

Research Article

Ceruloplasmin and serum MDA levels in hypothyroid patients

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Abstract

The present study was designed to investigate the relationship between serum levels of MDA which is a marker of oxidative stress and Ceruloplasmin with thyroid hormone status in hypothyroid females pre & post treatment. The study group comprised of 46 patients with primary hypothyroidism. The patients were reevaluated after 6 months of L-thyroxine therapy. The patients were compared with equal number of normal healthy controls. Serum MDA and Ceruloplasmin were measured according to an enzymatic spectrophotometric method.

Keywords: MDA, Ceruloplasmin, hypothyroid, free radicals

1. Introduction

Oxidative Stress is defined as the unbalancing between production of free radicals, molecules characterized by high reactivity due to one or more unpaired electrons in the external orbital and antioxidant defenses in the biological systems. Presently it is considered an important pathogenic mechanism in different diseases.¹

Free radicals may cause lipid peroxidation & damage to macromolecules & cellular structures of the organism, endothelium and erythrocytes. There are several biochemical markers showing the extent of ongoing oxidative stress in the body. Among them MDA is a lipid peroxidation marker used to assess oxidative stress on the other hand Ceruloplasmin (Cp) is considered as a protective antioxidant due to its ability to react and scavenge free radicals.²

Thyroid hormones have well-known effects on mitochondrial oxygen consumption but data regarding the role of hypothyroidism on oxidative stress is not adequate^{3,4}. In this study, we aimed to detect the correlation between the level of MDA, a lipid peroxidation marker and Ceruloplasmin in hypothyroidism with special reference to therapy.

1.1 Aims & Objectives

To estimate the changes in serum MDA & Ceruloplasmin levels in hypothyroid patients before & after treatment & to detect the correlation between MDA and Ceruloplasmin, in hypothyroid patients.

2. Materials & Methods

The determinations were performed on serum samples collected from 3 groups. Only female patients in the age group between 15-45 years were included in the study.

a) Adult control subjects-all female patients of similar age group.

b) Adult untreated hypothyroid subjects

c) Adult hypothyroid subjects after 6 months of treatment. All the patients were treated with Levothyroxine in varying doses (50-150µgm) according to the thyroid status. All the patients were attending the Biochemistry & Endocrinology Departments of IPGME & R for investigations & treatment.

Blood samples were collected by venous puncture in additive free vials and the serum was separated by centrifugation at 1000g for 15 minutes.

The levels of serum thyroid stimulating hormone (TSH), total triiodothyronine (T3), free thyroxine (FT4), and free triiodothyronine (FT3) were measured by ELISA method.

MDA was measured in serum by the method based on Pasha & Sadasivadu procedure⁵. MDA reacts with Thio barbituric acid to generate a coloured product, which can be measured spectrophotometrically. In acidic solution, the product absorbs light at 530 nm. TBA test detects only free MDA and measures the amount of free MDA in peroxidising lipid system. The molar extinction coefficient of MDA-TBS product is 1.54×10^5 at 530 nm and it is used to calculate the amount of MDA formed.

Reagents needed

40% Trichloroacetic acid (TCA), 67% Thiobarbituric acid (TBA)

1 ml of serum added to 1 ml of 40% TCA followed by addition of 2 ml 0.67% TBA. The mixture was then kept for 10 min. in a boiling water bath. It was cooled immediately in ice cold water bath. The mixture was then centrifuged at 6,000rpm for 30 s and absorbance of the supernatant was read at 530 nm.

$$E = KCL \text{ Or } C = E/KL \text{ nmol/dl}$$

Where K=Molar extinction coefficient (extinction offered by 1 M solution) i.e., 1.5×10^5 .

E=Extinction /absorbance; C=Concentration in moles/litre; L=Length of cuvette used (1 cm)

Ceruloplasmin is a copper containing enzyme which has Oxidizing activity. Its catalytic power to oxidize p-phenylenediamine is used to measure its activity in serum⁶. Heparinised/oxalated plasma may also be used. Dark lavender colour was read at 530 nm using control tube as blank. Concentration of Ceruloplasmin in mg/dl is absorbance x87.5.

1. 1 M Acetate buffer, 2. Sodium azide solution 0.5% in water.

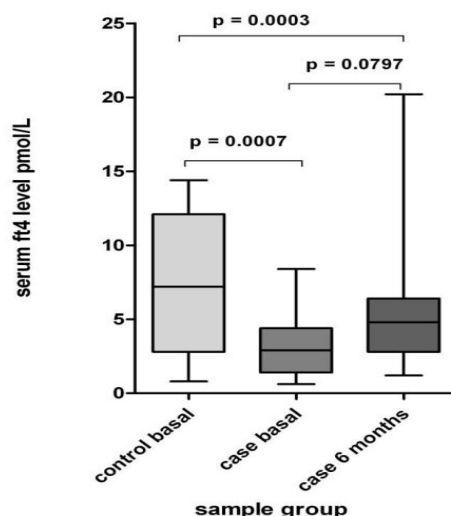
3. P-Phenylenediamine hydrochloride solution, 0.5% in water. .

Perform 2 series of tests, one with test serum and another with normal serum. For each series arrange 3 tubes marked T1, T2 and B for blank, duplicate test done for all the test samples. Then 0.1 ml of serum was taken in 3 test tubes marked as T1, T2 and B. 0.1 ml of sodium azide is added in the test tube marked as B. 8 ml of acetate buffer was added to all the 3 tubes 0.1 ml of P-Phenylyne diamine solution was added to all the 3 test tubes. The solutions were well mixed and kept at 37°C for 1 hour. Then 1 ml of Sodium azide solution was added to all the test tubes. They were shaken well and kept at 4° to 10°C for 30 minutes. Then T1 and T2 against B were read with yellow green filter (530nm). The mean of the 2 readings were taken and the result of the test series was compared with that of the series with normal serum. Normal values 0.2-0.5 (normal O.D)

3. Results

Box and Whisker plot of figure.1 represents median along with minimum to maximum range of serum ft4 level among control and in case subjects at baseline and after 6 months of therapy.

Figure-1: Showing represents median along with minimum to maximum range of serum ft4 level among control and in case subjects at baseline and after 6 months of therapy

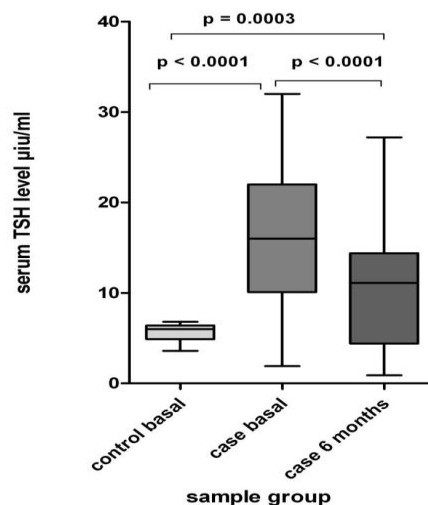


Serum ft4 was diminished remarkably among case subjects compared to control subjects at basal level(p=0.0007). Moreover, the level remains still lower after 6 months among case subjects compared to control individuals(p=0.0003).

But, basal level and after 6 months value of serum ft4 remains insignificant among case subjects (p=0.0797).

Box and Whisker plot of figure.2 represents median along with minimum to maximum range of serum TSH level among control and case subjects at base line and after 6 months only in case of case subjects.

Figure-2: Showing represents median along with minimum to maximum range of serum TSH level among control and case subjects at base line and after 6 months only in case of case subjects

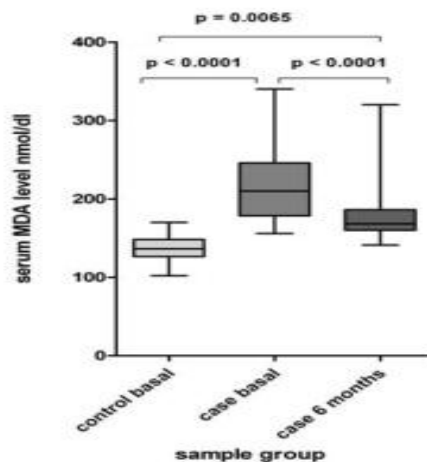


Serum TSH was high in cases when compared to control at basal level (p<0.0001).

Serum TSH was significantly high in patients at basal level compared to those after 6 months of Eltroxin Therapy ($p < 0.0001$) indicating a decrease post treatment.

Box and Whisker plot of figure.3 represents median along with minimum to maximum range of serum MDA levels among control and case subjects at baseline and after 6 months only in case of case subjects .

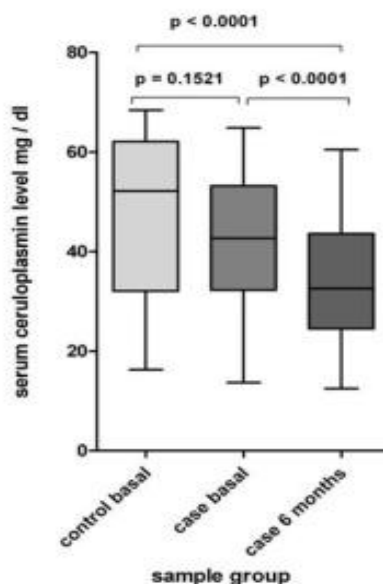
Figure-3: represents median along with minimum to maximum range of serum MDA levels



Serum MDA levels at base levels of cases were significantly higher in comparison to controls at basal levels ($p < 0.0001$), whereas serum MDA values of cases at 6 months of therapy was significantly lower than the MDA values at basal levels of cases ($p < 0.0001$).

Box and Whisker plot of figure.4 represents median along with minimum to maximum range of serum Ceruloplasmin levels among control and case subjects at baseline and after 6 months only in case of case subjects.

Figure-4: Showing median along with minimum to maximum range of serum Ceruloplasmin levels among control and case subjects at baseline and after 6 months only in case of case subjects.



Serum Ceruloplasmin was diminished among case subjects compared to control subjects at basal level but statistically not significant ($p = 0.1521$). But, basal level and after 6 months value of serum Ceruloplasmin remains significant among case subjects ($p < 0.0001$).

The figure is indicating a further decrease in Cp levels in cases after 6 months when compared to cases basal level- mention the findings and explain the decrease in discussion also.

4. Discussion

ROS have been reported to induce oxidative damage to membrane lipids, proteins, and DNA and might result in cell death by necrosis or apoptosis⁷.

Ceruloplasmin (Cp) is a α_2 -Globulin that contains approximately 95% of the total copper found in serum. The primary physiological role of Cp involves plasma redox reactions. It can function as an oxidant or antioxidant depending on other factors, such as the presence of free ferric ions and ferritin binding sites. Ceruloplasmin is also important in the control of membrane lipid oxidation – probably by direct oxidation of cations – thus preventing their catalysis of lipid peroxidation⁸.

Ceruloplasmin is a major defense against harmful effects of ROS in cells and in cultured erythrocytes, with a high capacity to degrade exogenous hydrogen peroxide⁸.

The extent of lipid peroxidation marker MDA was significantly increased in hypothyroid patients when compared to healthy controls. Resch *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 atherosclerosis⁹.

It has been suggested that hypothyroidism leads to oxidative stress and to a reduction of antioxidant defenses although the pathophysiological consequences of the decelerated antioxidant levels are not yet elucidated. This biochemical change is thought to be a physiological adaptation and a response to hypothyroidism. In agreement with previous findings, thyroid hormones are involved in combating the toxicity of oxidative stress in humans¹⁰. 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.

The depletion of antioxidants observed in hypothyroid individuals may reflect 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, due to a decrease of antioxidants like Ceruloplasmin. A high oxidative state in hypothyroid people has metabolic and biochemical characteristics such as increased mitochondrial enzyme activity. Thus, it is likely that patients' cells are damaged by prolonged oxidative stress that far exceeds the capacity of the patients' organs to synthesize antioxidant molecules or to synthesize them from extracellular sources¹¹.

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.

Finally, from all the aforementioned observations it can be concluded that increased generation of reactive oxygen species and concomitant impairment of the antioxidant system occurs in patients with hypothyroidism. These findings indicate that thyroid hormones have a strong impact on oxidative stress and the antioxidant system.

In our study, the mean MDA was high in hypothyroid patient compared to control group similar to that seen in study by Dumitriu. While ceruloplasmin was significantly lower than in controls. It was concluded that in hypothyroid patients, even after treatment for 6 months, a still raised levels of lipid peroxidation and its consequences are probably aggravated by the low serum Cp level. Hypothyroid patients' level of MDA was higher than that of healthy subjects. Our data showed a significantly decreased Ceruloplasmin activity in hypothyroid patients compared to controls.

Can treatment affect oxidative stress? -explain whether your findings are in line with findings of other studies post treatment and the effect of treatment in hypothyroidism in context of oxidative stress.

our study was done only for a short period of 6 months but ' long term treatment with antioxidants along with levothyroxine therapy will definitely be beneficial as thyroid hormones have a strong impact on oxidative stress and the antioxidant system giving rise to higher Ceruloplasmin level and simultaneous lowering of serum MDA.

5. Conclusion

It will be a better study if we use a larger population for a longer period of time with substitution of antioxidants like vitamin E in hypothyroid patients along with thyroid hormones to know whether it will be beneficial to combat the stress caused by hypothyroidism,

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