ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 05 | May - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ROLE OF THYROID STIMULATING HORMONE AS INDUCERS OF OXIDATIVE STRESS IN PATIENTS OF HYPOTHYROIDISM.

Biochemistry

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ABSTRACT

Hypothyroidism disorders have been found to be associated with increased oxidative stress. Excess thyroid stimulating hormone (TSH) is known to directly produce oxidative stress. Increased lipid peroxidation is known to facilitate protein carbonylation. However, the associations between lipid and protein oxidation and elevated TSH levels have not been studied. Thyroid profile, lipid peroxidation as malondialdehyde (MDA) levels and protein carbonylation as protein carbonyls (PCO) were estimated in patients of hypothyroidism consisting of 50 patients, in comparison to 50 euthyroid controls. We also determined the associations between TSH, MDA, and PCO levels in both groups. Increased oxidative damage was evidenced through significant elevations in the concentrations of MDA and PCO in hypothyroid group compared to controls ($p<0.01$). Both TSH and MDA levels were positively associated with PCO in hypothyroid group. The results indicate that there is a simultaneous oxidative damage to lipids and proteins leading to increased MDA and PCO levels in hypothyroid patient groups.

KEYWORDS

Hypothyroidism, MDA levels, PCO levels, Age group

INTRODUCTION

Protein carbonyls (PC) and malondialdehyde (MDA) are generated due to protein and lipid peroxidation, respectively. Oxidative stress in newly diagnosed hypothyroidism met with conflicting data in the research. And, a clear relationship between oxidative stress and early hypothyroidism is very limited and obscure. Therefore, we aimed to investigate the association between levels of MDA vs. thyrotropin (TSH) and PC vs. TSH among freshly diagnosed hypothyroid subjects[1].

Hypothyroidism is a chronic autoimmune inflammatory disease of the thyroid gland. The T and B lymphocytes that are stimulated against thyroglobulin (TG) and thyroid peroxidase (TPO) cause thyroid destruction and inflammation in HT. The T and B lymphocytes may cause the excessive production of reactive oxygen species (ROS) through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) enzyme when they are stimulated [2,3]. Malondialdehyde is an end-product of lipid peroxidation and is frequently measured as an index of these processes. Lipid peroxidation is associated with a wide variety of toxic effects, including decreased membrane fluidity and function, impaired functions of the mitochondria and Golgi apparatus and inhibition of enzymes [4]. Proteins carbonyl is an early marker of oxidative stress. It has been depicted in the literature that oxidative modification of protein occurs whenever lipid peroxidation products such as 4-hydroxy nonenal, MDA, etc. bind covalently to amino acid residues on proteins [5]. Several defence mechanisms have evolved to protect against oxidant injury. Central to these defences are the antioxidant enzymes [6]. The antioxidant enzyme superoxide dismutase, participates in the detoxification of reactive oxygen species by catalysing the dismutation of superoxide radicals. Three SOD isoenzymes are known in humans including CuZn-SOD found in the cytoplasm and nucleus, a mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) [7]. Free radical-scavenging enzymes such as SOD and CAT are the first line of cellular defence against oxidative injury, decomposing O2 − and H2O2 before interacting to form a more reactive hydroxyl radical (OH). These enzymes protect the red cells against O_2- and H_2O_2-mediated lipid peroxidation [8].

We have observed an increased activity of super oxide dismutase [9]. Oxidants or free radicals are atoms or molecules that are capable of having an independent existence that contain one or more unpaired electrons. These unpaired electrons are highly reactive species that may damage the cells. Antioxidants are the antidote for these free radicals as they quench them and transform them into less reactive forms [10,11].

MATERIAL & METHODS

Physical body assessment and biochemical analysis

Weight (kg) and height (m) were taken using a height and weight machine and used to compute the body mass index (BMI).

Sample Collection. Blood samples were collected after 12 h overnight fasting by venous puncture into grey and red top Vacutainers (Benesphera, India) tubes. The samples were centrifuged for 15min at 2500×g, and aliquots of serum were kept at −20◦C for maximum of 4 weeks. An aliquot of whole blood was collected into sodium citrate (3.2%) and diluted 1 : 10 in saline solution for measurement of CAT and SOD activities.

Assessment of thyroid function

Thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were determined by ELISA (Enzyme linked immune assay) in the department of biochemistry using kit manufactured from Benesphera. Normal thyroid function (euthyroidism) was defined as normal TSH levels (0.39–6.19 µU/L), FT4 (0.8–2.00 ng/dl) and FT3 (1.40–4.20 pgm/dl).

Evaluation of oxidative stress

Determination of malondialdehyde: Plasma malondialdehyde (MDA) levels, a marker of lipid peroxidation, were determined by the reaction of MDA with thiobarbituric acid (Sigma Aldrich kit; St. Louis, MO, USA).

Determination of Carbonyl Proteins**:** Plasma carbonyl proteins (marker of protein oxidation) were determined by the derivatization of protein carbonyl groups with 2, 4-dinitrophenylhydrazine leading to the formation of stable dinitrophenyl hydrazone adducts (Sigma Aldrich kit).

STATISTICALANALYSIS

Data are presented as mean and standard deviation (SD). The Whitney U-test was used to compare differences between groups. Spearman correlation was assessed to evaluate the correlations between the variables. Statistical significance was assumed at $P < 0.05$. Data were analysed using SPSS version 11.0 software (SPSS Inc., USA).

RESULTS

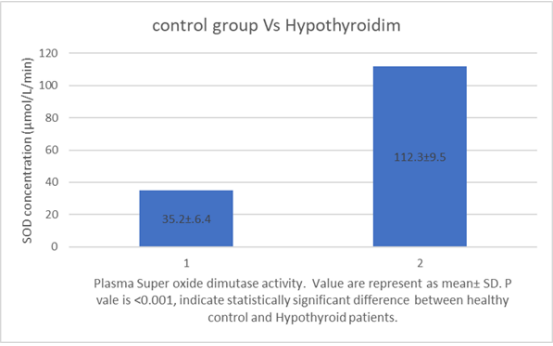
The mean ± SD of the parameters studied are presented in Table 1. T3 and T4 were significantly lower ($p < 0.01$) and TSH was significantly higher ($p < 0.01$) in OHT group compared to SHT group and controls. In comparison to controls, TSH was significantly higher ($p < 0.01$) in

SHT group with no significant difference in T3 and T4 levels. Mean ± SD of the general and biochemical characteristics of hypothyroid (HT) patients, and euthyroid controls.

Parameters	Hypothyroid groups (n=50)	Controls (n=50)
Age (years)	45.4 ± 20.0	36.9 ± 15.5
BMI (kg/m2)	25.6 ± 3.8	23.2 ± 1.2
Systole (mmHg)	110.6 ± 12.7	113.8 ± 8.4
Diastole (mmHg)	72.1 ± 8.5	74.6 ± 5.5
FT3 (pg/ml)	1.18 ± 0.36	1.34 ± 0.41
FT4 (ng/ml)	85.6 ± 19.0	85.9 ± 20.3
TSH (μIU/L)	13.30 ± 3.1	3.20 ± 1.1
MDA (lmol/L)	1.8 ± 1.0	0.9 ± 0.18
PCO (nmol/mg protein)	1.45 ± 0.2	0.72 ± 0.3

The antioxidant SOD activity in plasma and was significantly high in patients (figure 1).

Compared to controls, there was a significant increase ($p < 0.01$) in serum MDA and PCO among HT patients. PCO showed a significant association with both MDA and TSH in HT group.



The results of this study showed an increase in oxidative stress in hypothyroid patients may be because of low level of thyroid hormone (fT3/fT4). To diminished this effect the activity of superoxide dismutase is increased.

DISCUSSIONS

In the present study increased OXS was evidenced through increased MDA and PCO levels hypothyroid group compared to euthyroid controls, indicating increased oxidative damage to lipids and proteins, respectively. We also observed a significant increase in these oxidative damage markers in hypothyroid group suggesting that OXS increased significantly with the disease severity as assessed by serum TSH levels [12]. Increased lipid peroxidation results in the excess generation of reactive aldehydes as end products. Increased MDA level observed in this study is one such end product of lipid peroxidation which in turn facilitates alteration in protein structure and function. It has been reported that MDA can bind irreversibly to proteins via covalent bonds and increase the formation of PCO [13].

We observed significant correlations between MDA and PCO in hypothyroidism in the present study. Therefore, it was thought that increased lipid peroxidation could be attributable to increased PCO observed in both patient groups. However, our finding of significant association of TSH with PCO observed in both patient groups suggests that excess TSH might also be responsible for the increased PCO levels observed. As we found both TSH and MDA influencing the PCO levels in both patient groups, the correlation was repeated after nullifying the effect of one factor at a time (Table 3).

SUMMARY & CONCLUSIONS

Both lipid peroxidation and protein oxidation were significantly increased in hypothyroid group. Both excess TSH and increased lipid peroxidation are cumulatively responsible for increasing PCO levels in the patient group. Both MDA and PCO are efficient markers of OXS in hypothyroid group. In addition to routine monitoring of hypothyroid patients for their TSH levels, MDA might be considered for evaluation of oxidative injury. Supplementation of antioxidants may be a useful adjunct in the management of these patients. Our findings evoke the need for such clinical studies on hypothyroid patients.

REFERENCES:

1. Kailash Chandra and Johar Iqbal, Protein carbonylation in freshly diagnosed hypothyroidism is independent of thyrotropin levels, J Lab Med 2018; 42(5): 177–181
2. Ihsan Ates et al. The relationship between oxidative stress and autoimmunity in Hashimoto's thyroiditis., European Journal of Endocrinology (2015) 173, 791–799.
3. Jackson SH, Devadas S, Kwon J, Pinto LA & Williams MS. T cells express a phagocyte-type NADPH oxidase that is activated after T cell receptor stimulation. Nature Immunology 2004 5 818–827. (doi:10.1038/ni1096).
4. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 1990;186:421–31.
5. Dalle-Donne I, Rossi R, Giustarini D. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta. 2003;329:23–38.
6. Chattopadhyay S, Sahoo DK, Subudhi U,et al. Differential expression profiles of antioxidant enzymes and glutathione redox status in hyperthyroid rats: a temporal analysis. Comp Biochem Physiol. C. 2007;146:383–391.
7. Fukai T, Ushio-Fukai M. Superoxide Dismutases: role in redox signalling vascular function, and diseases. Antioxidants & Redox Signalling. 2012; 15:1583–1606.
8. S. Senthil, R. M. Veerappan, M. Ramakrishna Rao, and K. V. Pugalendi, "Oxidative stress and antioxidants in patients with cardiogenic shock complicating acute myocardial infarction," Clinica Chimica Acta, vol. 348, no. 1-2, pp. 131–137, 2004.
9. Adriana Santi,Marta et al., Association of Lipids with Oxidative Stress Biomarkers in Subclinical Hypothyroidism, Hindawi Publishing Corporation , International Journal of Endocrinology Volume 2012, Article ID 856359, 7 pages doi:10.1155/2012/856359.
10. Das K, Chainy G.B.: Thyroid hormone influences antioxidant defence system in adult rat brain. Neurochem. Res 2004; 29 (9): 1755-1766.
11. Sarandol E, Tas S, Dirican M, Serdar Z. Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: Effect of vitamin E supplementation. Cell Biochem. Funct 2005; 23 (1): 1-8.
12. A.N. Torun, S. Kulaksizoglu, M. Kulaksizoglu, B.O. Pamuk, E. Isbilen, N.B. Tutuncu, Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. Clin. Endocrinol. 70, 469–474 (2009)
13. P.A. Grimsrud, H. Xie, T.J. Griffin, D.A. Bernlohr, Oxidative stress and covalent modification of protein with bioactive aldehydes. J. Biol. Chem. 283, 21837–21841 (2008)