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Original Research Paper

Biochemistry

"ASSOCIATION BETWEEN LIPID PEROXIDATION AND VASCULAR DYSFUNCTION IN OVERT HYPOTHYROIDISM"

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ABSTRACT

Background and Aim: Oxidative stress is most commonly seen in Hypothyroidism. This raging oxidative stress might be responsible for cardiac dysfunction, which had not been studied extensively especially in overt hypothyroidism (OHT). The present study intends to explore the interrelationship between coronary artery disease and lipid peroxidation in overt hypothyroidism. **Material and methods:** Malondialdehyde (MDA) as a prognostic indicator of oxidative stress while plasma nitrates and asymmetric dimethyl-arginine (ADMA) were assessed as coronary arterial disease indicators in 175 overt hypothyroid patients compared to 175 euthyroid controls. **Results:** ADMA levels were seen to be markedly elevated along with MDA levels in overt hypothyroids whereas plasma nitrates were distinctly low in them when compared against euthyroids ($p<0.01$). To add to it, a positive and noteworthy association was seen between ADMA and MDA levels in the hypothyroids. **Conclusions:** In our conclusion, it was observed that there was an elevated lipid peroxidation due to the overproduction of ROS in clinical hypothyroid patients. Moreover, lower plasma nitrates and augmented ADMA levels substantiate coronary arterial dysfunction and disease. To add to it further, a significant relationship between ADMA and MDA specifies a tough influence of oxidative stress on the coronary arterial disease in overt hypothyroidism.

KEYWORDS : Oxidative stress, Thyroid disorders, Malondialdehyde, Dimethyl-arginine, Nitrates

INTRODUCTION

The endothelial vasculature of the human body is understood to discharge a lot of vasodilators like prostacyclin endothelium-derived factors, vasoconstrictors, and nitric oxide (NO). By regulating vascular tone, platelet aggregation as well as muscle cell proliferation, NO function as the key to successful endothelial health. [1]. Coronary arterial dysfunction is thought to be induced due to decreased bioavailability of NO. Dimethyl-arginine (ADMA), an L-arginine analog, competitively blocks NO synthesis [2] while oxidative stress inactivates NO action. Again it is well understood that bioactivity of NO is weakened by superoxide anions [3]. Thus, oxidative stress may be one of the major reasons for coronary artery diseases. Shreds of evidence have shown that increased oxidative stress is a major trigger for atherosclerosis in research animals [4]. Both cardiac dysfunctions along with overwhelming oxidative stress pose a great risk to cardiovascular events. This connects the idea of oxidative stress contributing to coronary arterial diseases.

Endothelial abnormalities are evidenced in coronary artery disease [5] along with elevated ADMA levels which forecasts general cardiovascular risk [6]. Elevated ADMA in plasma had also been observed in diabetes mellitus [8], renal failure [2, 9], hypercholesterolemia [7], and atherosclerosis [10]. Thyroid hormone diseases also lead to endothelial malfunction; although the correct mechanism is still unclear. However, studies of rapid relaxation of smooth muscles by thyroid hormones were studied in rats [11], and recently it was demonstrated that endothelium is specific targets for thyroid hormones [12]. Amplified ADMA levels along with low NO production have been documented in hyperthyroid [13] and hypothyroid [13, 14]. Although oxidative stress [15] and cardiovascular risk [16] was stated to be elevated in hypothyroids, there were very inadequate studies in coronary artery diseases related to hypothyroidism. Furthermore, the relation between oxidative stress as well as its impact on blood nitrates and ADMA levels still hasn't been confirmed. Hence, the goal of our research was to determine the levels of blood nitrate and ADMA as a marker of coronary arterial disease and hit upon its inter-relationship with oxidative stress, measured as blood malondialdehyde (MDA) concentration in clinical hypothyroids against healthy controls.

MATERIAL AND METHODS

Subjects

One seventy-five subjects identified with clinical hypothyroidism were enrolled from the OPD of Medicine and Endocrinology Department of Prasad Medical College (PIMS), Lucknow, Uttar Pradesh, India. Thyroid-stimulating hormone (TSH) levels greater than 15 mU / L and total thyroxine (T4) values lower than 55 µg / L was considered to be guidelines for the assessment of clinical hypothyroidism. On the other hand control group also had one hundred and seventy-five healthy euthyroid subjects with normal TSH value. Diabetics, smokers, alcoholics, hypertensives, malignants, and pregnants were excluded from both groups.

Biochemical analysis

Blood samples were collected and prepared by centrifugation and were stored in a refrigerator until analysis. Free fT4 and TSH were estimated by ELISA kits. MDA was measured by the TBARS method for identifying oxidative stress related lipid peroxidation [17]. The plasma nitrate levels were calculated using the Griess' method [18] to classify cardio-vascular diseases. The ADMA calculation was based on HPLC (19).

Statistical Evaluation

All values obtained were entered in an excel sheet and represented as mean and SD (Mean ± SD). Testing of the discrepancy between patient and control groups was done using the independent student t-test. All associations of lipoxidation and arterial dysfunction were studied by Pearson correlation analysis. The statistical significance of a p-value of < 0.05 was determined. Statistical analysis was done by IBM SPSS statistics 23 software for Windows.

RESULTS

Table 1 outlined the blood parameters for thyroid activity, lipoxidation, and coronary arterial dysfunction in patients and control groups. MDA levels were seen to be significantly increased in overt hypothyroids as contrasted with euthyroids.

The hypothyroid finding also indicates a pronounced increase in ADMA levels, while plasma nitrates were considerably decreased compared with control. Analysis of individual association was performed to infer the interrelationship between levels of MDA, ADMA, and nitrate. We observed a clear connection between proportions of MDA and ADMA ($r=0.472$, $P=0.036$) in patients with hypothyroids

Table 1: Compared with euthyroids, age, thyroid profile, and biochemical parameters of overt hypothyroid patients.

Parameter	Overt hypothyroid group	Euthyroid control group	p-value
Age (years)	35.0 ± 9.6	34.9 ± 7.9	NS
fT4 (μg/L)	25.0 ± 12.4	81.0 ± 20.3	0.000*
TSH (mU/L)	58.2 ± 30.7	2.1 ± 1.1	0.000*
MDA (μmol/L)	2.9 ± 0.6	0.9 ± 0.1	0.000*
Nitrates (μmol/L)	26.46 ± 4.62	63.11 ± 1.99	0.001*
ADMA (μmol/L)	1.72 ± 0.23	0.27 ± 0.06	0.001*

NS(non-significant), *(significant, $p<0.01$)

DISCUSSION

According to our study, the level of MDA was seen to be notably augmented in hypothyroids compared to euthyroids. The plasma nitrate level was also markedly low, whereas ADMA levels were radically high in the patient group (Table 1). Apart from it, a distinct relationship was observed between MDA with ADMA levels. These ultimately indicated increased oxidative stress along with a clear inter-relationship with coronary artery disease in overt hypothyroidism. In literature, increased lipoxidation in clinical hypothyroidism had been reported against euthyroids [15, 20]. Although, the association of oxidative stress with coronary artery disease had been weakly stated.

It has been previously stated that arterial dysfunction could crop up either due to the low bioavailability of NO or in the under-production of NO. Many more factors might also be accountable for coronary artery diseases like high oxidative stress, hyperglycemia, hypertension, hyperhomocysteinemia, and dyslipidemia. In our study, the inter-relationship between cardiovascular dysfunction and oxidative stress has been assessed in clinical hypothyroidism. One clear reason for endothelial dysfunction which was understood to be amplified oxidative stress, which again inactivates the action of NO. The reduced NO production and augmented ROS synthesis may be an answer to the impairment of endothelium-dependent vasodilatation in cardiovascular patients. It was previously understood that oxidative stress along with arterial dysfunction leads to atherosclerosis development [21]. We documented higher MDA levels which reflected oxidative damage due to high free radicals. It had been reported that ROS mainly targets endothelial cells of vessels [22]. By lipoxidation and transcription factor activation the functions of endothelium get tailored thereby interfering with NO availability [23]. NO reacts with molecular oxygen or superoxide radicals to produce nitrates or peroxynitrite. Hence oxidative stress, through NO destruction by superoxide reduces endothelium-dependent vasodilatation [24].

Intracellular methylated proteins degradation and enzyme dimethyl-arginine-dimethyl-amino-hydrolase (DDAH) on another side produce di-methyl-arginine which gets eliminated via urinary excretion. [25]. A drop in kidney filtration, boosted ADMA synthesis or a decline in enzymatic hydrolysis triggers an increased plasma ADMA level. Since kidney function had been seen to be normal in hypothyroids, the high ADMA level may be responsible for decreased kidney excretion. As the kidney is known to eliminate only 20% of ADMA, the majority of 80% are degraded by enzymatic hydrolysis [25]. Hence, decreased enzyme hydrolysis may be the key answer to our documented high ADMA levels. Oxidative stress, by diminishing DDAH activity, induces ADMA accumulation endogenously [26]. In our investigation, the distinct relationship seen between ADMA and MDA in clinical hypothyroids further supports the idea. In coronary artery malfunctions, the increased ADMA in hypothyroidism has been stated to be an impending factor. A minor elevation of ADMA levels may result in an exponential transformation in NOS activity [27]. By pinning down the NO release from the endothelium, ADMA, and arginine analogs augment the peripheral vessels tone. As documented in our study, oxidative stress in hypothyroids might lead to the inactivation of NO. Moreover, high oxidative stress by hindering DDAH activity leads to elevated ADMA levels and ultimately diminishes NO production.

CONCLUSION:

Summing up, higher MDA levels in hypothyroidism reflect overwhelming oxidative stress, low plasma nitrates, and higher ADMA levels. These factors ultimately lead to endothelial dysfunction. From our study, it is realized that greater development of ROS, as well as oxidative stress, compromises arterial function by inactivating NO and greater rates of ADMA production. Coronary arterial malfunction indicates early incidence in the pathology of atherosclerosis. From confirmations of higher oxidative stress along with coronary risk in hypothyroidism, a routine evaluation of arterial impairment in hypothyroid patients might be beneficial in the sensing of cardiovascular alterations. It is also recommended to focus on the arterial dysfunction and cardiovascular outcome aspect of oxidative stress.

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