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Original Article

Comparative Evaluation of Oxidative Stress Among Hyper and Hypothyroidism in a Tertiary Care Hospital at Kolkata

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ABSTRACT

Background: A large number of patients are regularly attending the thyroid outdoor department. Many of them are suffering from complications of thyroid disorders. Oxidative stress among hyper and hypothyroid patients may be a causative factor for thyroid related complications. **Materials and methods:** 40 hyperthyroid, 40 hypothyroid and 40 age and sex matched control subjects were included in the study. Malondialdehyde, a lipid peroxidation product, and protein carbonyl, a protein oxidation product were estimated in them spectrophotometrically. **Statistical analysis used**- Is done by SPSS 20 software for windows. **Results-** ANOVA shows significant increase ($p<0.001$) of oxidative stress parameters among the three groups. Bonferroni correction reveals significantly higher values of those parameters in hyperthyroids from hypothyroid and control groups ($p<0.001$) The hypothyroid patients had also significantly higher values than controls ($p<0.0001$). **Conclusion:** Hyperthyroidism produces more oxidative stress than hypothyroidism. Assesment and control of oxidative stress at early stage of hyper and hypothyroidism can prevent or delay the grave consequences by the same.

Keywords: Hyperthyroidism, Oxidative stress, carbonyl

1. INTRODUCTION

Thyroid diseases are the commonest endocrine disorders in the world. The prevalence is obvious, in India also. It has been estimated that about 108 million people in India are suffering from endocrine and metabolic disorders, of which thyroid disorders contribute about 42 million.[1] Oxidative stress (OS) is a state of disequilibrium between the free radical

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generation and antioxidant status. It has been described in several pathologies including thyroid disease.[2] Free oxygen radicals is responsible for oxidative damage of the molecules, furthermore they have a role in the etiopathogenesis of several diseases including neurodegenerative disorders, diabetes mellitus, cardiovascular diseases and different cancer types. The reactive structure of oxygen participates in autoimmune diseases of endocrine glands like some thyroid diseases. Grave's disease is one of them. Oxidative stress plays a major role in the pathogenesis of this disease. [3]

The oxidative damage of membrane phospholipids (lipid peroxidation) was observed in the liver cell, and was associated with the action of the thyroid hormone.[4] Some authors already suggested that the hypermetabolic state in hyperthyroidism is associated with increased free radical production and lipid peroxide levels. [5, 6, 7] Results on hypothyroidism, however, yield conflicting views. Some researchers concluded that the hypometabolic state induced by hypothyroidism is associated with a decrease in free radical production [4, 8, 9] and in lipid peroxidation products,[10] but the opposite trend,that hypothyroidism does induce oxidative modification of proteins and lipids, is also increasing.[11, 12, 13]

Once the oxidative damage sets in, a chain of events follow, ultimately leading to cellular derangements. Elevated levels of Reactive Oxygen Specis (ROS) cause severe damage to cellular proteins and membranes as well as to nucleic acids. They damage the cell membranes and membranes of cellular organelles by causing peroxidation of membrane lipids.[14, 15] Modification of proteins by ROS leads to denaturation of critical enzymes.[16, 17] Strand breaking in DNA by ROS leads to mutagenesis and significantly affects gene expression.[18, 19]

Accumulating all these facts,we can assume that variations in the levels of thyroid hormones may contribute to cellular oxidative stress. A study conducted regarding possible mechanisms of cardiovascular derangements in thyroid disorder states that hyper and hypothyroidism induce cellular events that play a significant role in the development and progression of myocardial remodelling and heart failure.[20]

Oxidative stress markers therefore will give an idea about different organ affections due to thyroid disorders. Hence, periodic measurements of a few oxidative stress markers can enable us to monitor the overall picture of thyroid disorder-induced complications.The protein damage by oxidative stress is reflected by protein carbonylation products. Lipid damage is evidenced by Malon-di-aldehyde (MDA), the major component of the thiobarbituric acid reacting substances (TBARS).

MDA and protein carbonyls are already reported to be significantly raised in a wide array of diseases that precipitate oxidative stress e.g. Diabetes,[21] Alzheimer's disease [22] and Psoriasis [23]

We could find only a few studies related to oxidative stress induced damages to cellular proteins and membrane lipids in thyroid disorders that have been carried out in India.[24, 25, 26]

Under these circumstances much scope remains for research in this field, especially in India.

The estimation of oxidative end-products like MDA and protein carbonyl is easy, cost-effective, and applicable for extensive use in Government setups, where the majority of the patients come from a low socio-economic background.

Considering all these facts, we estimated MDA and protein carbonyl in hyperthyroid and hypothyroid patients, and compared the same with controls.

2. MATERIALS AND METHODS

Study design- Cross sectional, observational study without any type of intervention.

Duration of study- The duration of study was two months (1.7.2013 to 31.8.2013).

Selection of cases and controls- A total of 40 hyperthyroid (16 males and 24 females, aged 30 to 60 years) and 40 hypothyroid (16 males and 24 females, aged 30 to 60 years) patients were selected for the study. Written informed consent was taken from them. 40 age and sex matched controls were also selected, with consent, from apparently healthy persons who were not suffering from any diseases.

Inclusion criteria: Selection was done according to method of convenience. All patients for estimation of thyroid status according to the advice of physicians and Surgeons of different departments of the institution.

Exclusion criteria:

a) Patients suffering from any other endocrine disorder like diabetes mellitus.

b) Any other disease which may cause increased MDA or protein carbonyl levels.

c) Antenatal mothers and psychiatry patients.

d) Smokers and tobacco chewers.

Ethical Clearance- Before commencement of the work, Ethical Clearance was obtained from the Institutional Ethics Committee, according to the Helsinki Declaration. Written informed consent was taken from cases and control subjects.

Methods for analysis of test parameters: The assessment of cases and controls was done under 3 headings, history,clinical examination, and biochemical assay. For biochemical assay, 5 ml of blood from the subjects was collected aseptically using standard protocols. The serum was separated by centrifugation (3000 rpm for 5 min) immediately and analysis was done. 20 µl serum was used for total protein estimation by Biuret method. The rest was used for the estimation of MDA and protein carbonyl.

Estimation of MDA by Thiobarbituric Acid Reactive Substance (TBARS) assay method. [27] Protein is precipitated from serum, treated with H_2SO_4 and boiled. Next colour is extracted by adding butanol and centrifuged.The OD of supernatant was measured (532 nm).Concentration was calculated in nmol/ml.

Estimation of Protein carbonyl by DNPH spectrophotometric method. [28] Protein is precipitated from serum and centrifuged.DNPH is added to precipitate.After incubation and repeated mixing,TCA is added and centrifuged.Then the precipitate was washed,incubated and centrifuged.OD of supernatant was measured(370nm) against 2M HCI as blank.

The conversion of unit from nmol/L to nmol/ mg protein was done using the formula: Carbonyl content (nmol/mg of protein)= carbonyl nmol/ml / protein mg/ml

Total protein was estimated by Biuret method. [29]

Statistical Analysis : comparison of mean values of test parameters in three groups was done using Analysis of Variance Test (ANOVA), with Bonferroni correction. Analysis was done by SPSS software.

Table 1: Descriptive Statistics of the study parameters

	N	Minimum	Maximum	Mean	Std. Deviation
MDA in control group	35	4.12	8.84	5.9743	1.25110
PC in control group	35	.43	1.40	.8000	.20580
MDA in hyperthyroid	40	24.58	50.04	36.5565	5.43710
PC in hyperthyroid	40	2.10	4.55	3.2558	.62173
MDA in hypothyroid	40	18.14	42.50	31.8285	5.54580
PC in hypothyroid	40	1.74	4.10	2.8815	.67033
Valid N (listwise)	35				

Table 1:- shows mean values of MDA and PC in controls,hypothyroids and hyperthyroid patients. MDA is measured in nmol/ml whereas PC is measured in nmol/mg of protein.

Table 2: Group Statistics (ANOVA) among the hypothyroid, hyperthyroid and control groups.

		Mean Square	F	Sig.
Protein carbonyl in nmol/mg of protein	Between Groups	64.181	213.059	p<0.001
	Within Groups	.301		
MDA in nmol/ml	Between Groups	9917.233	461.723	p<0.001
	Within Groups	21.479		

ANOVA shows significance of difference between controls, hypothyroids and hyperthyroid groups (p<0.0001)

Table 3: Post hoc ANOVA with Bonferroni correction was done among the hypothyroid, hyperthyroid and control groups.

(I) grouping	(J) grouping	Protein carbonyl.			MDA		
		Mean Difference (I-J)	Std. Error	Sig	Mean Difference (I-J)	Std. Error	Sig
.00	1.00	-2.08150*	.12631	.000	-30.58221*	1.07268	.000
	2.00	-2.45575*	.12703	.000	-25.85421*	1.07268	.000
1.00	.00	2.08150*	.12631	.000	30.58221*	1.07268	.000
	2.00	-.37425*	.12198	.008	4.72800*	1.03631	.000
2.00	.00	2.45575*	.12703	.000	25.85421*	1.07268	.000
	1.00	.37425*	.12198	.008	-4.72800*	1.03631	.000

The mean difference is significant at the 0.05 level.

Grouping : Control -.00
Hypothyroid -2.00
Hyperthyroid -1.00

To analyse the difference between individual group,post hoc ANOVA with Bonferroni correction was done.

3. RESULTS

Results showed that the MDA and PC values were highest in hyperthyroid group,and significantly higher from hypothyroid and control group.The hypothyroid patients had also significantly higher MDA and PC values than controls.

4. DISCUSSION

We observed significant rise of MDA and PC in hyperthyroid patients in comparision to controls and hypothyroids. Significant rise of the same parameters were also found in hypothyroids when compared to controls (Table 3)

Hyperthyroidism leads to hypermetabolic state which ultimately gives rise to increased free radical production and lipid peroxide levels, suggested by some authors. [5, 6, 7] Our study corroborates with them. Studies on hypothyroidism, however, yield variations. Some studies concluded that the hypometabolic state induced by hypothyroidism is associated with a decrease in free radical production[8, 9, 10] and in lipid peroxidation products, but the opposite trend was also observed we found increased oxidative stress in hypothyroidism as indicated by increased MDA and protein carbonyl adducts.[12, 13]

The oxidative damage to lipids and proteins in hyperthyroidism is contributed by the increased mitochondrial Reactive Oxygen Species (ROS) generation due to the enhanced levels of electron carriers, by which hyperthyroid tissues increase their metabolic capacity. [30, 31]

In hyperthyroidism,the events of biochemical changes happen due to thyroid hormones increase tissue susceptibility to oxidative challenge, which exacerbates the injury and dysfunction in stressful conditions. However, antioxidant defence system showed controversial results. On the other hand, previous articles differ in their opinions regarding the exact mechanism of oxidative stress in hypothyroidism. Hypothyroidism-associated oxidative stress is a consequence of increased production of free radicals and reduced capacity of the anti-oxidative defence stated by some authors. [32] Hypothyroidism decreases mitochondrial inner membrane cation permeability [33], thus significantly reducing the “proton leak” from mitochondria. Since a major function of proton leak is to decrease the production of harmful free radicals [34],

thyroid hormone deficiency will result in free radical excess and subsequent oxidative stress. Metabolic disorder from autoimmune-based hypothyroidism can also increase oxidative stress. [35]

It is now understood that hypothyroidism is characterized by reduced oxidative metabolism and markedly increased lipid and lipoprotein plasma levels due to metabolic suppression caused by lower thyroid hormones. [4, 36, 37] Hypothyroid patients also show significant higher Low Density Lipoproteins (LDL) content in the lipid peroxides and higher oxidation rate. In one study, in comparison to normal subjects, there was a significant reduction in the Vitamin A content and elevation of β-carotene levels, which can be accounted by a blockage of β-carotene conversion to Vitamin A due to lack of thyroid hormones.[38] As a result of this possible pro-oxidant activity due to elevated β-carotene LDL content and lack of effective antioxidant protection, the higher-than-normal LDL oxidation in hypothyroid patients can be explained. [39] Hypothyroid patients also suffer from hypercholesterolemia due to decreased fractional clearance of LDL by a reduced number of LDL receptors, in addition to decreased receptor activity.[40] There is evidence of lowering oleic to linoleic acid ratio which is inversely proportional to oxidative stress in altered thyroid states and could account for the high oxidation rates. [41]

A study conducted on the possible mechanisms of cardiovascular derangements in thyroid disorder states that hyper- and hypothyroidism induce cellular events that play an important role in the development and progression of myocardial remodelling and heart failure. [20]

Studies indicated that the tissues in the body vary in terms of their susceptibility to the thyroid disorder induced oxidative stress. In one particular study, male wistar rats (60 days old), were randomly assigned to one of three groups: euthyroid, hypothyroid, and hyperthyroid. Hypo and hyperthyroidism were induced. In tissues of hypothyroid rats the lipid peroxidation was not modified, whereas in hyperthyroid rats, lipid peroxidation increased in liver and heart but not in skeletal muscle. In all rat groups the whole antioxidant capacity of tissues decreased, but significantly only in liver and heart. The results obtained, after studying the response to oxidative stress in vitro indicated that the susceptibility to oxidative challenge was increased in all tissues of hyperthyroid rats and in heart and muscle of hypothyroid animals. These results are explainable in terms of tissue variations in haemoprotein content and/or of antioxidant capacity. Since it has been reported that hypothyroidism offers in vivo protection against free radical damage, the study suggested that such an effect could be due to greater effectiveness of cellular defence systems different from antioxidant ones.[42, 44]]Lack of similar experiments on the human population limits our knowledge of the existence of a similar phenomenon among mankind. Furthermore these facts can explain our observations where hyperthyroid patients showed significantly higher MDA and PC levels than hypothyroids and controls.

ROS generation was found to be 3-folds higher in hyperthyroids as compared with euthyroids and hypothyroids in one study and this was not found to be gender specific.[43]

5. CONCLUSIONS

Our study proved that oxidative stress parameters like Protein carbonyls and MDA are significantly elevated in hypo and hyperthyroidism, those parameters were significantly increased in hyperthyroidism than hypothyroid patients and controls, whereas significant increase of same parameters were found in hypothyroid patients when compared to controls. This study was done in Calcutta National Medical College and

Hospital, Kolkata, which serves the people of low socio-economic status. The patients lacked proper education and health awareness. Regular estimations of those parameters may be done for evaluation of their oxidative status. Such an intervention could prove to be quite beneficial, since it is an indicator of ongoing oxidative damage in the body, and prompts a system-wise evaluation. In our opinion,analysis of oxidative stress parameters which are not too costly, have significant relevance to healthcare of India; as thyroid disorder is a common occurrence in our country.

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