that malondialdehyde (MDA) level is increased and lipid profile is altered (characterized by increase in serum TC, LDL and TG and decrease in HDL-cholesterol) in primary hypothyroid patients. The study revealed that treatment with levothyroxine decrease MDA levels significantly but not up to the normal level. The treatment improves the disease process by decreasing oxidative stress. As hypothyroidism induces dyslipidaemia or expedite the process of existing dyslipidaemia, and LT_4 therapy corrects dyslipidaemia to a significant level, the latter can be given to reduce the risk of atherosclerosis in this cohort. Supplementation with antioxidants and addition of lipid lowering drugs with traditional LT_4 treatment of hypothyroid patients should be undertaken in further studies.

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