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Original Research Article

# A STUDY OF OXIDATIVE STRESS AND STATUS OF SERUM ZINC & COPPER IN SUBJECTS WITH HYPOTHYROIDISM

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## Abstract

**Background:** Hypothyroidism is a common endocrinopathy which remains associated with free radical generation resulting in oxidative damage to various organs. Zinc and Copper are two essential trace elements for the human body, principally because of its role in antioxidant mechanism. Since oxidative damage has been reported to be related to the development of complications of hypothyroidism, it is prudent to identify the condition, in an attempt to retard the progress of the complications of hypothyroidism. The aim & objective is to estimate serum Malondialdehyde (MDA), Zinc and Copper levels in Primary hypothyroid subjects and compare it with euthyroid subjects. **Materials and Methods:** A cross sectional observational study was conducted in Dept. of Biochemistry, Nalanda Medical College, Patna, Bihar from March 2022 to February 2023. The study recruited 80 primary hypothyroid subjects and 80 age and sex matched healthy controls. Serum TSH and T4 was measured by Chemiluminescence method, while MDA, Zinc and Copper were measured in spectrophotometer. **Result:** Serum MDA was significantly higher in Primary hypothyroid (7.00 ± 3.93) nmol/ml in comparison to controls (1.88 ± 0.56) nmol/ml. Values of serum zinc and Copper found to be significantly lower in Primary hypothyroid cases compared to euthyroid subjects. (P value < 0.05). **Conclusion:** In this study it was observed Serum MDA was significantly higher in Primary Hypothyroid subjects along with decreased Zinc and Copper level. Considering the results of this study as well as those done earlier, underlying oxidative damage needs to restricted. The potential benefits of supplementing Zinc and Copper in Primary Hypothyroid subjects needs to be evaluated further.

## INTRODUCTION

Hypothyroidism is a very common Endocrinopathy worldwide including India where about 42 million Indians are reported to be suffering from spectrum of thyroid disorder, mainly hypothyroidism.[1] The physiological effects of thyroid hormones is primarily related to functions of all the tissues of body involving modulation of gene transcription resulting in alteration of basal metabolic rate and energy metabolism affecting carbohydrate, lipid and amino acid metabolism.[2]

Free radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism Free radicals may contribute towards lipid per oxidation and damage macromolecules and cellular structure of the organism, endothelium and erythrocytes. Serum Malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of lipid per oxidation.[3,4] Free radicals are eliminated from the body by their interaction with various non-enzymatic and enzymatic antioxidants such as uric acid, albumin, bilirubin, vitamins E, C, A, Glutathione peroxidase, Superoxide dismutase (SOD) and Catalase.[5] Previous clinical and experimental studies showed an altered free radical level (with different results) in hypothyroidism. Some of the studies showed an increase,[6,7] while some other showed decrease,[8] or no significant differences.[9,10] Micronutrients like zinc, copper and selenium are also important molecules involving in removal of free radicals through various mechanism namely antioxidant enzyme like Superoxide dismutase, Catalase, Peroxidase etc. Defence against free radical toxicity is related to zinc as a component of SOD.[11] There are reports suggestive of transport of zinc from the intestine & renal tubules are dependent on thyroid hormones as observed in rat model.[12] Copper in


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excess is found to be related to oxidative injury in other study.[13]

**Aim & Objective**

The aim of this study was to investigate the effect of hypothyroidism on lipid per oxidation and evaluate possible links between trace element concentrations (copper, zinc) and thyroid hormones in comparison to euthyroid subjects.

## MATERIALS AND METHODS

This was a cross- sectional hospital-based study carried out over 12 months from March 2022 to February 2023 in the Dept. of Biochemistry, Nalanda Medical College, Patna, Bihar. The study commenced after obtaining proper Institutional Ethics Committee approval.

The selected cut-off value for accepting subjects under the study on Hypothyroidism was: TSH above 10.0 µIU/ml and FT4 below 0.8ng/dl. (Williams). Patients suffering from Diabetes Mellitus, chronic renal diseases, chronic infection, pregnancy and patients taking Antioxidant, Zinc or Copper supplementation were excluded from the study

**Inclusion Criteria**

Patients diagnosed with TSH above 10.0 µIU/ml and FT4 below 0.8ng/dl.

**Exclusion Criteria**

Patients suffering from Diabetes Mellitus, chronic renal diseases, chronic infection, pregnancy and patients taking Antioxidant, Zinc or Copper supplementation were excluded from the study

The required sample size was calculated as 80 cases and 80 controls rounding to the next nearest number, using the formula suggested for case-control studies.

Primary Hypothyroidism is very common where there is low FT4 and high TSH. Secondary Hypothyroidism is less common variety where levels of TSH & FT4 decreases. Primary hypothyroid subjects in the age group between 18-70 years and age and sex matched normal subjects were selected after obtaining informed consent from all the subjects. Each individual enrolled in the study underwent a detailed history, clinical examination and laboratory investigation designed for the study. Five ml of fasting blood sample was collected from each individual in sterile plain vial. The blood was allowed to clot and then centrifuged at 3000 rpm for 10 minutes. Serum was separated and stored at -20˚C until analysis. Serum TSH and fT4 were measured by Chemiluminescence method (Siemens Immulite 1000). Serum Zinc and Copper was estimated using commercial kits in semiautoanalyser (ERBA Chem. V+ v2). Serum MDA assay based on its reaction with thiobarbituric acid by SATHO and others (14) in double beam UV-VIS Spectrophotometer (EC-PCI). Data was analysed using Microsoft office Excel and SPSS 20.0 software.

## RESULTS

The required sample size was calculated as 80 cases and 80 controls rounding to the next nearest number, using the formula suggested for case-control studies. There were 28 (56%) females and 22 (44%) males in the cases, whereas 26(52%) females and 24 (48%) males in controls.

**Table 1: Distribution of patients according to sex.**

| Sex | Test group | Percentage | Control group | Percentage | p value |
|---|---|---|---|---|---|
| Male | 34 | 42.5% | 38 | 47.5% | 0.212 --? |
| Female | 46 | 57.5% | 42 | 52.5% | |
| Total | 80 | 100% | 80 | 100% | |

**Table 2: C Baseline and demographic characteristics of the study group.**

| Biochemical Tests | | Test group (n=80) | Control group(n=80) |
|---|---|---|---|
| MDA | Mean±SD | 7.00±3.93 | 1.88±0.56 |
| | Median | 6.58 | 1.96 |
| | IQR | 4.1 | 0.75 |
| Zn | Mean±SD | 46.59±31.88 | 148.68±70.67 |
| | Median | 36.62 | 111 |
| | IQR | 55.66 | 99 |
| Cu | Mean±SD | 105.54±67.16 | 130.65±30.40 |
| | Median | 72.56 | 133 |
| | IQR | 112.40 | 22 |

SD= Standard deviation, IQR= Inter quartile range Table 1 describes the central tendency of MDA, Zinc & Copper levels in the two groups studied.

**Table 3: Test of normality for data of MDA, Zn and Cu level.**

| | Kolmogorov-Smirnova | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statics | df | Sig. | Statics | df | sig |
| MDA | 0.179 | 150 | 0.000 | 0.816 | 150 | 0.000 |
| Zn | 0.161 | 150 | 0.000 | 0.880 | 150 | 0.000 |
| Cu | 0.083 | 150 | 0.006 | 0.945 | 150 | 0.000 |

A. Lilliefors Significance Correction


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The results of both the normality tests, Kolmogorov-Smirnov and Shapiro-Wilk, are significant for all three data sets, i.e. MDA, Zn and Cu levels. These indicate that, these data are not normally distributed. So, parametric test like unpaired t-test were not done to compare the values of these three parameters between test and control groups. Instead, nonparametric test (MANN WHITNEY U TEST) was done.

**Table 4: Hypothesis test summary for MDA, Zn and Cu level across test and control groups.**

| S.No | Null Hypothesis | Test | Sig. | Decision |
|---|---|---|---|---|
| 1 | The distribution of MDA is the same across categories of group | Independent-sample Mann-Whitney U Test | 0.000 | Reject the null hypothesis |
| 2 | The distribution of Zn is the same across categories of group | Independent-sample Mann-Whitney U Test | 0.000 | Reject the null hypothesis |
| 3 | The distribution of Cu is the same across categories of group | Independent-sample Mann-Whitney U Test | 0.000 | Reject the null hypothesis |

**The significance level is 0.05**

As the data is not normally distributed as previously shown in [Table 2], nonparametric test (Mann-Whitney U test) was performed to check whether there is significant difference between two groups. It has been found that there is significant difference in distribution of MDA, Zn and Cu levels between the two groups (p value <0.05).

## DISCUSSION

The data presented in this study show that lipid per oxidation is markedly higher in hypothyroidism as compared to euthyroid subjects (p value < 0.05). In previous studies, different interpretations were given. Venditti et al,[15] in an experimental study on hypothyroid rats showed that Malondialdehyde (MDA) levels did not differ significantly from euthyroid values. Mano et al,[16] found that the concentration of lipid peroxides did not change in hypothyroid rats in comparison with euthyroid animals. Gredilla et al,[17] demonstrated that in vivo and in vitro lipid peroxidation was not altered in the hypothyroid state. Dumitriu et al,[18] showed that the mean malondialdehyde level was significantly higher in hypothyroid patients in comparison to the control group. Yilmaz et al,[19] showed that MDA level of hypothyroid rats was increased in liver, but levels were decreased in the tissues of the heart and thyroid. Sawant et al,[20] demonstrated that the tissue lipid peroxidation level significantly increased in hypothyroid rats. The result of our study is in conjunction with the results of studies showing that the level of MDA is significantly increased.[17,21] But present results are not in agreement with the other studies.[10,16,17,19] The increase in reactive oxygen species induced by thyroid hormone may contribute towards an oxidative stress condition in some tissues with a consequent lipid peroxidative response. Possible sources of elevated free radicals in hypothyroid patients include increased production of radical oxygen species, especially from lipid peroxidation processes and probably decreased antioxidant defence systems.

In our study significant low Zinc levels were observed in hypothyroid subjects as compared to euthyroid subjects. This is in support with other studies.[22,23] This could be possibly caused due to either impaired gastrointestinal absorption of zinc or altered zinc distribution leading to sequestration of zinc in liver and other tissues in hypothyroid subjects. Oliveri et al,[24] reported that thyroid hormones did not correlate with indices of zinc status in hypothyroid subjects; although they had observed decreased iodothyronine levels in case of zinc deficiency.

Copper deficiency can exert both a direct effect on the metabolic process and an indirect one disturbing iodine metabolism, and sharply decreasing protein-bound iodine production by the thyroid gland. Our results showed statistically significant decreased serum Cu levels in patients with hypothyroidism when compared with control group (P < 1%), which is diffusible.[20,21] Thyroid hormones enhance the synthesis of lysosomal enzymes in muscle and are necessary for the catabolic response to a variety of stimuli in this tissue and it also contribute towards increase in the concentration of free amino acids in plasma.[28,29] These findings may provide an explanation for our data that concentrations of serum Cu was lower in patients with hypothyroidism; however we could not estimate plasma ceruloplasmin levels and erythrocytes Cu concentrations.

## CONCLUSION

So, the observed findings hypothesize that Hypothyroidism is often associated with deficient Zinc and Copper levels in serum and the index of Oxidative stress is also significant. It can be concluded that hypothyroidism is associated with Oxidative Stress as also too decreased zinc and copper levels.

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