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

Study of antioxidant status and oxidative stress in cases of Hypothyroidism

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ARTICLE HISTORY

Received: 08.07.2016

Accepted: 16.09.2016

Available online: 30.09.2016

Keywords:

Hypothyroidism, TBARS, SOD, Alpha tocopherol, Oxidative stress, Antioxidants.

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ABSTRACT

Hypothyroidism leads to hypometabolic state and low oxygen consumption. This study was undertaken to assess the oxidative stress and antioxidant protective activity in different hypothyroid states. 50 hypothyroid 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. Hypothyroid patients had mean T4 value 2.02 ± 1.0 µg/dl (in euthyroid cases 7.95 ± 0.55, p = 0.000), mean TSH 24.4 ± 8.9 µIU/ml (in euthyroid 2.55 ± 0.95, p = 0.000). Mean serum TBARS level in hypothyroid cases 2.76 ± 0.18 nmol/ml (in euthyroid 2.89 ± 0.19 nmol/ml, p = 0.001), Mean plasma SOD in hypothyroids 7.74 ± 0.51 IU/ ml (in euthyroid 7.76 ± 0.69, p > 0.15), Mean serum alpha tocopherol 12.01 ± 0.45 mg/L (in euthyroid 12.11 ± 0.26, p > 0.72). Comparison between mean plasma SOD value and mean serum TBARS and between vitamin E level and TBARS level in hypothyroid patients show no significant correlation. Hypothyroidism is free of any oxidative stress related damages. Hence supplementation with antioxidants like Vitamin E is not required.

INTRODUCTION

Thyroid hormones are the only known iodine-containing compounds with biological activity in living systems. Hypothyroidism is the most common disorder of thyroid function. It is a hypometabolic state due to deficiency of thyroid hormone secretion. The defect may primarily due to disorder of thyroid gland itself or secondarily due to impaired regulation of hypothalamo-pituitary-thyroid axis. Thyroid hormones maintain the optimal level of metabolism in virtually all the tissues in mammalian species. They stimulate oxygen consumption, thermogenesis and many anabolic processes [1]. They also participate in protein, carbohydrate, lipid metabolism and bone mineral turnover [2]. These hormones target tissues like skeletal muscle, heart, liver and kidney [2]. In aerobic cells reactive oxygen species (ROS) like superoxide anion, hydrogen peroxide, and hydroxyl radicals are generated as a byproduct of oxidative metabolism. They are very toxic to biomembranes and other vital cellular components and lead to peroxidation of membrane phospholipids and tissue damage [3, 4]. Several compounds or anti-oxidants which include reduced glutathione, ascorbic acid, vitamin E, NADPH etc scavenge ROS, suppress their formations and oppose their actions. Free radical mediated oxidative damage of molecules has been implicated in the pathogenesis many human diseases including thyroid disorders, neurodegenerative disorders, diabetes mellitus, vascular and heart diseases, and different types of cancers [5 9]. Hypothyroidism is a hypometabolic state and leads to decrease in free radical production and lipid peroxide levels [10, 11]. There are several conflicting studies on the influences of the thyroid hormones on lipid peroxidation and antioxidant protective system in liver [3, 12, 13], heart [3, 14, 15], brain [16], muscle [3, 14], lymphoid organs [17] and blood [18, 19] in hypothyroid states. Keeping all these facts in mind, the present study was undertaken to assess the oxidative stress and antioxidant protecting system activity in different thyroid disorder states and to determine if any

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

	Hyporthyroid	Euthyroid
Serum Total T4 (µg/dl)	2.03 ± 0.9*	7.95 ± 0.55*
Serum TSH (µIU/ ml)	24.6 ± 8.7*	2.55 ± 0.95*

*P = 0.000

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

Parameters	Hypothyroid cases	Euthyroid cases	p values
Serum TBARS (nmol/ml)	2.74 ± 0.2	2.89 ± 0.19	0.001
Plasma SOD (IU/ml)	7.74 ± 0.51	7.76 ± 0.69	0.15
Alpha tocopherol (mg/L)	12.01 ± 0.45	12.11 ± 0.26	0.72

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

Correlation coefficient	Hypothyroid cases	Euthyroid cases
r_{SOD}	0.187 (p = 0.187)	0.027 (p = 0.862)
$r_{VITAMIN\ E}$	- 0.165 (p = 0.251)	- 0.345 (p = 0.014)

changes in mitochondrial oxidative metabolism due to thyroid dysfunction lead to modification of peroxidative process and antioxidant defense system activities in different thyroid disorders. Serum TBARS, a stable end product of lipid peroxidation was selected as a marker of the free radical mediated oxidative damage. Plasma SOD and serum alpha tocopherol were selected as markers of the antioxidant status.

MATERIALS & METHODS

It is a hospital based case control study. 50 hypothyroid subjects and 50 age and sex matched euthyroid controls have been taken as per their clinical history and serum levels of total T4 and TSH in 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. 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. Vials without any anticoagulant were used for serum samples and fasting plasma samples were taken in heparinised vials. MDA and vitamin E in serum and SOD in plasma were measured. MDA is estimated as follows: - 0.5 ml of fresh serum was mixed with 2.5 ml of trichloroacetic acid (20mg/dl) in a test tube and the tube was allowed to stand for 10 minutes at room temperature (mixture 1). 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 poured to mixture 1 and stirred extensively (mixture 2). 3.5 ml of TBA reagent [prepared by mixing 100ml of 2M sodium sulphate solution (sodium sulphate solution was prepared by dissolving 28.4gm of anhydrous sodium sulphate and making up to 100 ml by double distilled water) with 200mg of TBA] was mixed to the mixture 2 (mixture 3). Heating of mixture 3 in boiling water bath for 30 minutes results in the coupling of lipid peroxide with TBA. 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. Further separation of the organic phase was facilitated by centrifugation at 3000 rpm for 10 minutes. The supernatant organic phase was pipetted into a fresh test tube. Its absorbance was measured at 532 nm by spectrophotometer (spectronic 21). n-butabol was used as blank to adjust zero reading in spectrophotometer. The OD (optical density) was documented and MDA (expressed in nmol/ml) was calculated from standard curve (multiple 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).

α tocopherols in serum was estimated by Baker and Flank in 1968 [20]. It can be measured by reducing ferric to ferrous ions which then form a red colour complex with α dipiridyl. Tocopherols being lipid soluble were first extracted into xylene and the OD was read at 520 nm. 1.5 ml each of water as blank, serum as test, and standard (DL α tochopherol standard solution 10 mg/L in ethanol) were taken into respective 'B', 'T' and 'S' marked stoppered tubes. Further 1.5 ml of ethanol to 'T' and 'B' tubes and 1.5 ml of water to 'S' tube were added. 1.5 ml of xylene was added to all three tubes. Tubes were stoppered and the

contents were thoroughly mixed and centrifuged. 1 ml of each xylene layer was transferred in to a fresh stoppered tube. 1 ml of α, α' dipiridyl reagent (1.2 gm/L in 1-propanol) was added to each tube, stoppered and mixed thoroughly. 1.5 ml of the mixtures were pipetted into colorimeter cuvettes and absorbance were 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 properly. Reading of absorbance was noted at 520 nm against blank.

SOD was measured by mixing 1.2 ml of sodium pyrophosphate buffer (pH 8.3, prepared by dissolving 1.154 gm 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) 0.3 ml of NBT (Nitroblue tetrazolium 26 mg/dl), 50 µl of plasma and 1.35 ml of double distilled water in a centrifuge tube. 0.2 ml of NADH solution (2mg /ml) was poured to it to start the reaction. After incubation at 39ºC for 90 seconds the reaction was stopped by addition of 1ml of glacial acetic acid and 4 ml of n-butanol and then vortexing the mixture vigorously. The mixture was then 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 similarly without adding plasma. Calculation was done by percentage inhibition in mixture with sample in comparison 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.

RESULT

From table 1 it was evident that in hypothyroid patients, the serum level of thyroxine was significantly lower and TSH level is significantly higher than control group. In Table 2 showed the effect of changes in thyroid status in lipid peroxidation and antioxidant activity in blood. TBARS level was significantly lower in hypothyroid cases than control group. But there was no significant differences in SOD activity and vitamin E level between hypothyroid and euthyroid group. In Table 3, no significant correlation found between TBARS and Vitamin E level as well as TBARS and SOD activity.

DISCUSSION

In hypothyroid cases, the TBARS levels in serum were found to be decreased significantly in comparison with euthyroid values. In hypothyroidism, metabolic suppression brought about by low levels of thyroxine should give rise to reduced generation of reactive oxygen species from the mitochondrial electron transport chain and the microsomal fraction. Reduced generation of reactive oxygen species thus led to decreased production of lipid peroxidation end product in hypothyroidism. Similar results were found with some previous investigations [14, 15]. The differences between the hypothyroid cases and control group were insignificant and there was no positive correlation between the SOD activity and TBARS levels in the hypothyroid cases. TBARS levels were significantly reduced in hypothyroid cases indicating decreased oxidative stress. Due to absence of oxidative stress, there is no compensatory increase in SOD activity. Similarly differences in vitamin E level between cases and control were insignificant. Bivariate correlation analysis shows no correlation between TBARS level and vitamin E level in hypothyroid cases. As the generation of reactive oxygen species was decreased, there was no consumption of antioxidant vitamin E level in hypothyroid cases. So, there is no significant difference in the level of vitamin E in hypothyroid cases from that of euthyroids.

CONCLUSION

Hypothyroidism is free of any free radical damage or oxidative stress. Hence, regular assessment of TBARS level, SOD activity and vitamin E level is not at all required in hypothyroidism. Supplementation with vitamin E is not beneficial in these patients.

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