Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Assessment of oxidative stress and antioxidant status in hyperthyroidism

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ARTICLE HISTORY

Received: 14.02.2016

Accepted: 12.03.2016

Available online: 30.03.2016

Keywords:

Hyperthyroidism, TBARS, SOD, Vitamin E.

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ABSTRACT

Hyperthyroidism leads to hypermetabolic state with stimulation of high oxygen consumption. This study was undertaken to assess the oxidative stress and antioxidant protective activity in different hyperthyroid states.50 hyperthyroid subjects and 50 age and sex matched euthyroid controls have been taken. Serum TBARS (thiobarbituric acid reacting substances), as a marker of the free radical mediated oxidative damage was measured. The activity of free radical scavenging enzyme, plasma SOD (Superoxide dismutase), and the serum alpha tocopherol, were measured as markers of the antioxidant status. Hyperthyroid patients had mean T4 value 17.1 ± 1.2 µg/dl (in euthyroid cases 7.99 ± 0.58, p = 0.000), mean TSH 0.14 ± 0.08 µIU/ml (in euthyroid 2.57 ± 0.98, p = 0.000). Mean serum TBARS level in hyperthyroid cases 5.76 ± 0.34 nmol/ml (in euthyroid 2.91 ± 0.22 nmol/ml, p = 0.001), Mean plasma SOD in hyperthyroids 8.24 ± 0.98 IU/ ml (in euthyroid 7.75 ± 0.70, p = 0.01), Mean serum alpha tocopherol 10.28 ± 0.33 mg/L (in euthyroid 12.1 ± 0.27, p = 0.000). Comparison between mean plasma SOD value and mean serum TBARS in hyperthyroid patients show positive correlation and between vitamin E level and TBARS level shows strong negative correlation. Assessment of disturbances in the antioxidant systems might be useful indicator of the susceptibility of patients with hyperthyroidism to free radical damage and suggests usefulness of supplementation with antioxidants in order to prevent oxidative damage in these patients.

INTRODUCTION

Thyroid hormones are only known iodine-containing compounds with biological activity in living systems. Disorders of thyroid functions are one of the most common endocrinal disorders. Hyperthyroidism is a hypermetabolic disorder caused by excessive production and secretion of free T_4 and T_3. The disorder may be due to primary defect in the thyroid gland itself or secondarily due to a deranged regulation of hypothalamo-pituitary-thyroid axis. Acceleration of basal metabolic rate and the energy metabolism of tissues in several mammalian species represent one of the major functions of thyroid hormones [1]. It maintains the optimal level of metabolism in virtually all tissues in mammalian species. Their major effects include stimulation of oxygen consumption and thermogenesis [1]. In aerobic cells, reactive oxygen species (ROS) like superoxide anion, hydrogen peroxide and hydroxyl radicals are generated as a by-product of oxidative metabolism. These reactive oxygen species are toxic to biomembranes and other vital cellular components and unless removed by free radical scavenging enzymes or antioxidants, lead to peroxidation of membrane phospholipids and tissue damage [2, 3]. Free radical mediated oxidative damage of molecules has been implicated in the pathogenesis of a large number of human diseases, diabetes mellitus, neurodegenerative disorders and thyroid disorders [4 8]. Accumulating evidences have suggested that hypermetabolic state in hyperthyroidism is associated with an increase in free radical production and lipid peroxide levels [2, 9]. However it was not clear whether thyroid hormone induced increase in lipid peroxidation was generalised or confined to some tissues only. Also the response of the antioxidant protective

system to free radical mediated oxidative damages in hyperthyroidism was unclear. Keeping these facts in mind, this study was undertaken to assess the oxidative stress and antioxidant protective activity in different hyperthyroid states and to determine if changes in mitochondrial oxidative metabolism due to thyroid dysfunction lead to modification of peroxidative process and antioxidant defence system activities in hyperthyroidism.

MATERIALS AND METHODS

It is a hospital based case control study. 50 hyperthyroid subjects and 50 age and sex matched euthyroid controls have been taken on the basis of their clinical history and serum levels of total T4 and TSH in their fasting blood samples. Patients and controls were not suffering from any other disorder at the time of presentation. Total T4 and TSH levels were measured by ELISA method using kits supplied by Transasia Bio-Medicals Ltd. Serum TBARS, a stable end product of lipid peroxidation was selected as a marker of the free radical mediated oxidative damage. On the other hand, the activity of free radical scavenging enzyme, plasma SOD, and the serum alpha tocopherol, a non-enzymatic antioxidant were selected as markers of the antioxidant status. Fasting plasma samples were taken in heparinised vials for plasma and in vials without any anticoagulant for serum. Plasma and serum were separated by centrifugation. Then SOD in plasma and MDA and vitamin E in serum were measured. For measurement of SOD 1.35 ml of double distilled water, 50 µl of plasma, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, prepared by dissolving 1.154gm sodium pyrophosphate in up to 100 ml of distilled water and pH adjusted by adding NaOH and HCl), 0.1 ml of PMS (N-methyl phenazonium methosulphate 6mg/ 10ml) and 0.3 ml of NBT (Nitroblue tetrazolium 26 mg/dl), were mixed in a centrifuge tube. 0.2 ml of NADH solution (2mg /ml) was added to it to initiate the reaction. After incubation at 39ºC for 90 seconds the reaction was terminated by adding 1ml of glacial acetic acid and 4 ml of n-butanol was added and mixed vigorously by vortexing. Then the mixture was centrifuged at 4000 rpm for 10 minutes and the absorbance of the upper butanol layer was measured at 560 nm. For the comparison blank was prepared in the same way except addition of the plasma. Calculation was done by percentage inhibition in mixture with sample in respect of blank and expressed as unit of enzyme per µl of plasma. 1 unit of SOD activity was defined as the amount of enzyme that inhibits rate of reactions by 50% under specified conditions.

MDA is measured in the following manner: - to 2.5 ml of trichloroacetic acid (20mg/dl), 0.5 ml of freshly prepared serum was added and the tube was allowed to stand for 10 minutes at room temperature. Then 2.5 ml of sulphuric acid (of 0.05 M prepared by adding 2.8 ml of H_2SO_4 to 997.2 ml double distilled water) was added and stirred thoroughly. 3.5 ml of TBA reagent (it was prepared by mixing 200mg of TBA with 100ml of 2M sodium sulphate solution and sodium sulphate solution was prepared by dissolving 28.4gm of anhydrous sodium sulphate and making up to 100 ml by double distilled water) was added to this. The coupling of lipid peroxide with TBA was carried out by heating in boiling water bath for 30 minutes. It was then cooled in water. 4.0 ml of n-butanol was added and chromogen was extracted to organic phase by vigorous shaking and vortexing. Separation of the organic phase was facilitated by centrifugation at 3000 rpm for 10 minutes. The supernatant organic phase was pipetted into a clean test tube. Its absorbance was measured at 532 nm wave length by spectrophotometer (spectronic 21). n-butabol was used as blank to assure zero reading. The optical density was noted and level of MDA was calculated from standard curve (standard solutions were prepared of 5nmol/ml, 7.5 nmol/ml, 10 nmol/ml, 12.5 nmol/ml, 15 nmol/ml, 17.5 nmol/ml, and 20 nmol/ml using lipid peroxide solution 1,1,3,3 tetrahydroxypropane). MDA values were expressed in nmol/ml. in case of serum.

Estimation of α tocopherols in serum was done by Baker and Flank method in 1968[23]. Serum tocopherols can be measured by reduction of ferric to ferrous ions which then form a red colour complex with α dipiridyl. Tocopherols being lipid soluble are first extracted into xylene and the absorbance is read at 520 nm. 1.5 ml each of serum (test), standard (DL α tochopherol standard solution 10 mg/L in ethanol) and water as blank were taken into respective stoppered test tubes. 1.5 ml of ethanol was taken to the test and blank tubes and 1.5 ml of water was added to standard tube. 1.5 ml of xylene was added to all test tubes. Tubes were stoppered and mixed the contents and then centrifuged. 1 ml of each xylene layer was transferred in to a clean stoppered tube. 1 ml of α, α' dipiridyl reagent (1.2 gm/L in 1-propanol) was added to each tube, stoppered and mixed. 1.5 ml of the mixture was pipette into colorimeter cuvettes and absorbance was read at 460 nm against blank. 0.33 ml of ferric chloride solution (1.2 gm of $FeCl_3$, $6H_2O$ in 1 L ethanol kept in brown bottle) were added to all these test tubes excluding blank and mixed. Reading of absorbance was taken at 520 nm against blank.

RESULT

The increase in extracellular Cu^+Zn^+ SOD activity was significantly positively correlated to the increase in TBARS levels in hyperthyroidism in comparison to euthyroid cases. Decrease in vitamin E level was also significantly correlated to the increase in TBARS levels in comparison to euthyroid cases.

DISCUSSION

In the present study an effort was made to analyse alterations of the free radical activity and antioxidant defence systems in hyperthyroid cases compared to the euthyroid subjects. Previous few studies have reported that hyperthyroidism tended to enhance

Table 1. : Mean ± SD for thyroid parameters in euthyroid and hyperthyroid subjects

	Hyperthyroid	Euthyroid
Serum Total T4 (µg/dl)	17.1 ± 1.2*	7.99 ± 0.58*
Serum TSH (µIU/ ml)	0.14 ± 0.08*	2.578 ± 0.98*

*P = 0.000

Table 2. : Comparisons of means for different parameters in hyperthyroid and euthyroid subjects

Parameters	Hyperthyroid cases	Euthyroid cases	*p* values
Serum TBARS (nmol/ml)	5.76 ± 0.34	2.91 ± 0.22	0.000
Plasma SOD (IU/ml)	8.24 ± 0.98	7.75 ± 0.70	0.01
Alpha tocopherol (mg/L)	10.28 ± 0.33	12.1 ± 0.27	0.000

Table 3. : Bivariate correlation between different parameters in hyperthyroid and euthyroid patients

Correlation coefficient	Hyperthyroid cases	Euthyroid cases
r_{SOD}	0.988 (p = 0.000)	0.026 (p = 0.861)
$r_{VITAMIN\ E}$	- 0.901 (p = 0.000)	- 0.344 (p = 0.013)

the lipid peroxidation product contents in blood and most tissues based on the measurement of the end products of this process [2, 9, 10, 11, 12, 13]. These end products of lipid peroxidations were used as marker of oxidative stress in hyperthyroid patients in some studies [14, 15,16, 17, 18]. TBARS, the index of lipid peroxidation process, was selected as the marker of oxidative stress in this study. A significant rise ($p = 0.00$) in TBARS level was found in serum of hyperthyroid cases in comparison with euthyroid values. These changes were accompanied by a significant decrease in vitamin E levels and significant increase in SOD activity in hyperthyroid cases comparing the euthyroid parameter. The results of the present study corroborate to the opinion that free radicals, mainly superoxide anion radicals, play a significant role in hyperthyroidism. Increased oxidative stress in heperthyroidism is most probably due to an induction in hypermetabolic state. Increased generation of reactive oxygen species leading to an oxidative stress brought about by hyperthyroid state and impairment of cellular and extracellular antioxidant system potential in blood of hyperthyroid cases where also demonstrated in earlier studies [12, 13]. The reactive oxygen species, which can be inhibitors of free radical chain reactions leading to oxidative damage to biomembrane lipids and other structures, are generated in various subcellular sites like mainly mitochondrial respiratory chain. One of the major effect of thyroid hormone is to increase mitochondrial respiration [19, 20] by increasing the number and activity of mitochondrial respiratory chain components [10]. It has also been suggested that accelerated mitochondrial electron transport, brought about by a thyroid hormone induced hypermetabolic state, results in the increased generation of superoxide anion at the site of ubiquinone [21]. Even it is seen in experimental hyperthyroid state created by thyroid hormone treatment also results in increased rate of $O_2^{\cdot-}$ generation by hepatic microsomal fraction, concomitantly with an enhancement in NADPH oxidase activity and decreased cytochrome P-450 content associated with an elevated level of TBARS in livers which exhibits increased respiration and univalent reduction of oxygen [9]. Now superoxide radicals can lead to formation of many other reactive species, including hydroxyl radicals, which can readily start the free radical process of lipid peroxidation [22]. In this study, SOD activity was increased significantly ($p < 0.01$) and vitamin E level was found to be decreased significantly ($p < 0.01$) in all hyperthyroid cases in comparison to control values. The significantly low level of vitamin E in the hyperthyroid cases most probably resulted from its increased consumption due to significantly increased production of lipid peroxidation products compared to the euthyroid state. The bivariate correlation study supported this where vitamin E levels were significantly negatively correlated to the TBARS levels in both hyperthyroid cases and controls. As alterations in antioxidant systems may play an important role in influencing tissue susceptibility to per oxidative process, the results of this study could be attributed to modifications in the antioxidant defence system activity in hyperthyroid cases. The decrease in the extracellular fluid antioxidant potential, represented by significantly reduced levels of vitamin E in hyperthyroidism might indicate that serum antioxidants are the agents involved in the defence of the organism against oxygen free radical activity that were used up most rapidly. Increased lipid peroxidation in hyperthyroid cases suggested an imbalance between production of oxygen free radicals and antioxidant defences which were significantly reduced. I n this study, the increased lipid peroxidation levels in spite of increase SOD activity could be due to increased production of oxygen free radicals in excess of the capacity of antioxidant enzyme to metabolize them.

CONCLUSION

Assessment of disturbances in the antioxidant systems might be useful indicator of the susceptibility of patients with hyperthyroidism to free radical damage and suggests usefulness of supplementation with antioxidants in order to prevent oxidative damage in these patients.

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