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Comparison Between Oxidant/Antioxidant Levels In Blood And Tissues Of Patients With Thyroiditis And Thyroid Adenocarcinoma.

Enas A. Oraby*.

Department of Toxicology, Mansoura Hospital, Faculty of Medicine, Mansoura University, Mansoura, 35516, Egypt.

ABSTRACT

Oxidative stress may result from overproduction of free radicals or oxidants as well as the deficiency of antioxidant defense system which destroy these harmful species. The present study aims to compare the total oxidant/antioxidant levels in both blood and tissues of thyroiditisand thyroid adenocarcinoma patients. Methods:The study included 145 subjects divided into three groups thyroiditis patients (n=59), thyroid adenocarcinoma patients (n=56) and healthy control subjects (n=30). Different antioxidant activities were evaluated, these include, Superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) as well as total antioxidant capacity (TAC). Oxidative stress was evaluated by measuring the level of lipid peroxidation end product malondialdehyde (MDA) in both blood and tissues of thyroid patients. Results: blood evaluation in both thyroiditis and thyroid adenocarcinoma patients showed a significant decrease in SOD, GSH, CAT, and TAC while MDA showed a significant increase when compared with blood of control healthy subjects. The evaluation of antioxidant enzyme activities in tissue samples also showed a significant decrease especially CAT enzyme and a significant increase in MDA in thyroid adenocarcinoma patients when compared to tissues of thyroiditis patients. Conclusions: The present study suggests a failure of antioxidant defense mechanisms with enhanced lipid peroxidation this oxidant/antioxidant imbalance may act as an indicator for the development of thyroid carcinoma. It is recommended to evaluate antioxidants level especially CAT enzyme in thyroiditis as a predictive marker for thyroid carcinoma development.

Keywords: Thyroid, Superoxide dismutase, Catalase, Glutathione, Total antioxidant capacity, Malondialdehyde, Adenocarcinoma.

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Corresponding author

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INTRODUCTION

Oxidative stress was defined as the disturbance in balance between excessive productions of reactive oxygen species (ROS) and the deficiency of antioxidant defense system [1]. ROS are highly reactive species; consequently they are harmful and may induce oxidative damage in cellular large molecules as proteins, lipids, and DNA [2]. The cell contains different enzymatic and non-enzymatic antioxidants that capable of reducing and eliminating these free radicals and protecting cell from harmful effects such as catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD) [3], ROS levels are controlled by these antioxidants therefore oxidative stress develops when ROS increase and the antioxidant capacity decrease [4].Malondialdehyde (MDA) is the lipid peroxidation end product which used as a marker for oxidative damage of cells [5]. To prevent oxidative damage there must be a balance between the formation and removal of lipid peroxides, this balance could be disrupted due to the lack of cellular defense mechanisms and increased peroxidation reactions [6].

It is postulated that any variations in thyroid hormones or thyroid dysfunctions may associated with enhanced oxidative stress which as a consequence may induce inflammation [7,8]. Oxidative stress can participate in the pathogenesis and complications of many diseases including cancer [9]. Thyroid adenocarcinoma is one of the common endocrine cancers; hence an interest has grown in studying the role played by oxidative stress in thyroid carcinogenesis [10].

Thyroid hormones organize the biosynthesis of both enzymatic and non-enzymatic antioxidants [11]. Enzymatic antioxidants are such ascatalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-disulfide reductase (GSR), and non-enzymatic antioxidants are such as vitamin E and C and glutathione (GSH), the changes in these enzymatic and non-enzymatic antioxidants affect the reduction-oxidation balance in the body cells and, in turn, regulates thyroid function [12]. One of the important roles of thyroid hormones is to increase mitochondrial respiration, which results in excessive increase of ROS, leading to oxidative damage to membrane lipids [13].

When thyroid hormones produced, oxidants are generated and oxidative stress increased as a part of the process. Under normal reduction-oxidation balance, the ROS are eliminated by antioxidant systems, thus limiting oxidative damage. However, when abnormal conditions such as thyroiditis as well as thyroid carcinoma may induce imbalance between ROS and antioxidant levels [14] subsequently leading to oxidative damage [15].

This work is aimed to compare the total oxidant/antioxidant levels in both blood and tissue between thyroiditisand thyroid adenocarcinoma patients.

SUBJECTS AND METHODS

Subjects

The study included 145 subjects divided into three groups thyroiditis patients (n=59), thyroid adenocarcinoma patients (n=56) and healthy control subjects (n=30) All thyroid subjects under study were recruited from Oncology hospital, Mansoura University.Consent was received from thyroid and healthy volunteer participants.

Methods

Preparation of samples

Blood samples were collected from all patients immediately before thyroidectomy. Under aseptic precautions, 2ml blood was drawn from the site. After sampling and centrifugation, the serum samples were stored at -20ºC until analysis. After thyroidectomy thyroid tissues samples of both thyroiditisand thyroid adenocarcinoma patients were homogenate using phosphate buffer saline (PBS).

All kits were obtained from Biodiagnostic and were measured spectrophotometrically(SpectraMax M5, Molecular Devices) according to the work pamphlets of each enzyme. The activity of superoxide dismutase (SOD) was estimated by Nitro blue tetrazolium (NBT) method [16], glutathione (GSH) was estimated

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by 5,5'dithiobis (2-nitrobenzoic acid) (DTNB) method [17], catalase (CAT) was estimated by H_2O_2 consumption method [18], total antioxidant capacity (TAC) was estimated by 3,5 dichloro -2- hydroxy benzensulphonate method [19] and the level of lipid peroxidation product malondialdehyde (MDA) was estimated by thiobarbituric acid reactive substances (TBARS) method [20].

Statistical analysis

Data were evaluated using the Statistical Package for Social Sciences (SPSS) software, version 20 for Windows (IBM Corp., Armonk, NY, USA). All results are expressed as the mean &S.E. When P values <0.05 the results were considered statistically significant.

RESULTS

The results of this study are shown in the following tables and figures. In table (1) and figures (1,2) the level of antioxidants SOD,CAT,GSH, and TAC showed a significant decrease in blood of both thyroiditis and thyroid adenocarcinoma patients compared with healthy control subjects (P<0.05), while MDA shows a significant increase in blood of both thyroiditis and thyroid adenocarcinoma patients compared with healthy control subjects (P<0.05).

Blood Parameters	Control	Thyroiditis	Thyroid Carcinoma
GSH	2.37±0.12	1.98±0.41*	1.7±0.04*
CAT	504.22±63.34	184.96±12.73*	153.61±15.37*
SOD	252.29±24.62	122.59±13.23*	115.54±10.76*
TAC	4.82±0.24	1.89±0.04*	1.50±0.03*
MDA	0.57±0.07	1.79±0.22*	2.28±0.12*

Results are presented as mean ± SE.

*Significant difference between blood samples of thyroiditis and thyroid carcinoma compared with blood samples of control group P<0.05

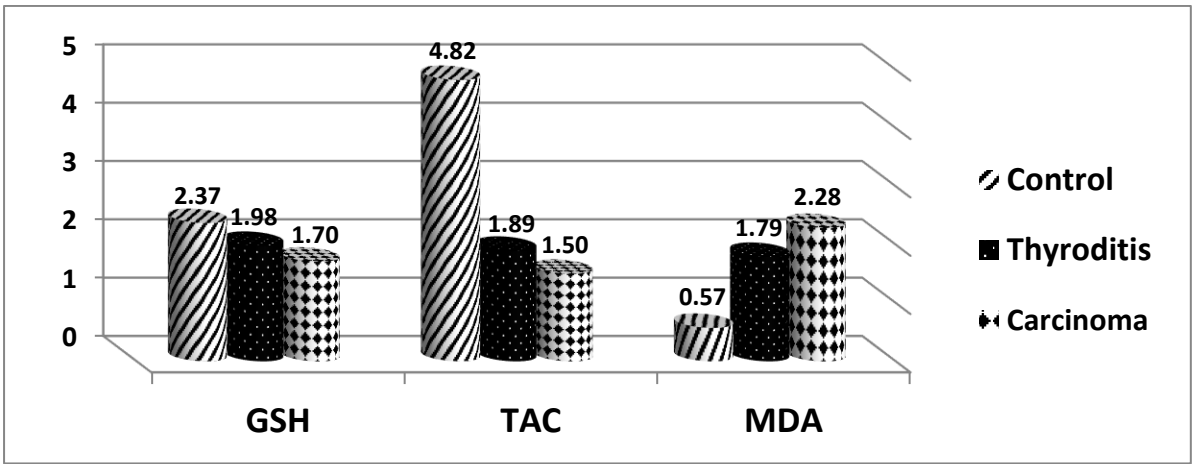

Figure (1): Illustrating the antioxidant glutathione (GSH), total antioxidant capacity (TAC) and the oxidative stress malondialdehyde (MDA) in blood of thyroiditis and thyroid adenocarcinoma patients compared with blood of healthy control.

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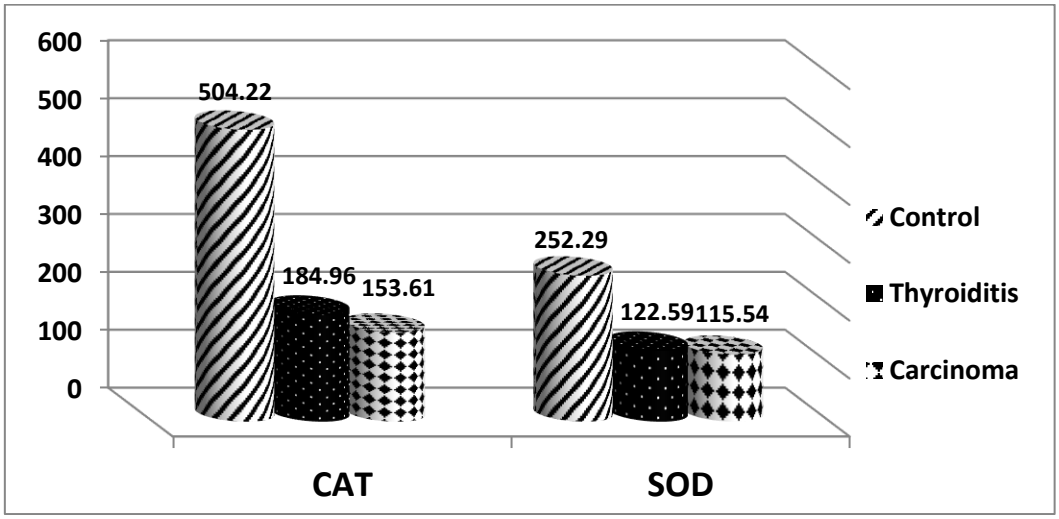

Figure (2): Illustrating the antioxidants catalase (CAT) and superoxide dismutase (SOD) in blood of thyroiditis and thyroid adenocarcinoma patients compared with blood of healthy control.

In table (2) and figures (3,4) the level of antioxidants SOD,CAT,GSH, and TAC showed a significant decrease in tissues (T) of thyroid adenocarcinoma patients compared with thyroiditis patients (P<0.05), while the lipid peroxidation MDA shows a significant increase in tissues of thyroid adenocarcinoma patients compared with thyroiditis patients (P<0.05).

Tissue Parameters	Thyroiditis	Thyroid Carcinoma
GSH	2.24±0.09	1.55±0.08*
CAT	95.0±12.62	37.03±4.68*
SOD	183.27±21.45	148.57±10.42*
TAC	1.68±0.05	1.50±0.04*
MDA	1.74±0.08	2.67±0.23*

Results are presented as mean ± SE. *Significant difference between tissue samples of thyroid carcinoma patients compared with tissue samples of thyroiditis patients P<0.05.

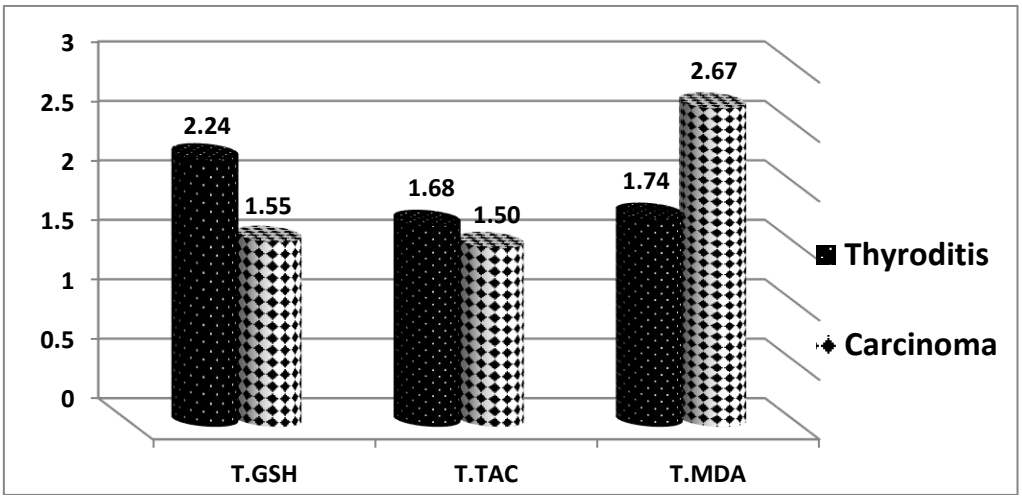

Figure (3): Illustrating the antioxidant glutathione (GSH), total antioxidant capacity (TAC) and the oxidative stress malondialdehyde (MDA) in tissues (T) of thyroid adenocarcinoma patients compared with tissues (T) of thyroiditis patients.

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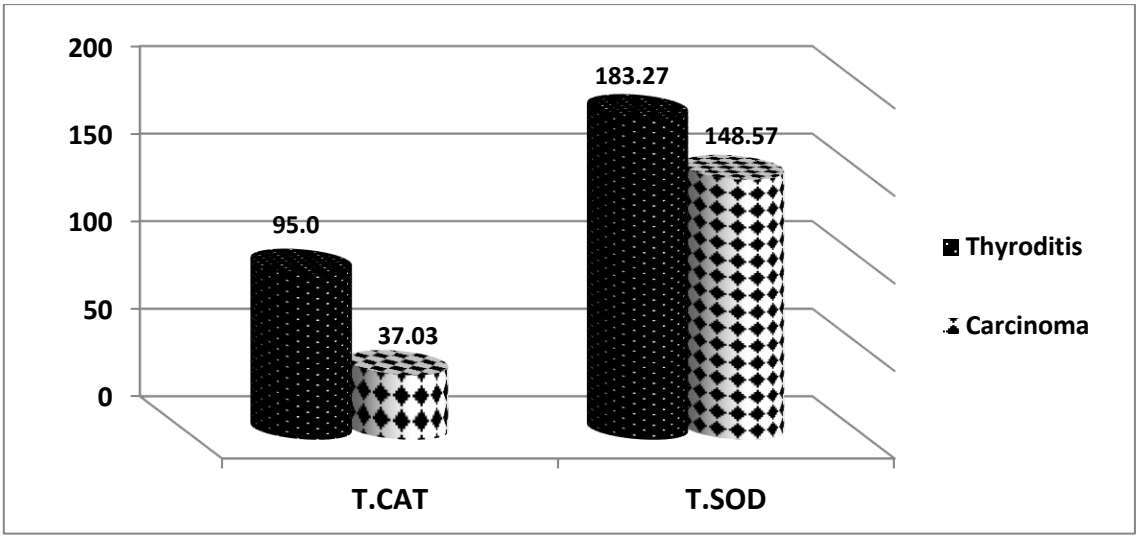

Figure (4): Illustrating the antioxidants catalase (CAT) and superoxide dismutase (SOD) in tissues (T) of thyroid adenocarcinoma patients compared with tissues (T) of thyroiditis patients.

In table (3) blood and tissues were compared in both thyroiditis and thyroid adenocarcinoma patients. The level of antioxidants SOD, CAT, GSH, and TAC showed a significant decrease in both blood and tissues of thyroid adenocarcinoma patients compared with blood and tissues of thyroiditis patients ($P<0.05$), while the lipid peroxidation MDA shows a significant increase in blood and tissues of thyroid adenocarcinoma patients compared with blood and tissues thyroiditis patients ($P<0.05$).

	Thyroiditis	Carcinoma	Thyroiditis	Carcinoma
	Blood	Blood	Tissues	Tissues
GSH	1.98±0.41	1.7±0.04*	2.24±0.09	1.55±0.08**
CAT	184.96±12.73	153.61±15.37*	95.0±12.62	37.03±4.68**
SOD	122.59±13.23	115.54±10.76*	183.27±21.45	148.57±10.42**
TAC	1.89±0.04	1.60±0.03*	1.68±0.05	1.50±0.04**
MDA	1.79±0.22	2.28±0.12*	1.74±0.08	2.67±0.23**

Results are presented as mean ± SE.

*Significant difference between blood samples of thyroid carcinoma patients compared with blood samples of thyroiditis patients $P<0.05$.

** Significant difference between tissues samples of thyroid carcinoma patients compared with tissues samples of thyroiditis patients $P<0.05$.

DISCUSSION

Reactive oxygen species (ROS) when associated with deficiency of the antioxidant defense system have a pathogenic effect on human tissues and the development of several diseases as cancer [21]. Measuring antioxidant activities and MDA levels are important indicators of the oxidant/antioxidant status.

Free radicals and oxidants which increased in thyroid cancer condition are supposed to be due to the increased lipid peroxidation and the deterioration of antioxidant defense system [5]. In our study, MDA levels were found to be higher in both blood and tissues of thyroiditis and thyroid adenocarcinoma patients than those in controls; also MDA was found to be higher in thyroid adenocarcinoma patients than thyroiditis

patients that indicating increased free radical generation. Similar to our studies [4,5,22,23]found that MDA showed significantly higher concentrations in tissues of thyroid carcinoma than normal thyroid tissues .

The elevation of free radicals in thyroid cancer is explained by the increased lipid peroxidation and damage of the antioxidant system [24]. In a study of Al-Sowdani [25] considered that an increase of MDA was observed along with an increase of SOD activity which may suggest that SOD activity as antioxidant is insufficient to destroy lipid peroxidation. Considering euthyroid status of patients under study, it can be assumed that there could be a shift to the oxidant pathway. As a result, evaluation of MDA levels may be important after thyroidectomy to confirm the treatment success in thyroid carcinoma patients. Thus, there is a need for other researches.

Sugawara [26] and Durak [27] showed that SOD activity in thyroid goiter and other thyroid disorders including thyroid cancer tissues showed a significant decrease. These results are in agreement with our study, in both blood and tissues of thyroiditis and thyroid carcinoma SOD activity decrease significantly compared with control patients.

Our findings showed that MDA levels were increased, while serum TAC, GSH, and CAT levels were decreased in thyroiditis and thyroid adenocarcinoma patients. These results indicate disturbance in balance between oxidants and antioxidants and this disturbance associated with thyroid disorders. Free radicals and ROS are participating in many pathogenic processes occurred in the thyroid gland. Recently, many evidences proved that free radicals have a role in the mechanisms of initiation and development of neoplastic transformations in tissues and in the stimulation of malignant genes. Most of these evidences result from the fact that the agents that destroy free radicals or disrupt the chain of events induced by free radical can inhibit the process of neoplastic formation on both its cellular and molecular levels [28]. Free radicals induce harmful effects on most of the cell components such as DNA, protein, lipid, and carbohydrates, and especially lipids are the most sensitive component [29].

Dong [10] evaluates the total oxidant (TO) and the total antioxidant status (TAS) in thyroid cancer patients and found that TAS levels were decreased in patients with thyroid cancer. Increased levels of oxidants and decreased levels of antioxidants may provide evidences for thyroid patients who were exposed to potent oxidative stress. Also in other previous investigations [5,15,30,31,32] TAC, GSH and CAT levels were decreased in Thyroid cancer patients. In our study, we found that TAC, GSH and CAT levels decreased in blood and tissues of both thyroiditis and thyroid adenocarcinoma patients compared with control and they are lower in carcinoma than thyroiditis patients.

In fact, measuring of antioxidant enzyme activities as CAT, GSH, SOD and the evaluation of TAC in blood are good indicators for the oxidant/antioxidant status but do not specifically indicate their real changes occurring in the thyroid directly, because many factors are involved and can modify the concentration of blood antioxidant enzymes so in our study we measure all parameters in thyroid tissues to be more precise.

Ramli [33] reported that the malignancy of the thyroid gland may increase the generation of high levels of ROS, which consequently increase oxidative stress which in turns lowers the antioxidants activity.

Catalase is an antioxidant enzyme which catalyzes the hydrogen peroxide decomposition, the increase of hydrogen peroxide concentration oxidizes cellular components so its elimination is vital for cells [34,35]. In this study, it was observed that catalase may act as a good indicator for thyroid carcinoma that it was decreased significantly in blood and tissues of thyroid adenocarcinoma patients.

CONCLUSIONS

The present study suggests a failure of antioxidant defense mechanisms with enhanced lipid peroxidation this oxidant/antioxidant imbalance may act as a predictor of the risk factor of thyroid carcinoma development. It is recommended to evaluate antioxidants level in thyroiditis as a predictive marker especially CAT for thyroid carcinoma development.

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