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 triiodothyronine (T3) 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 (H2O2) is crucial for thyroid hormone biosynthesis, acting at different steps of the process. Additionally, H2O2 is believed to participate in the Wolff-Chaikoff’s effect, undergoing in conditions...
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-tetrahydrobipterine (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 concentration of NO produced by eNOS and nNOS are cytoprotective whilst supraphysiological concentration 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 T3 (TT3) and Total T4 (TT4) 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 (HO3SC6H2NH2) to form a diazoniumcation (HO3SC6H2N= − N=N) which subsequently couples to the aromatic amine 1-naphthylamine (C10H7NH2) to produce a red-violet coloured (λmax = 540 nm), water-soluble azo dye (HO3SC6H2−N=NN−C10H7NH2) [8]. Serum free triiodothyronine (FT3) and free T4 (FT4) 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), TT3 (70-200 ng/dL), TT4 (5.5-13.5 µg/dL), FT3 (2.3-4.2 pg/mL) and FT4 (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 (36.2±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**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Healthy Control</th>
<th>Hypothyroid group</th>
<th>p value</th>
<th>Hypothyroid group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>TT$_3$ (ng/dL)</td>
<td>129.88±32.69</td>
<td>97.88±37.51</td>
<td>0.178</td>
<td>209.04±155.54</td>
<td>0.004*</td>
</tr>
<tr>
<td>TT$_4$ (µg/dL)</td>
<td>8.58±2.49</td>
<td>5.04±2.77</td>
<td>0.626</td>
<td>12.49±5.47</td>
<td>0.019*</td>
</tr>
<tr>
<td>TSH (µIU/mL)</td>
<td>1.78±1.67</td>
<td>42.53±46.08</td>
<td>0.000**</td>
<td>0.12±0.03</td>
<td>0.000**</td>
</tr>
<tr>
<td>FT$_3$ (pg/mL)</td>
<td>3.01±0.46</td>
<td>2.15±0.84</td>
<td>0.025*</td>
<td>5.53±3.93</td>
<td>0.001*</td>
</tr>
<tr>
<td>FT$_4$ (ng/dL)</td>
<td>1.31±0.21</td>
<td>1.02±0.43</td>
<td>0.006*</td>
<td>2.17±1.78</td>
<td>0.001*</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>36.24±7.61</td>
<td>57.6±15.8</td>
<td>0.002*</td>
<td>6.4±3.8</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

*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 isofrom in young rats and by an increase in caveolins expression in adult rats [16].

Similar results have been reported in hypothyroidism by other researchers. 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 vitro 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.
Increased 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 endothelial 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-1-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.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


