

Research Article

Protein Oxidation in Hypothyroidism: A Prospective Study in Eastern India

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Abstract: Prevalence of subclinical hypothyroidism is increasing in community. Oxidative stress is a common occurrence in hypothyroidism. Studies regarding oxidative stress in subclinical & overt hypothyroidism are limited and controversial. Aim of the study was to estimate and compare the malondialdehyde (MDA) and protein carbonyl (PC) as oxidative stress parameters, in control, subclinical and overt hypothyroid groups, furthermore to find out any correlation that exist between the same parameters. 71 hypothyroid patients (36-subclinical, 35-overt hypothyroidism) were selected for the study. Age and sex matched control subjects were also selected. Hypothyroid patients were selected on the reports of hormonal assay (TSH & T₄). MDA was estimated by TBARS assay method spectrophotometrically and PC by DNPH spectrophotometric method. SPSS 20 software was used for statistical analysis. ANOVA showed significant increase ($p < 0.001$) of MDA & PC between all the groups. Regression analysis showed TSH was dependent on PC but not on MDA in overt hypothyroid group ($p < 0.0001$). Oxidative stress was increased in hypothyroidism. TSH was positively correlated with PC in overt hypothyroidism. Assessment and control of oxidative stress at subclinical level of hypothyroidism can prevent or delay the grave consequences of hypothyroidism.

Keywords: Subclinical hypothyroidism, Overt hypothyroidism, Oxidative stress, MDA, PC.

INTRODUCTION

Hypothyroidism is one of the common occurrence of endocrine disorders in India. Thyroid diseases in India has increased upto 42 million by the year 2000 in a nationwide survey [1]. Oxidative stress (OS) occurs as a result of disequilibrium between the free radical generation and antioxidant status. It has been implicated in several pathologies including thyroid disease [2]. Hypothyroidism is a clinical event resulting from the deficiency of thyroid hormones or, more rarely from their impaired activity at the tissue level. The term subclinical thyroid disorders is applied to patients who show an abnormal serum thyroid-stimulating hormone (TSH) concentration but thyroxine and triiodothyronine levels within their reference ranges. Subclinical hypothyroidism occurs in 4% to 10% of the general population. According to the studies, subclinical hypothyroidism has been found in 0.6-16% of the population [3]. Different opinions were found in a limited number of studies regarding overt and subclinical hypothyroidism [2]. Oxidative stress was also reported in hypothyroidism [4]. Nanda N *et al.* suggested association of OS with hyperlipidemia and increased lipid risk factors of cardiovascular disease in hypothyroid patients. According to their opinion, OS may be one of the plausible mechanisms for future atherosclerotic complications in these patients [5]. Oxidative stress produced in hypothyroid rats in the

form of protein carbonyl (PC) can affect their antioxidant defense system of testes, found by some authors [6]. Furthermore, hypothyroidism can exacerbate the oxidative stress evidenced by increased protein carbonyl in rats, interestingly this oxidative stress could not be reversed by T3 treatment [7].

A number of oxidant and antioxidant markers were selected by different authors related to hypothyroidism [8, 9]. Some have chosen Thiobarbituric Acid Reacting Substances (TBARS), popularly known as Malondialdehyde (MDA) as a marker of oxidative stress, furthermore they found that MDA level has increased significantly in Thyroperoxidase Antibody (TPO Ab) positive hypothyroid patients [10]. Some have chosen PC as markers of oxidative stress [8, 9]. Variable observations were found by them in oxidative stress induced by hypothyroidism [11]. Other studies also suggested that the lipid peroxidation levels represented by MDA were decreased in hypothyroidism [12].

We found a limited number of studies exist regarding associations between lipid and protein oxidation and altered TSH levels [8].

A large number of patients are regularly referred to Biochemistry department for estimation of thyroid

hormones in our institution; many of them are diagnosed as hypothyroids. We have estimated TSH, T4, MDA and protein carbonyl in subclinical and overt hypothyroidism patients, compared and tried to assess the link for any observed value of significance and correlation.

MATERIALS AND METHODS

Study design

Cross section, observational, hospital based non interventional study.

Place of Study

The study was carried out at the Department of Biochemistry, Calcutta National Medical College and Hospital (CNMCH).

Duration of study

The duration of study was January 2014 to June 2014.

Selection of cases and controls

Hypothyroid patients were selected according to the report of the hormonal assay (TSH and T₄). A total of 71 (40 female, 31 male) hypothyroid patients (36 of Subclinical hypothyroid patients having normal T₄, but high TSH levels), and 35 of overt hypothyroidism(Low T₄, and high TSH levels), aged 30 to 60 years were selected for the study. Written informed consent was taken from them. 35 age and sex matched healthy control subjects were also selected for the study. Written informed consent was taken from all study subjects.

Inclusion criteria

All patients diagnosed as hypothyroid according to the hormonal assay at Biochemistry department of Calcutta National Medical College and Hospital, Kolkata as advised by the physicians and surgeons of different departments of the institution.

Exclusion criteria

- Patients who are suffering from any acute illness.
- Patients who are on antithyroid drugs.
- Patients who are on any drugs which can increase the oxidative stress

- Patients suffering from any other endocrine disorder like diabetes mellitus.
- Any other disease which may cause increased MDA or protein carbonyl levels.
- Antenatal mothers and psychiatry patients.
- Smokers and tobacco chewers.

Ethical clearance

Written informed consent was taken from the study subjects. The study was approved by the Institutional Ethics Committee, according to the Helsinki declaration.

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 [13].
- Estimation of Protein carbonyl by DNPH spectrophotometric method [14].

The conversion of unit from nmol/L to nmol/ mg protein was done using the formula:

$$\text{Carbonyl content (nmol/mg of protein)} = \frac{\text{Carbonyl nmol/ml}}{\text{Protein mg/ml}}$$

- Total protein was estimated by Biuret method [15].
- Statistical Analysis was done by SPSS 20

RESULTS

Table 1 shows the demographic profile of the study. Table 2 ANOVA shows significant variation in serum TSH-PC-MDA level in between and within 3 groups (Control, Subclinical & overt). Table 3 describes regression analysis, according to which TSH is dependent on PC in but not on MDA on overt group whereas in control and subclinical group there is no dependence of TSH on either PC or MDA.

Table1: Demographic profile of various study groups

Parameter	Control (n=35)	Subclinical hypothyroid (n=36)	Overt Hypothyroid (n=35)
TSH(microIU/ml) Mean ± SD	2.24±0.37	9.44±1.64	12.01±2.53
Protein Carbonyl (nmol/mg of protein) Mean ± SD	0.73±0.18	2.42±0.41	3.1±0.86
MDA(nmol/ml) Mean ± SD	5.90±1.1	28.12±3.40	31.86±1.91

Table 2: ANOVA analysis between different groups for TSH, PC, MDA

Parameter		Mean Square	F	Sig
TSH(microIU/ml)	Between groups	954.301	314.500	.000
	Within groups	3.034		
	Total			
PC(nmol/mg of protein)	Between Groups	57.558	182.226	.000
	Within groups	.316		
	Total			
MDA(nmol/ml)	Between Groups	7344.940	1254.146	.000
	Within groups	5.857		
	Total			

Table 3: o-Regression analysis Coefficients of PC and MDA dependent variable TSH in overt hypothyroidism groups

Model	t	Sig.
(Constant)	2.054	.048
PCOVERT	20.161	.000
MDAOVERT	-.635	.530

DISCUSSION

ANOVA shows significant increase of MDA and PC in overt group in comparison to subclinical hypothyroidism (Table 2). We also observed positive TSH dependence on PC in overt group in regression analysis.

Yilmaz *et al.* found that MDA levels in muscle and liver tissue of experimental hypothyroid rats are higher than the control group [16]. In another study it was stated that lipid peroxidation levels were higher in hypothyroidism [17]. Those findings were similar to our study. Karabag F [11] found high protein carbonyl level in hypothyroid patients in comparison to controls, but the difference is significant only in the subclinical hypothyroid group. They didn't find any statistically significant increase between the disease groups (subclinical and overt). Sahoo DK [7] found in transient hypothyroidism, the oxidative stress is prevailed as marked by decreased antioxidant enzymes like Superoxide Dismutase (SOD), CAT, GPx and Glutathione Reductase levels and that might be responsible for triggering germ cell apoptosis in transient hypothyroid rats results in reduction in sperm count. Such type of altered testicular physiology by hypothyroidism is reflected in adulthood with hampered fertility as evidenced by reduced total viable germ cells [18] and sperm counts [19]. In agreement to our study Haribabu A *et al.* found TSH and MDA levels were positively correlated with PC in overt hypothyroidism [8]. Nanda M found MDA, and PC levels were higher in hypothyroid patients, they also found positive correlation between MDA and PC [9]. In another study he found the rise of those oxidative stress parameters are much higher in thyroperoxidase antibody positive hypothyroid patients [10]. Ozturk O observed increased MDA and PC in serum and LDL fraction of overt hypothyroid patients but partly in serum and not

in LDL fraction of subclinical hypothyroid patients [20]. Reddy VS found the antioxidant defense is poor in overt hypothyroids than subclinical hypothyroids [2]. Coria MJ found increased nitric oxide concentration in overt hypothyroidism than subclinical and euthyroidism Nitric oxide (NO) is a free-radicals released in oxidative stress [21]. Rostami R found substantial reduction of GSH status in Hushimotos thyroiditis [22].

In agreement with them, we can explain the positive correlation between TSH and PC in overt hypothyroid group but not in subclinical hypothyroid group.

Hamid WJ concluded that tissue undergo several biochemical and histological changes in hypothyroidism, which predispose them to oxidative damage. So hypothyroid patients may get benefit from supplements of antioxidants [23].

Reactive oxygen species (ROS) cause oxidative damage in protein, carbohydrate, and lipid molecules [24, 25]. The reactive oxygen species induced by the effects of thyroid hormones have influences on lipids, proteins, nucleic acids, and carbohydrates. Lipid peroxidation and carbohydrate oxidation products make modifications in amino acid content of proteins and increase plasma protein carbonyl contents. The damage caused by ROS, is related to the imbalance between toxic molecules and antioxidant capacity [25]. Overt (OHT) and subclinical hypothyroid (SHT) disorders have been found to be associated with increased oxidative stress. Excess thyroid stimulating hormone (TSH) is known to directly produce oxidative stress. Simultaneous oxidative damage to lipids and proteins leading to increased MDA and PC levels in these two patient groups. Either of the excess TSH and increased MDA levels are involved in combination with the elevation of PC in hypothyroidism [8]. Enhanced lipid

peroxidation could be a plausible contributor for accelerated glycation of protein [9]. In a recent study, on a group of subclinical hypothyroid patients, there was no increase in C- Reactive Protein (CRP) and other markers of coronary heart disease (CHD), no association between these markers with increased TSH [26]. Some researchers [27] found strong association of ultra sensitive CRP (USCRP) and cardiovascular risk in hypothyroidism and positive correlation of USCRP with TSH and oxidative stress parameters like MDA and PC, and negative correlation with GSH. So degree of inflammation increases with development of hypothyroidism and may contribute to the increased oxidative stress. They proposed that inflammation can exacerbate OS in hypothyroid patients and those factors can exert an additive effect on the risk of atherosclerosis and cardiovascular disease in hypothyroid patients.

Hypothyroidism is a treatable endocrine dysfunction. However, it is a disease that requires lifelong drug supplementation with careful monitoring of thyroid profile to avoid iatrogenic side effects [27]. Often the drug therapy takes long to normalize the thyroid profile [28]. In another report it was found that despite treatment in primary hypothyroidism, patients are still at increased risk of morbidity associated with various circulatory disease, ischemic heart disease, dysrhythmias and cerebrovascular diseases [29]. In our study we have found the positive correlation of TSH and protein carbonyl. Gradual increase of TSH is an indicator of permanent damage of proteins. Assessment of oxidative stress early in the disease process as in subclinical stage of hypothyroidism can prevent the grave consequences. This could be vital while selecting the modified treatment protocols especially for Indian hypothyroid patients who appear to be more vulnerable to OS and inflammation. Moreover, in view of the socio-economical status of our country, measurement of MDA and PC are beneficial as they are simple and not too costly.

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