e ISSN 2277 - 3290
Print ISSN 2277 - 3282

Journal of Science **Medicine**

www.journalofscience.net

THYROID DISORDERS AND NITRIC OXIDE LEVELS

Monica Verma*[1], Kiran Dahiya[2], Veena Singh Ghalaut[3], Shashi Seth[4], Prasanta Saha Roy[5], Atreyee Basu[6], Abhishek Soni[7]

[1]Senior Resident, Department of Biochemistry, Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana- India.
[2]Professor, Department of Biochemistry, Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana- India.
[3]Senior Professor and Head, Department of Biochemistry, Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana- India.
[4]Senior Professor, Department of Biochemistry, Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana- India.
[5]Biochemist, Department of Biochemistry, Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana- India.
[6]Ex Resident, Department of Biochemistry, Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana- India.
[7]Senior Resident, Department of Radiotherapy, Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana- India.

ABSTRACT

Nitric oxide has been regarded as a marker of oxidative stress in various diseases. There is a lot of controversy regarding levels of nitric oxide in thyroid disorders. The present study included 50 diagnosed hypothyroid, 50 hyperthyroid and 50 healthy controls. The nitric oxide levels were estimated by Griess reaction. The results were compared statistically. Nitric oxide concentration was found to be significantly low in hyperthyroid patients (6.4±3.8 µmol/L) as compared to control subjects (36.24±7.61 µmol/L) ($p < 0.05$), while it was significantly raised in hypothyroid patients (57.6±15.8 µmol/L) ($p < 0.001$). Estimation of nitric oxide levels in thyroid disorders may aid in understanding the etiopathogenesis of thyroid disorders.

Keywords: Hyperthyroidism, Hypothyroidism, Nitric oxide, Total triiodothyronine, Total thyroxine, Thyroid stimulating hormone.

INTRODUCTION

Thyroid hormones are the most important humoral factors involved in setting the basal metabolic rate on a long-term basis in target tissues such as liver, heart, kidney and brain. Thyroid disease in its various forms is common, affecting around 5% of the population. Hypothyroidism, or under activity of thyroid gland, results from either reduced secretion of thyroxine and trioidothyronine (T_3) that may be correlated with increased secretion of thyroid stimulating hormone (TSH) from pituitary. Hyperthyroidism is defined as a hypermetabolic condition caused by excessive production of thyroid hormones [1-2].

Thyroid hormones from the thyroid gland are necessary for the normal development of body organs. It has been demonstrated that nitric oxide (NO) participates in the regulation of thyroid function. NO brings about oxidation reactions which will produce free radicals, and can start chain reactions that damage cells. This leads to the production of ROS (reactive oxygen species). These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or oxidizing DNA or proteins [3].

Reactive oxygen species (ROS) play an important role in physiological processes, but cause oxidative damage to molecules. Under physiological conditions, the process of production and detoxification of ROS is almost balanced. ROS and free radicals participate in physiological and pathological processes in thyroid gland also, e.g. hydrogen peroxide (H_2O_2) is crucial for thyroid hormone biosynthesis, acting at different steps of the process. Additionally, H_2O_2 is believed to participate in the Wolff-Chaikoff's effect, undergoing in conditions

Corresponding Author:-**Monica Verma** Email:-monisoni26@gmail.com

of iodide excess in the thyroid [4].

NO is synthesized by endothelial cells from L-arginine and oxygen. Blood flow and laminar shear stress induce the activation through phosphorylation of NO synthase (NOS), that catalyzes the conversion reaction from L-arginine to citrulline and NO, through two cofactors: calmodulin and pteridin-tetrahydrobiopterine (BH4). There are at least three isoforms of constitutive NOS: the endothelial form (eNOS), the neuronal form (nNOS) and the inducible form (iNOS); eNOS, the calcium-dependent form of the enzyme, is in many cellular types and is responsible for NO production in healthy blood vessels. nNOS is a special type of NOS, expressed in the central nervous system. iNOS, a form induced by immunological stimuli, is expressed in the myocytes, in the macrophages and in the endothelial cells. NOS are formed by two distinct catalytic subunits, as terminal C-reductase and terminal N-oxygenase domain. In the presence of sufficient amounts of BH4, these domains work together and synthesize NO. Otherwise in case of increased oxidative stress they cause these production of peroxynitrites ($ONOO^-$) which are highly reactive free radicals [5].

Current evidence suggests that lower concenteration of NO produced by eNOS and nNOS are cytoprotective whilst supraphysiological concenteration produced by iNOS triggers cell death. This paradox may be explained by the free radical nature of NO and the ease with which it reacts with other radicals, particularly ROS, to form various NO related species in vivo, e.g as cytotoxic $ONOO^-$ is formed when NO reacts with superoxide anion from inflammatory cells [6]. The NO produced induces guanylate cyclase to produce cGMP from GTP. cGMP is responsible for cellular hyperpolarization due to the activation of the potassium channels. These reactions cause the inhibition of the entrance of calcium and, thus the vasodilatation in the cardiovascular system [7].

There is a lot of controversy regarding levels of NO in thyroid disorders. Some studies indicate an increase in levels of NO in hypothyroid and decreased levels in hyperthyroid patients while in most of the studies, a decreased level of NO was observed in hypothyroid patients. The present study was, therefore, aimed to assess the levels of NO in hypothyroid and hyperthyroid patients.

MATERIALS AND METHODS

This study was conducted on 50 diagnosed hypothyroid and 50 hyperthyroid patients. A total of 50 healthy volunteers served as controls with thyroid profile in normal range. To eliminate the factors which might affect free radical antioxidant activity, we excluded all chronic smoking and alcoholic subjects. All individuals suffering from chronic diseases, such as diabetes mellitus, diseases of the liver, kidney, cardiac and other endocrine and immunological disorders were also excluded from both patient groups and healthy controls with the help of suitable investigations.

After obtaining informed consent from the subjects venous blood was collected from median cubital vein aseptically. Serum was separated and stored at -20°C until analysis. The serum Total T_3 (TT_3) and Total T_4 (TT_4) were estimated by radioimmunoassay and TSH levels were estimated by immunoradiometric assay (IRMA) to group them as normal subjects, hypothyroid and hyperthyroid patients. The NO level (measured as nitrite-plus-nitrate (NO(x)) concentration) was estimated by Griess reaction method. In this method nitrite reacts under acidic conditions with sulfanilic acid ($HO_3SC_6H_4NH_2$) to form a diazoniumcation ($HO_3SC_6H_4–N\equiv\mathbf{N^+}$) which subsequently couples to the aromatic amine 1-naphthylamine ($C_{10}H_7NH_2$) to produce a red–violet coloured ($\lambda_{max} \approx 540$ nm), water-soluble azo dye ($HO_3SC_6H_4–N=\mathbf{N}–C_{10}H_6NH_2$) [8]. Serum free triiodothyronine (FT_3) and free T_4 (FT_4) were assayed by a chemiluminescent assay method using Advia centaur CP analyzer with original kits obtained from Siemens healthcare diagnostics ltd. (Bayswater Victoria, Australia).

Normal ranges of different parameters used in the study are as following: TSH (0.3-5.0 µIU/mL), TT_3 (70-200 ng/dL), TT_4 (5.5-13.5 µg/dL), FT_3 (2.3-4.2 pg/mL) and FT_4 (0.89-1.76 ng/dL).

All statistical analysis were performed using the Statistical Package for the Social Sciences (SPSS) version 20 for windows. Values shown in the text, tables and figures are mean ±SD. Student t test were applied for comparison of means of study groups. p value < 0.05 were considered significant. Correlations between groups were analyzed using Pearson correlation coefficient (r) formula.

RESULTS

The mean age of the patients in hypothyroid group was 37.92±13.61 (17-65) years while in hyperthyroid group, the mean age was 44.40±14.48 (18-68) years. Out of 50 patients, 2 were males and 48 was females in hypothyroid group while there were 6 males and 44 females in hyperthyroid group. Women was overrepresented in both groups of patients (96% in the hypothyroid group and 88% in the hyperthyroid group) and therefore, the control group was gender-matched by the inclusion of more control women (92%) than men. The mean age of the control group was 38.16 ± 11.8 (20-57) years. The biochemical parameters are shown in table I.

NO concentration was significantly lower in hyperthyroid patients (6.4±3.8 µmol/L) than in control subjects (36.24±7.61 µmol/L) ($p < 0.05$) while it was significantly higher in hypothyroid patients (57.6±15.8 µmol/L) ($p < 0.001$). In hypothyroid group, the plasma

NO levels were found to be negatively correlated with TT_3 (r= -0.474, $p < 0.05$) and TT_4 (r= -0.457, $p < 0.05$) values as per Pearson's correlation coefficient and the correlation was statistically significant. With FT_3 and FT_4 also correlation was negative and significant (r= -0.599, $p < 0.05$, r= -0.589, $p < 0.05$ respectively). With TSH correlation was found to be positive (r= 0.341) but not significant ($p > 0.05$). In hyperthyroid group, a positive correlation of NO was found with TSH (r= 0.109) and a negative correlation with TT_3 (r= -0.302), TT_4 (r= -0.268), FT_3 (r= -0.307) and FT_4 (r= -0.353) but it was not significant statistically.

Table 1. Thyroid profile and NO levels in patients with hypothyroidism, hyperthyroidism, and healthy controls

Groups	Healthy Control	Hypothyroid group	p value	Hyperthyroid group	p value
Number	50	50	-	50	-
TT_3 (ng/dL)	129.88±32.69	97.88±37.51	0.178	209.04±.155.54	0.004*
TT_4 (µg/dL)	8.58±2.49	5.04±2.77	0.626	12.49±5.47	0.019*
TSH (µIU/mL)	1.78±1.67	42.53±46.08	0.000**	0.12±0.03	0.000**
FT_3 (pg/mL)	3.01±0.46	2.15±0.84	0.025*	5.53±3.93	0.001*
FT_4 (ng/dL)	1.31±0.21	1.02±0.43	0.006*	2.17±1.78	0.001*
NO (µmol/L)	36.24±7.61	57.6±15.8	0.002*	6.4±3.8	0.000**

*Significant result, ** Highly significant result; all values are in mean ± SD.

DISCUSSION AND CONCLUSION

The present study showed that there is an increased level of NO in hypothyroid group and decreased level in hyperthyroid group as compared to healthy controls.

Increased NO in hypothyroidism

TSH levels are generally, found to be raised in hypothyroidism due to removal of feedback inhibition. The presence of functional TSH receptors has been demonstrable in bone marrow cells, in cardiomyocytes, in human coronary artery smooth muscle cells and in human endothelial cells. It has also been reported that TSH directly induces TNF-α (tumour necrosis factor) secretion by bone marrow cells and IL-6 (interleukin-6) by adipocytes. It has been proven by various other studies that inflammatory cytokines like IL-2, IL-6, IL-15 are increased in hypothyroidism. TSH at a higher concentration may induce secretion of inflammatory cytokines and decrease the antioxidant status [9]. TNF-α is a pivotal NO controlling cytokine. Elevated TNF-α and other cellular cytokines may promote the expression of inducible nitric oxide synthase enzyme (iNOS). Activity of iNOS is long lasting and lead to the production of a lot of NO, since its activation is not Ca^{2+} and calmodulin dependent. If the enzyme is induced, the production of NO lasts for hours, even days [10-12].

In another experimental study on animals it was demonstrated that at low levels of T_3 in hypothyroidism, nNOS mRNA levels is increased by three fold and nNOS translocation to mitochondria was favoured with concomitant increase in mitochondrial NOS expression and activity [13]. In fact, it has recently been reported [14] that liver and skeletal muscle mitochondrial NOS is increased in hypothyroidism and is inversely correlated with serum T3, whereas in neural tissues hypothyroidism is associated with reduced NOS activity [15]. Another study reported an increase in ventricular and aortic NOS activity in young and adult hypothyroid rats and it was due to an increase in inducible NOS isoform in young rats and by an increase in caveolins expression in adult rats [16].

Similar results have been reported in hypothyroidism by other researchers.[17-19] Hypothyroidism associated oxidative stress is the consequence of both increased production of free radicals and reduced capacity of the antioxidant defense [20-22]. Variation in the levels of thyroid hormones can be one of the main physiological modulators of in *vivo* cellular oxidative stress due to their known effects on mitochondrial respiration. In particular, it has been suggested that the increase in reactive oxygen species induced by a deficiency of thyroid hormones can lead to an oxidative stress condition in liver and heart and some skeletal muscles with a consequent lipid peroxidative response. Metabolic disorder from autoimmune-based hypothyroidism can also increase oxidative stress [20, 23]

Hypothyroidism-induced dysfunction of the respiratory chain in the mitochondria leads to accelerated production of free radicals (i.e., superoxide anion, hydrogen peroxide, and hydroxyl radicals as well as lipid peroxides), which consequently leads to oxidative stress (OS). By stimulating enzymes that control active transport pumps, demand for cellular oxygen increases, and as ATP production goes up, heat is produced [24-25].

So, two things are clear that in hypothyroidism there is increased oxidative stress and increased iNOS as we can see from our results also which shows increased NO levels in hypothyroidism. This increased supraphysiological concentration produced by iNOS will react with other radicals, particularly ROS to form various NO related species like peroxynitrites and will lead to damage to cells.

Decreased NO in hyperthyroidism

In the present study, NO levels were found to be significantly lower in hyperthyroidism than in control subjects. It has been seen that hyperthyroidism is associated with tachycardia, systolic hypertension, atrial fibrillation, heart failure, and evidence of increased probability of cardiovascular and cerebrovascular mortality [26]. Clinical studies revealed that endothelial dysfunction seems to be the possible cause of such complications and the most important mechanism for endothelium dysfunction is the reduction in NO availability [7].

Evidence has accumulated in recent years that the endothelial production and release of nitric oxide (NO) plays a crucial role in the maintenance of physiological vascular tone and structure. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthase. It inhibits vascular NO production at concentrations found in pathophysiological conditions, and also causes local vasoconstriction when infused intra-arterially [27]. Thus, decreased NO levels can be explained by elevated ADMA levels which have been proved by various studies in hyperthyroid patients. Previous studies have also reported increased ADMA and decreased NO levels in hyperthyroidism [28-30].

Several lines of evidence indicate that ADMA is synthesized from the degradation of methylated proteins rather than from the methylation of free arginine. The specific enzyme arginine *N*-methyltransferase (protein methylase I) has been shown to methylate internal arginine residues in a variety of polypeptides. Catabolism of these polypeptides generates *N* Gmonomethyl- l-arginine, ADMA (Asymmetric dimethyl arginine), and SDMA (Symmetric dimethyl arginine). Thyroid hormone up-regulates protein methylase I activity, leading to increased ADMA levels and finally decreased NO associated with hyperthyroidism. Also, it could be hypothesized that hyperthyroidism would decrease DDAH (Dimethyl arginine dimethyl aminohydrolase) activity through increased production of oxygen free radicals and increased lipid peroxidation which increases ADMA levels [29] leading to decreased NO synthesis. Moreover, the free radicals react with NO and lead to production of peroxynitrite. Peroxynitrite inhibits eNOS synthesis and also changes the mission of eNOS from synthesis of NO to synthesis of oxygen radicals resulting in decreased NO levels [31] so, all these reactions explain the decreased levels of NO in hyperthyroidism.

These findings may add some information to the literature in this field, in which a definite conclusion is yet to be reached. Moreover, estimation of NO levels in thyroid disorders may help in understanding its etiopathogenesis.

ACKNOWLEDGEMENT

The authors would like to acknowledge the laboratory technical staff for their cooperation and assistance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Bhimte B, Agrawal BK, Sharma VK. Oxidative stress status in hypothyroid patients. *Biomed Res*, 23, 2012, 286-288.
2. Karabag F, Sozbilir NB. Effects of caffeic acid phenethyl ester on plasma homocysteine, asymmetric dimethylarginine, nitric oxide levels in rats constituted hyperthyroidism. *J Appl Biol Sci*, 5, 2011, 1-5.
3. Suchetha K, Sandhya N, Gowda K. Oxidative Stress in Hypo and Hyperthyroidism. *Al Ameen Journal of Medical Sciences*, 4, 2011, 49.
4. Karbownik M, Lewinski A. The role of oxidative stress in physiological and pathological processes in the thyroid gland; possible involvement in pineal-thyroid interactions. *Neuro Endocrinol Lett*, 24, 2003, 293-303.
5. Tentolouris C,Tentolouris C, Tousoulis D. L-Arginine in coronary atherosclerosis. *Int J Cardiol*, 75, 2000, 123-128.
6. Kim YM, Bombeck CA. Nitric oxide as a bifunctional regulator of apoptosis. *Cir Res*, 84, 1999, 253-256.
7. Raddino R, Caretta G, Teli M. Nitric oxide and cardiovascular risk factors. *Heart International*, 3, 2007, 18-26.
8. Dimitrios T. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Greiss reaction in the L-arginine/ nitric oxide area of research. *J Chromatography*, 851, 2007, 51-70.
9. Yilmaz S, Ozan S, Benzer F. Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. *Cell BiochemFunct*, 21, 2003, 325-330.
10. Dardano A, Ghiadoni L, Plantinga Y. Recombinant human thyrotropin reduces endothelium dependent vasodilation in patients monitored for different thyroid carcinoma. *J Endocin and Metab*, 91, 2006, 4175-4178.
11. Licino J, Prolo P, McCann SM. Brain iNOS: current understanding and clinical implications. *Mol Med Today*, 5, 1999, 225-232.
12. Kokcam I, Dertlioglu SB. The levels of nitric oxide in Bechets disease. *Turk J Med Sci*, 41, 2011, 587-594.
13. Franco MC, Valeria G, Arciuch A. Hypothyroid phenotype is contributed by mitochondrial complex I inactivation due to translocated neuronal nitric oxide synthase. *J of Biol Chem*, 281, 2006, 4779-4786.
14. Carreras MC, Peralta JG, Converso DP. Modulation of liver mitochondrial NOS is implicated in thyroid-dependent regulation of O2 uptake. *Amr J Physiol*, 281, 2001, H2282–2288.

15. Ueta Y, Levy A, Chowdrey HS. Hypothalamic nitric oxide synthase gene expression is regulated by thyroid hormones, *Endocrinology*, 136, 1995, 4182–4187.
16. Sarati LI, Martinez CR, Artes N. Hypothyroidism: age-related influence on cardiovascular nitric oxide system in rats.*J Metabol*, 61, 2012, 1301-1311.
17. Coria MJ, Pastran AI, Gimenez MS. Serum oxidative stress parameters of women with hypothyroidism. *Acta Biomed*, 80, 2009, 135-139.
18. Wang J, Van Praag A, Hamilton E. serum xanthine oxidase: Origion, regulation and contribution to control of Trypanosome parasitemia. *Antioxid Redox Signal*, 4, 2002, 161-178.
19. Erdramar H, Demirci H, Yaman H. The effect of hypothyroidism, hyperthyroidism and their treatment on parameters of oxidative stress and antioxidant status. *Clin Chem Lab Med*, 46, 2008, 1004-1010.
20. Carmeli E, Bachar A, Barchad S. Antioxidant status in the serum of persons with intellectual disability and hypothyroidism: A pilot study. *Res Development Disab*, 29, 2008, 431-438.
21. Das K, Chaing GB. Thyroid hormone influence antioxidant defense system in adult rat brain. *Neurochem Res*, 29, 2004, 1755-1766.
22. Sarandol E, Tas S, Dirican M. Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: Effect of vitamin E supplementation. *Cell Biochem Funct*, 23, 2005, 1-8.
23. Nanda N, Bobby Z, Hamide A. Association of thyroid stimulating hormone and coronary lipid risk factors with lipid peroxidation in hypothyroidism. *Clinical Chem Lab Med*, 46, 2008, 674-679.
24. Kale M, Umathe SN, Bhusari KP. Oxidative stress and the thyroid. *Positive Health ecognitive.co.* 2006, 21- 27.
25. Venditti P, Balestrier M, Dimeo S. Effect of thyroid state on lipid peroxidation, antioxidants defenses, and susceptibility to oxidative stress in rat tissues. *J Endocrin*, 155, 1997, 151-157.
26. Fazio S, Palmieri EA, Lombardi G. Effects of thyroid hormone on the cardiovascular system. *N Engl J Med*, 344, 2001, 501-509.
27. Boger RH. Asymmetric dimethylarginine (ADMA) and cardiovascular disease: insights from prospective clinical trials. *Vascular Medicine*, 10, 2005, S19-25.
28. Hermenegildo C, Medina P, Peiro M. Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in hyperthyroid patients. *J Clin Endocrinol Metab*, 87, 2002, 5636-5640.
29. Arikan E, Karadag CH, Guldiken S. Asymmetric dimethylarginine levels in thyroid diseases. *J Endocrinol Invest*, 30, 2007, 186-191.
30. Gu LQ, Zhao L, Zhu W, Li FY. Relationships between serum levels of thyroid hormones and serum concentrations of asymmetric dimethylarginine (ADMA) and N-terminal-pro-B-type natriuretic peptide (NT-proBNP) in patients with Graves' disease. *Endocrine*, 39, 2011, 266-271.
31. Kuzkaya N, Weismann N, Harrison DG. Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase. *J Biol Chem*, 278, 2003, 22546-22554.