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Thyroid-stimulating hormone, Malonaldehyde and Protein Carbonyls in Hypothyroidism

Bagchi Debajit[1], Gupta Kiran[1], Dasgupta Amrapali[1], Simlai Sankha[2]

[1]Assistant Professor, Department of Biochemistry, IQ City Medical College & Hospital, Durgapur, West Bengal, India.
[2]Associate Professor, Department of Biochemistry, IQ City Medical College & Hospital, Durgapur, West Bengal, India.

Abstract

Background: Oxidative stress (SOX) has been frequently correlated with hypothyroidism. SOX are openly produced during the overproduction of TSH. Hence there may be higher lipoxidation and protein carbonylation. Although, the link between oxidation of lipid and protein has not been well illustrated in circumstances of elevated TSH levels.**Material and Methods:**Approximation of free T3 levels, free T4 levels, TSH levels, peroxidation of lipids like MDA levels, and carbonylation of proteins as PCO were projected in hypothyroidism. Around 175 patients in each diseased category, against 175 euthyroid controls were taken under study. The links between MDA, TSH, and PCO were also calculated.**Results:**Noteworthy rise in MDA and PCO levels in hypothyroidism reflected enlarged oxidative damage when compared with the euthyroid group ($p<0.01$). MDA and TSH levels had a significant relationship with PCO in both hypothyroid patients. Investigation of Pearson partial correlation exposed a mutual relationship of overloaded TSH levels and increased MDA levels in manipulating the increase of PCO in clinical and sub-clinical hypothyroidism.**Conclusion:**Parallel damage to MDA and PCO was seen due to enhanced oxidative stress in both hypothyroid patients leading to lipoxidation and protein carbonylation. Excess TSH and high MDA levels may be cumulatively caught up in the rise of protein carbonylation in hypothyroidism.

Keywords: Thyroid-stimulating hormone, Malonaldehyde, Protein Carbonyls and Hypothyroidism.

Corresponding Author: Dr.SankhaSimlai, Associate Professor, Department of Biochemistry, IQ City Medical College & Hospital, Durgapur, West Bengal, India. Email: sankha_simlai@yahoo.co.in

Introduction

A normal cellular metabolism creates a range of free radicals of which the most prominent is ROS reactive oxygen species. The conflict between antioxidant and free radicals maintain a balance in damages caused by scavenging free radicals and diminish their devastating effects. Any disproportion in this homeostasis favors free radical production and weakening of antioxidant status and ultimately leading to oxidative stress (SOX). SOX has the capability to disrupt every target like proteins, lipids, and nucleic acids. The implication of damage due to free radical-mediated oxidative stress had been perceived in several human diseases like thyroid gland disorders.[1,2] Currently, oxidative stress in hypothyroidism, characterized by high levels of thyroid-stimulating hormone (TSH) and low thyroid profile has grabbed much research curiosity. Light of association between SOX and hypothyroidism of both classes had been reported by researchers.[2] However, in overt hypothyroids (OHT), amplified lipid peroxidation and carbonylation of protein were predicted.[2,3] Whilst only a few documents were found demonstrating the peroxidation of lipids,[2-4] there was hardly any research on protein carbonylation (PCO) in subclinical hypothyroidism (SHT). It was evidenced that

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SOX alteration had been due to excess of TSH in circulation.[5] Nanda et al, in recent times, depicted the straight adaptation of TSH on enhanced malondialdehyde (MDA) and carbonylation of proteins (PCO) in hypothyroids.[3] Lipid peroxidation is also believed to aid supplementary free radical generation thereby altering the structure and function of proteins.[3,6] The commonly used indicator for protein oxidation understood as carbonylation of proteins formation (PCO) occurs either by free radicals oxidation or by lipid peroxidation reactive aldehydes like MDA.[7] PCO, regarded as a before-time marker of oxidative stress (SOX),[8] and is associated with the severity and progression of the disease.[9] In an isolated claim, an appreciable link between peroxidation of lipid and carbonylation of proteins (PCO) was shown in overt hypothyroid patients, except the part of the association between an overload of TSH and lipid peroxidation or PCO.[3] Moreover, reports demonstrating the interlink between MDA and PCO in subclinical hypothyroidism (SHT) has not been cleanly illuminated. Hence, it was unclear about the role of high TSH levels in circulation and increased lipid peroxidation in influencing the carbonylation of protein in hypothyroidism.
For that reason, in our study, we targeted to establish a relationship between the peroxidation of lipids and carbonylation of protein in both clinical and subclinical hypothyroidism against euthyroids. We also intended to assess the reason how protein carbonylation is affected by high lipid peroxidation and excess TSH levels in all patient groups.

Methodology

This study was performed in 175 clinical hypothyroid patients and 175 sub-clinical hypothyroid patients along with 175 healthy euthyroid controls. Patients seeking treatment at IQ City Medical college and Hospital, Durgapur, West Bengal, India, were therefore enlisted prospectively for the study. The clinical hypothyroid patients were identified and selected on the foundation of their blood TSH level >15 mU/L and T4 values <55µg/L. Subjests with T4 levels between 55-135 µg/L and augmented TSH >5 but <15 mU/L were diagnosed as SHT patients. The reference group constituted euthyroid subjects without any evidence or history of thyroid problems. Alcoholics smokers, chronic/acute disease patients, diabetics, hypertensives, hepatic diseased, renal diseased, inflammatory diseased, pregnant, postmenopausal women, endocrine diseased other than thyroidism, patients on lipolytic medications or antioxidant vitamins supplements were debarred from enrollment.
Sample Collection: Fasting blood was collected, centrifuged, and stored until analysis was to be carried out.

Materials and Methods: Free T3, free T4, and TSH was estimated using ELIZA kits. Measurement of MDA, by TBARS method, was performed [10]. The color was interpreted at 532 nm and expressed as µmol/L. Protein carbonylation in blood was evaluated by the DNPH method.[11] It was expressed as nmol/mg protein.

Statistic analysis: Data were presented as mean ± SD. The variation between the groups was tested with the independent student-t-test. Disparity was measured at $p<0.05$ significance. Relationship was assessed by the Pearson and partial correlation analysis. Statistic calculations were conceded out on IBM SPSS 23 software for Windows.

RESULTS

The interpretation of biochemical factors is obtainable from [Table 1]. FT3 and FT4 were drastically lesser ($p < 0.01$) and TSH was notably elevated ($p < 0.01$) in the clinical hypothyroid group when matched up to to sub-clinical hypothyroids and controls. In evaluation against the euthyroid controls, TSH was drastically high ($p < 0.01$) in the SHT group along with a momentous difference in fT3 than fT4 levels. Again compared to

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euthyroids, there was a notable increase ($p<0.01$) of blood MDA and PCO among clinical (OHT) and sub-clinical (SHT) patients; a significant disparity was observed between overt and sub-clinical groups, being higher in overt hypothyroid patients. However, on evaluation of PCO levels, it was evidenced that a significant association prevailed between MDA and TSH in both clinical hypothyroidism [Table 2] and sub-clinical hypothyroidism [Table 3]. However, when corrected for one of the factors, the associations vanished, presenting that both high TSH and elevated MDA levels were collectively responsible for the increase in PCO levels in hypothyroidism.

Table 1:Mean ± SD of General and Biochemical parameters in Hypothyroidism and Euthyroid control groups.

Parameter	Overt hypothyroid group	Subclinical hypothyroid group	Euthyroid controls
Age (years)	35.0 ± 9.6	36.5 ± 9.6	34.9 ± 7.9
BMI (kg/m^2)	28.6 ± 4.3	26.8 ± 4.2*	25.4 ± 4.1
Systole(mmHg)	111.2 ± 11.9 # +	114.3 ± 9.3	115.3 ± 7.6
Diastole(mmHg)	73.7 ± 7.5	73.5 ± 7.5	74.1 ± 5.1
fT3 (pmol/L)	0.4 ± 0.2#+	6.1 ± 0.08*	1.6 ± 1.3
fT4 (ng/dl)	25.0 ± 12.4, #+	84.6 ± 19.4*	81.0 ± 20.3
TSH (mIU/L)	58.2 ± 30.7 #+	10.7 ± 3.4*	2.1 ± 1.1
MDA (µmol/L)	2.9 ± 0.6# +	1.8 ± 0.1*	0.9 ± 0.1
PCO (nmol/mg protein)	2.2 ± 0.8# +	0.7 ± 0.1*	0.4 ± 0.1

*comparison with SHT and euthyroid controls, #denotes comparison of OHT with SHT patients. + denotes comparison of OHT with controls, $^{*,\#,+}$($p<0.01$), BMI: body mass index, MDA: malondialdehyde, PCO: protein carbonyls

Table 2: Correlations between MDA, TSH, and PCO in Overt hypothyroid patients

Parameters	Pearson correlation		Partial correlation Nullified by TSH	
MDA vs PCO	r	p	r	p
	0.39	0.01*	0.07	0.35
			Nullified by MDA	
TSH vs PCO	r	p	r	p
	-0.35	0.03*	0.01	0.86

Statistically significance *($p<0.05$).

Table 3: Correlations between MDA, TSH, and PCO in sub-clinical hypothyroid patients

Parameters	Pearson correlation		Partial correlation Nullified by TSH	
MDA vs PCO	r	p	r	p
	0.32	0.04*	0.13	0.06
			Nullified by MDA	
TSH vs PCO	r	p	r	p
	0.34	0.03*	0.03	0.69

Statistically significance *($p<0.05$).

DISCUSSION

Only from experimental studies, oxidative stress was evidenced in hypothyroidism that's too with controversial findings.[12,13] Most of the research on SOX in human hypothyroidism was focused on overt/clinical hypothyroidism. Hardly studies had been reported about SOX in sub-clinical hypothyroidism.[2] Although the machinery behind the increase of SOX in hypothyroidism has multiple factors and is also disputed. It was portrayed that lipid

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peroxidation levels, presented by MDA level, in hypothyroidism might be due to a slower clearance rate of MDA.[3] Decreased cholesterol clearance, hyperlipidemia, deficient antioxidant system, ineffective regulation of antioxidant enzymes, effects by thyroid hormones,[14] and overload in TSH levels,[5] were some of the other projected types of machinery connected with high SOX in hypothyroidism. Even in subclinical hypothyroidism where TSH levels were high in comparison to clinical hypothyroids also was associated with SOX.[2]

In our recent findings, an elevated SOX was seen when compared against euthyroid controls. This indicated increased PCO levels along with higher MDA levels in hypothyroids and also increased oxidative stress with harm to lipids and proteins. It was also evidenced from our study that an increase in oxidative damage markers in the OHT group versus the SHT group was indicative of increased SOX, which increased significantly with the severity of the disease, which again is assessed by serum TSH levels. In concordance with this, Torun et al.[2] demonstrated varied status for oxidative damage in clinical and sub-clinical hypothyroids. Our result of increased lipoxidation conformed with other previous studies in clinical hypothyroidism,[3,15] and also sub-clinical hypothyroidism.[2] On the other side similar to us, an increase in PCO levels was reported earlier in clinical hypothyroid patients.[3] We however also found increased PCO levels in sub-clinical hypothyroid patients.

We have found a prominent positive correlation of PCO with TSH and MDA in Overt/clinical hypothyroids[Table 2] and sub-clinical hypothyroids[Table 3]. It was established before that, high TSH levels may directly alter lipoxidation and carbonylation of protein leading to an increase in MDA and PCO levels.[3] It had also been reported previously that overload of TSH may increase SOX.[5] Moreover, excess TSH in circulation is also believed to interfere with the arterial wall,[16] and affect endothelium homeostasis,[17] adipocytes,[18] and lymphocytes resulting in oxidative stress.[19]

Surplus making of reactive aldehydes is the end product of increased lipid peroxidation. It was evident from our study that higher MDA levels facilitated protein structure and function modification. It was earlier stated that MDA permanently binds to proteins by covalent bonding to increase PCO formation.[7,8] In support of this idea, we observed in our study that a distinct association was present between PCO and MDA in cases of clinical hypothyroids[Table 2] and sub-clinical hypothyroid patient groups [Table 3]. Hence, it was believed that an increase in lipoxidation is a trait for the increase in PCO levels in both patient groups. As per our report of correlation between TSH and PCO in clinical and sub-clinical hypothyroid patient groups, it was evident that amplified lipid peroxidation single-handedly could not increase PCO levels and that the overload of TSH in circulation also had a primal role to enhance PCO levels.

It was observed in our study that both MDA and TSH had a significant association with PCO in both overt and sub-clinical patient groups, hence the influence of TSH on the connection between MDA and PCO was statistically canceling out. Similarly, the effect of MDA on the relationship between TSH and PCO in both OHT [Table 2] and SHT groups [Table 3] was also canceled out. It was observed that the significant correlations were lost after statistical nullification in both clinical and sub-clinical hypothyroidism groups. This confirms that together high TSH and MDA levels might be cumulatively forcing PCO increase in both clinical and sub-clinical hypothyroidism.

To wrap up the study, it was evident that there was a marked elevation in lipoxidation as well as oxidation of proteins in both clinical and sub-clinical hypothyroidism. Overload of TSH in circulation and enhanced lipoxidation are jointly mixed up in increasing plasma PCO levels in all patient groups. In addition to a scheduled check-up of TSH levels, MDA levels, and PCO levels in hypothyroidism, a regular evaluation of oxidative injury might be beneficial.

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Moreover, the relevance of lipoxidation scavengers and carbonylation of protein need to be attended to in future studies.

CONCLUSION

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