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Estimation of Oxidative stress parameters in thyroid dysfunction patients and effect after attainment of euthyroidism with treatment.

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ABSTRACT: Dysfunctions of thyroid gland are among the most common diseases of the endocrine gland in India. The thyroid hormones affect cellular activity of almost all the tissues of the body &are also known to influence free radical production. The study plans to assess the oxidant and antioxidant status in thyroid dysfunction patients and see the effect of treatment.The study was conducted on 43 newly diagnosed hypothyroid patient, 30 newly diagnosed hyperthyroid patients and 49 controls.The levels of oxidative stress markers i.e TBARS, SOD, Catalase (CAT) and Glutathione Peroxidase (GSH-Px) were estimated based on spectrophotometer determination method using commercial kit (Cayman chemical, USA) and Microplate Spectrophotometer (EPOCH-27) manufactured by BioTek Instrument.The data obtained was analyzed using SPSS version "23". Unpaired't' test was used for comparing parameters between controls & hypothyroid /hyperthyroid patient while Paired "t" test was used for comparing the subjects before and after treatment.

There was a decrease in antioxidant enzyme Catalase (CAT),superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in hypothyroid patients though statistically insignificant, which did not improve much with treatment. TBARS (MDA), was increased in hypothyroid patients which decreased with treatment. In hyperthyroid patients, there was a significant increase in levels of TBARS which further increased with treatment. Levels of Catalase and SOD were decreased which on treatment increases but not to that level of controls, while that of GSH-Px was higher in hyperthyroid patients.The study showed that there was a decrease in antioxidant enzymes in both hypothyroid and hyperthyroid patients indicating the presence of reduced antioxidants defense and intensification of oxidative processes in thyroid dysfunction patients, which to some extend improves with treatment.

KEYWORD:Oxidative stress, superoxide dismutase,catalase,glutathione peroxidase, TBARS

I.INTRODUCTION

Thyroid disorder are the most common among all the endocrine diseases in India [1].One of the major effects of thyroid hormone is increase in mitochondrial respiration by altering the components of electron transport chain as well as altering the redox state of the components [2]. This induced hypermetabolic state accelerate free radical production in the mitochondria and induce changes in the anti-oxidant defense system [3]. A free radical is an unstable molecule having one or more unpaired electron in its outer orbital and always tend to undergo a reduction reaction (gain one electron) or oxidation reaction (lose one electron) to attain stability [4]. These free radicals are biologically highly reactive and attack the nearby lipid, proteins and carbohydrates leading to structural and functional damages to nearby molecules [5]. In our body, each cell and its vicinity is furnished by various types of anti-oxidants which accepts the free radical and thus protect the cellular architecture. Therefore, leakage or overproduction of free radical and or decreased effective concentration of anti-oxidants can lead to oxidative stress [6].The term oxidative stress is defined as the stress that results from either excess production of free radicals or decreased effective concentration of anti-oxidants or both [7]. Cells contains enzymatic anti-oxidants likeglutathione reductase, glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and also non-enzymatic anti-oxidants like glutathione (GSH), vitamin E, vitamin C, β-carotene and flavonoids [8].

Association of oxidative stress with hyperthyroidism is clearly implicated by an

increased free radical generation and lipid peroxide levels which exceeds the antioxidant capacity [9].However, hypothyroidism does not support the same theory. In hypothyroidism, there is not only a decrease in free radical production because of metabolic suppression brought about by the decrease in thyroid hormone levels [10] but also there is depression of the anti-oxidant activity[11]. A reduction in the thyroid hormone activity involving decreased reactive oxidative stress production depresses the anti-oxidant activity both enzymatic and non-enzymatic[12].Thyroid stimulating hormone (TSH) directly has a role to produce oxidative stress so, in hypothyroidism due to increase in the level of TSH there is an associated oxidative stress. Therefore the effectiveness in bringing reduction in the levels of stress marker with treatment with L-Thyroxine has been postulated [13].Finding has been inconsistent in most of the studies done earlier. Some studies found normalization of the various parameters measured with attainment of euthyroidism.

Hence the present study aims to assess the oxidant-antioxidant status in hypothyroid and hyperthyroid patients and see the effect after attainment of euthyroidism with treatment.

II. MATERIALS ANDMETHODS

This study was conducted from September 2016 to September 2018 on 43 newly diagnosed hypothyroid patient, 30 newly diagnosed hyperthyroid patients and 49 controls, fulfilling the inclusion and exclusion criteria, in the Neurophysiology Lab of the department of Physiology of a tertiary hospital in Northeast India.The subjects were recruited from the Endocrine OPD and thyroid clinic of the hospitaland were diagnosed based on general history, clinical examination and serum levels of fT3, fT4 and TSH. The controls were taken from among the staffs of the hospital, based on clinical examination and general history only. TheInstitutional Ethical Committee clearance was taken and an informed written consent was taken from all the subjects and healthy volunteers after the procedure was explained to them.Patients suffering from any systemic disease like Diabetes mellitus, hypertension, and chronic smokers & alcoholics were excluded from the study.

The subjects were categorized into the following groups:

1. Controls (n = 49)
2. Hypothyroid I (n = 43) : newly diagnosed hypothyroid patients
3. Hypothyroid II (n = 34) : hypothyroid I after attaining euthyroidism
4. Hyperthyroid I (n = 30) : newly diagnosed hyperthyroid patients
5. Hyperthyroid II (n = 25) : hyperthyroid I after attaining euthyroidism

The quantitative estimation of serum fT3,fT4,TSH was done by Chemiluninescence Immunoassay (CLIA) using Benesphera Kit method (manufactured by Avantor). The levels of oxidative stress markers i.e TBARS, SOD, CAT and Glutathione Peroxidase (GSH-Px) were estimated based on spectrophotometer determination method, using commercial kit (Cayman chemical,USA) and Microplate Spectrophotometer (EPOCH-27) manufactured by BioTek Instrument.

The estimation of oxidative stress markers of the controls was done once while that of the hypothyroid and hyperthyroid patients were done before treatment and after 3 months of treatment with attainment of euthyroidism.

III. STATISTICAL ANALYSIS

The data obtained was analyzed using SPSS version "23". Unpaired 't' test was used for comparing parameters between the controls and hypothyroid I/hyperthyroid I. Paired "t" test was used for comparing the subjects before and after treatment i.e. between hypothyroid I and hypothyroid II and between hyperthyroid I and hyperthyroid II. All test were two tailed. Results were expressed as Mean± SD and 'P' value was calculated. P value < 0.05 was considered statistically significant.

IV. RESULTS

The results are based on the study conducted on 43 newly diagnosed hypothyroid patient, (mean age:36.65±10.27 years), 30 newly diagnosed hyperthyroid patients (mean age: 40.33±10.39 years) and 49 controls (mean age: 34.34±6.86 years). Table 1 & Table 2 shows the comparison of serum levels of fT3, fT4, TSH between controls & Hypothyroid I and between controls & Hyperthyroid I.

In our study, as shown in Table 3& 4, there was a decrease in antioxidant enzyme CAT, SOD and GSH-Px in hypothyroid patients though statistically insignificant, which did not improve much with treatment. TBARS (MDA), which is one of the biomarkers for lipid peroxidation was increased in hypothyroid patients which decreased with treatment though it did not reach the control level.

In the hyperthyroid patients, there was a significant increase in the levels of TBARS which further increased with treatment. Levels of CAT

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and SOD were decreased which on treatment increases but not to that level of controls, while that of GSH-Px was higher in hyperthyroid patients. (Table 5&6)

Table 1: Comparison of serum levels of TSH, fT3, fT4 in Control and Hypothyroid I

	Control (Mean±SD)	Hypothyroid I (Mean±SD)	t	p value
TSH(m IU/L)	2.62±0.67	12.82±6.24	-10.66	0.000***
fT3 (pg/ml)	2.89±0.55	1.56±0.70	9.81	0.05
fT4 (mg/dl)	1.22±0.35	1.19±0.98	0.18	0.02*

*p<0.05 ***p<0.001

Table 2: Comparison of serum levels of TSH, fT3, fT4 in Control and Hyperthyroid I

Parameters	Control (Mean±SD)	Hyperthyroid I (Mean±SD)	t	p value
TSH(m IU/L)	2.62±0.67	0.24±0.59	15.44	0.072
fT3 (pg/ml)	2.89±0.55	9.05±5.45	-7.379	0.000***
fT4 (mg/dl)	1.22±0.35	7.12±3.39	-11.382	0.000***

***p<0.001

Table 3: Comparison of oxidative stress markers in Control and Hypothyroid I

Parameters	Control (Mean±SD)	Hypothyroid I(Mean±SD)	t	p-value
TBARS (MDA)(µ mol/litre)	9.82±3.78	11.38 ±6.05	-1.455	0.149
Catalase (nmol/min/ml)	27.42±9.50	26.54±11.16	0.389	0.698
SOD(U/ml)	0.64±0.13	0.61±0.15	1.229	0.223
GSH-Px(µ mol/litre)	5.02±2.57	4.42±2.89	1.00	0.320

Table 4: Comparison of oxidative stress markers in Hypothyroid I and Hypothyroid II

Parameters	Hypothyroid I(Mean±SD)	Hypothyroid II(Mean±SD)	t	p-value
TBARS (MDA)(µ mol/litre)	11.38±6.05	12.13 ±7.08	-0.422	0.675
Catalase (nmol/min/ml)	26.54±11.16	26.53±10.17	0.003	0.998
SOD(U/ml)	0.61±0.15	0.61±0.16	-0.283	0.779
GSH-Px(µ mol/litre)	4.42±2.89	4.20 ±2.72	0.304	0.763

Table 5: Comparison of oxidative stress markers in Control and Hyperthyroid I

Parameters	Control (Mean±SD)	Hyperthyroid I(Mean±SD)	t	p-value
TBARS (MDA)(µ mol/litre)	9.82±3.78	10.91 ± 5.01	-1.055	0.027*
Catalase (nmol/min/ml)	27.42±9.50	24.20±9.30	1.39	0.510
SOD(U/ml)	0.64±0.13	0.61±0.16	1.018	0.585
GSH-Px(µ mol/litre)	5.02±2.57	5.20±3.33	-0.251	0.103

*p- value < 0.05 (significant)

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Table 6: Comparison of oxidative stress markers in Hyperthyroid I and Hyperthyroid II

Parameters	Hyperthyroid I(Mean±SD)	Hyperthyroid II(Mean±SD)	t	p-value
TBARS (MDA)(µ mol/litre)	10.91±5.01	11.90 ±5.73	-0.742	0.465
Catalase (nmol/min/ml)	24.21±9.30	28.09±9.65	-1.298	0.207
SOD(U/ml)	0.61±0.16	0.61±0.18	0.045	0.965
GSH-Px(µ mol/litre)	5.20±3.33	4.38±2.38	0.848	0.405

V. DISCUSSION

In our study there was a decrease in antioxidant enzyme Catalase (CAT), SOD and GSH-Px in hypothyroid patients,though statistically insignificant which did not improve much with treatment. TBARS (MDA), which is one of the biomarkers for lipid peroxidation was increased in hypothyroid patients which decreased with treatment though it did not reach the control level. Similar to our findings, Sumit et al,[14]also found higher level of MDA in treatment naïve primary hypothyroid patient which reduced significantly after treatment with L-Thyroxine.Carmeli et al.,[15]in their study found that patients with hypothyroidism have significantly lower level of SOD, Catalase, Glutathione peroxidase(GSH Px) and glutathione reductase as compared to healthy normal.

The decrease in antioxidant enzymes in hypothyroid may reflect the increased free radical production at the electron transport chain on the mitochondrial inner membrane. Hypothyroidism increases the oxidation of plasma cholesterol mainly because of an altered pattern of binding and to the increased levels of cholesterol, which presents a substrate for the oxidative stress [16]. Studies have shown a reduction of antioxidant defense in hypothyroidism leading to lipid peroxidation and this may play a role in the pathogenesis of atherosclerosis in hypothyroidism.

In the present study,there was a significant increase in the levels of TBARS which further increased with treatment in hyperthyroid patients. The levels of Catalase and SOD were decreased which on treatment increases but not to that level of controls, while that of GSH-Px was higher in hyperthyroid patients.Our findings are similar to that of Pasupathi P et al.,[17]and Sahoo DK et al., [18]where they found decreased CAT activity inboth hypothyroid and hyperthyroid patients. Contrary to our findings, Imran et al,[19] found increased level of SOD and CAT in female hyperthyroid patients as compared to male patients and control groups while GSH remains the same. Bharat et al.,[20] also foundincreased level of SOD while Glutathione Peroxidase activity was significantly lowered in hyperthyroidism as compared to normal.

In a study conducted by Saeed Naazeri et al, [21]the total antioxidant capacity in individuals with hyperthyroidism decreases as compared to healthy controls but individuals with hypothyroidism showed no significant differences as compared to the healthy controls. Catalase and superoxide dismutase activity in hypothyroidism and hyperthyroidism were significantly increased as compared with healthy controls.

In our study, there was a decrease in antioxidant enzymes in both hypothyroid and hyperthyroid dysfunction indicating presence of reduced antioxidant defense and intensification of oxidative process in thyroid dysfunction patients.Acceleration of the basal metabolic rate and the energy metabolism of tissue in several mammalian species represents one of the major functions of thyroid hormones [22]. Studies have suggested that the hypermetabolic state in hyperthyroidism is associated with increase in free radical production and lipid peroxide levels [23,24], whereas the hypometabolic state induced by hypothyroidism is associated with the decrease in free radical production [25] and in lipid peroxidation products [26].Literature reveals that susceptibility of tissues to oxidative challenge in both hypothyroid and hyperthyroid states is generally higher than in the euthyroid[27].

VI. CONCLUSION

Oxidative stress is an imbalance between the production of pro-oxidant substances and anti-oxidant defenses. Antioxidant defense mechanism plays a crucial role in reducing the increased level of free radicals generated by the thyroid gland dysfunction. In our study, there was a decrease in antioxidant enzymes in both hypothyroid and hyperthyroid patients & following treatment, oxidative stress takes longer duration to normalize than the thyroid profile which is seen in our study and thus may arise the need for supplementing antioxidant therapy in thyroid dysfunction patients.

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